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Synthesis and degradation characteristics of salicylic acid-derived poly(anhydride-esters)

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Abstract

A biodegradable poly(anhydride-ester) was synthesized by melt condensation polymerization of the acetylated monomer to yield a novel polymeric prodrug. The polymer we have synthesized is composed of alkyl chains linked by ester bonds to aromatic moieties, specifically salicylic acid—the active component of aspirin. With the medicinal properties attributed to salicylic acid and the ease of metabolism, the incorporation of this compound into a polymer backbone yields a polymeric prodrug that may have potential in a variety of applications (i.e., inflammatory bowel disease). For these reasons, we have designed a synthetic scheme that yields the desired poly(anhydride-ester). The *in vitro* hydrolytic degradation of these polymers has been performed and results indicate that the polymer degradation rate is pH-dependent. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In recent years, many polymeric and conjugated prodrugs have been developed which prolong the blood concentrations of the drug upon administration as compared to the free drug given alone [1–4]. This phenomenon is indicative of the prodrugs' ability to release the drug in a controlled manner ultimately allowing for site-specific drug targeting [5–7] and reduced side effects which may be incurred if the drug is released immediately. In two examples, salicylic acid-L-alanine conjugate and Naproxen coupled via lactic acid to a low molecular weight protein, significantly extend the drug lifetime in the blood relative to free drug [2–5].

One factor that has considerable impact when developing polymeric prodrugs is the amount of drug attached to the polymer. In most cases, drug is attached to a polymer via a linker molecule that limits the drug attachment, or drug loading. For example, when ibuprofen is covalently attached via side chains to a methacrylic polymer backbone, this polymeric prodrug has a maximum of 55 wt% of attached drug [1]. Other polymeric prodrugs include 5-aminosalicylic acid coupled to an

acrylic polymer backbone and iodoxuridine coupled to poly(D,L-lactic acid) in which the maximum drug content is 1 and 20 wt%, respectively [8,9]. With most polymer prodrug systems, the polymer backbone is water-soluble but does not degrade, which may be a drawback for some applications.

We designed a polymeric prodrug that allows for a higher percentage of deliverable drug (62 wt%) that is available as the polymer degrades. Unlike other polymeric prodrug systems, our polymers are unique in that the drug is chemically incorporated into the polymer backbone—not attached as a side group. This design allows for a maximum amount of drug to be incorporated within the polymer structure—up to 100 wt% is possible. An additional feature is that the polymer completely degrades because of the anhydride and ester linkages within the polymer backbone.

In this paper, we describe the synthesis of a poly(anhydride-ester) (I) composed of alkyl chains linked by ester bonds to aromatic moieties of salicylic acid (II). The polymer, by design, undergoes hydrolytic degradation to release salicylic acid (Scheme 1).

Salicylic acid (II) is an antipyretic, anti-inflammatory analgesic with a half-life of 2–3 h in low doses and 20 h in higher doses [10]. With the medicinal properties attributed to salicylic acid (II) and the ease of metabolism, the incorporation of this compound into a polymer

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chloride/petroleum ether yielded the pure diacid (m.p. 130–132°C, 98% yield) (**VII**). Analytically calculated for $C_{22}H_{26}O_8$ (418): C, 63.15; H, 6.26. Found: C, 64.50; H, 5.73. $^1\text{H NMR}$ (CDCl_3) δ : 8.15 (d, 2H, Ar-H), 7.60 (t, 2H, Ar-H), 7.35 (t, 2H, Ar-H), 7.15 (d, 2H, Ar-H), 2.60 (t, 4H, CH_2), 1.80 (t, 4H, CH_2), 1.45 (m, 8H, CH_2). IR (neat): 1748 cm^{-1} (C=O, phenyl ester), 1694 cm^{-1} (C=O, carboxylate group).

The diacid (**VII**) (2.00 g, 4.52 mmol) was acetylated using excess acetic anhydride (52.0 ml) heated to reflux temperature (145°C) for 2 h under dry nitrogen. Excess acetic anhydride was removed under vacuum while heating (55°C) to yield monomer (**VIII**) as a pale-yellow oil (quantitative yield). Analytically calculated for $C_{28}H_{30}O_{10}$ (526): C, 63.90; H, 5.70. Found: C, 64.82; H, 5.68. $^1\text{H NMR}$ (CDCl_3) δ : 8.05 (d, 2H, Ar-H), 7.55 (t, 2H, Ar-H), 7.35 (t, 2H, Ar-H), 7.20 (d, 2H, Ar-H), 2.60 (t, 4H, CH_2), 2.20 (s, 6H, CH_3), 1.75 (t, 4H, CH_2), 1.40 (m, 8H, CH_2). IR (neat): 1765 cm^{-1} (C=O, phenyl ester), 1792 cm^{-1} and 1736 cm^{-1} (C=O, anhydride).

2.4. Poly(anhydride-ester) synthesis

Melt condensation polymerization was performed at 200°C for 3 h under vacuum (2 mm Hg) using 1.00 g of monomer. Polymerization tubes were dried overnight in an oven (100°C). The reaction mixture was flushed with dry nitrogen every 15 min and the polymerization determined complete when the mixture became a hard solid. The polymer was isolated by precipitation into diethyl ether from methylene chloride, filtered, and dried under vacuum overnight to obtain a light brown solid (quantitative yield) (**I**). Analytically calculated for $C_{24}H_{24}O_7$ (425): C, 67.70; H, 5.64. Found: C, 67.57; H, 5.75. $^1\text{H NMR}$ (DMSO) δ : 8.20 (d, 2H, Ar-H), 7.95 (t, 2H, Ar-H), 7.75 (t, 2H, Ar-H), 7.40 (d, 2H, Ar-H), 2.20 (t, 4H, CH_2), 1.55 (m, 4H, CH_2), 1.25 (m, 8H, CH_2). IR (neat): 1765 cm^{-1} (C=O, phenyl ester), 1792 and 1736 cm^{-1} (C=O, anhydride). GPC: Mw 6000; PDI 1.2. T_g : 23.5°C, $T_{d1} = 397^\circ\text{C}$ (55%, mass loss) $T_{d2} = 441^\circ\text{C}$ (40%, mass loss).

2.5. Hydrolytic degradation of poly(anhydride-ester)

The poly(anhydride-esters) (**I**) were prepared by compression-molding (Carver Laboratory Press, Model M) 50 mg of polymer between two stainless-steel plates at room temperature and 1000 psi for 1 min to give 0.1 mm thick films. Each film was cut into 3 mm squares and placed into scintillation vials containing 10 ml of 0.1 M phosphate buffer saline at pH 3.5, 7, or 10. The vials were then incubated at 37°C for 90 days using an incubator-shaker (New Brunswick Scientific, Series 25, New Brunswick, NJ). Periodically, the degradation media was removed and replaced with fresh buffer solution. Analysis of the spent solutions were performed using HPLC with

UV detection (235 nm). Calibration curves were generated from known concentrations of salicylic acid (**II**) in buffered media. Experiments were performed in triplicate.

2.6. Model compounds

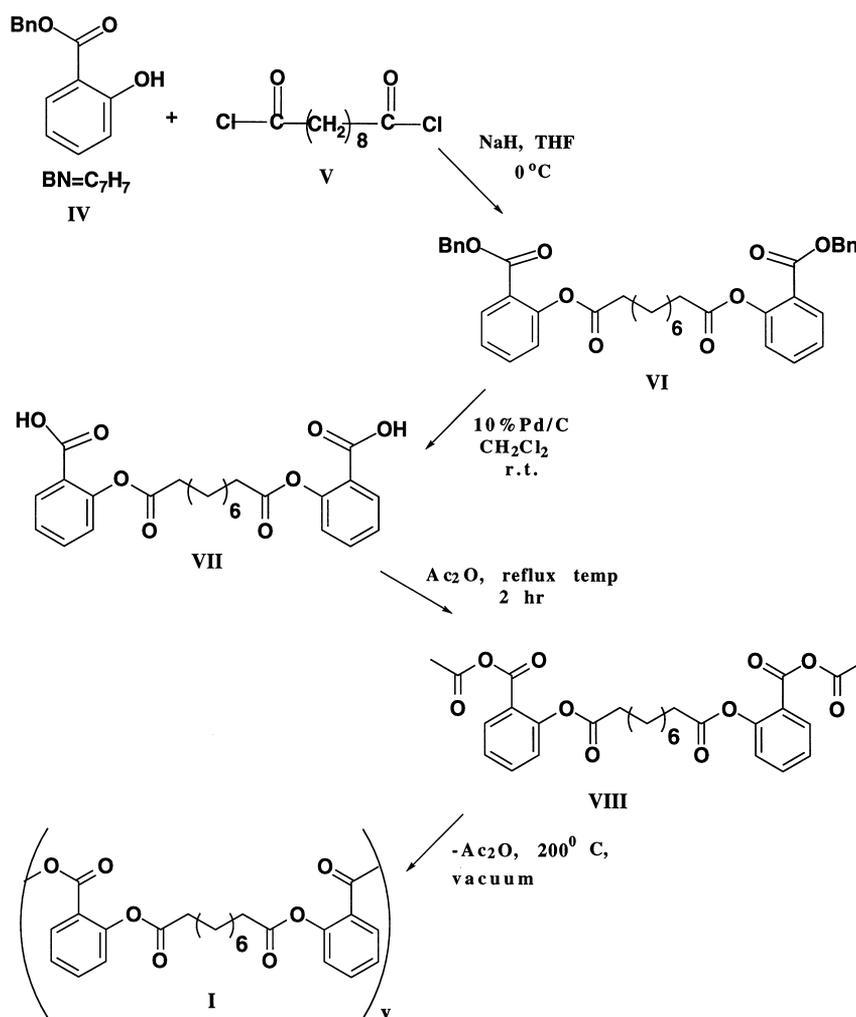
Phenol (**IX**) (0.592 g, 6.29 mmol), decanoyl chloride (1.09 ml, 5.24 mmol) and triethylamine (1.10 ml, 7.86 mmol) were dissolved in tetrahydrofuran (10 ml) at room temperature. Upon completion of the reaction, triethylamine-hydrochloride was removed by filtration, and the filtrate evaporated to dryness. The residue was re-dissolved in methylene chloride, then washed with water (2 \times) and dried over MgSO_4 . Phenyl decanoate (**X**) was isolated as an oil (76% yield). $^1\text{H NMR}$ (CDCl_3) δ : 7.39 (t, 1H, Ar-H), 7.23 (m, 2H, Ar-H), 7.08 (d, 2H, Ar-H), 2.54 (t, 2H, CH_2), 1.73 (m, 4H, CH_2), 1.3 (m, 12H, CH_2), 0.89 (t, 3H, CH_3).

Benzoic acid (**XI**) and phenyl decanoate (**X**) were placed into scintillation vials containing 10 ml of 0.1 M phosphate buffer saline at pH 3.5, 7, or 10. The vials were incubated at 37°C for 7 days using an incubator-shaker (New Brunswick Scientific, Series 25, New Brunswick, NJ). Periodically, the buffered media was removed and replaced with fresh buffer solution. Analysis of the solutions were performed using HPLC with UV detection (235 nm). Calibration curves of known concentrations of benzoic acid (**XI**) and phenol (**IX**) in buffered media were generated to compare the hydrolysis of benzoic anhydride (**XII**) and phenyl decanoate (**X**), respectively.

3. Results and discussion

3.1. Synthesis and characterization

In our initial experiments, we used salicylic acid (**II**) as the starting material because we wanted to incorporate this component into the polymer backbone. However, the insolubility of salicylic acid in organic solvents did not facilitate the coupling reaction with sebacoyl chloride (**V**). In addition, both the carboxylic acid and hydroxyl functional groups were reacting with the sebacoyl chloride under the reaction conditions. Triethylamine as base to remove the hydroxyl proton gave low yields, and complex purification methods were necessary to isolate the product. Alternately, sodium hydride was used as base but both the carboxylic acid and hydroxyl groups were deprotonated leading to incomplete conversions. By protecting the carboxylic acid functionality with a benzyl group to yield benzyl salicylate (**IV**), the reactivity of the phenol was significantly enhanced and the solubility of benzyl salicylate (**IV**) in various organic media gave homogenous reaction conditions.



Scheme 2. Synthesis of poly(anhydride-ester) (I).

An outline of the monomer and polymer synthesis is shown in Scheme 2. The phenolic group of benzylated salicylic acid (**IV**) reacted with the sebacoyl chloride (**V**) to give compound **VI**. The ^1H NMR spectra for compound **VI** indicated that the aromatic protons of **VI** resonate between 8.05–7.10 ppm, which is shifted downfield relative to the aromatic protons of the starting material, benzyl salicylate (**IV**) at 7.90–6.85 ppm. Concurrently, the aliphatic protons of **VI** resonating between 2.40–1.30 ppm shifted slightly upfield compared to the aliphatic protons of sebacoyl chloride (**V**) at 2.90–1.35 ppm. The IR spectra of **VI** also confirmed the presence of the phenyl ester and the benzoate ester: the $\text{C}=\text{O}$ stretches of the phenyl ester and benzoate ester were observed at 1764 and 1720 cm^{-1} , respectively. The benzylic protecting groups of **VI** were removed by catalytic hydrogenation to give the diacid, **VII**. This deprotection reaction must be closely monitored by TLC because the aliphatic ester bond can be cleaved after extended time periods. The ^1H NMR data for **VII** indicated complete removal of the benzyl group as indicated by disappear-

ance of benzylic proton resonance at 5.30 ppm. IR analysis of **VII** revealed absorptions at 1748 and 1694 cm^{-1} corresponding to $\text{C}=\text{O}$ stretches of the phenyl ester and the carboxyl group, respectively. The acid (**VII**) is activated with excess acetic anhydride to give the monomer (**VIII**). Acetylation of **VII** to give the monomer **VIII** was verified by the presence of the acetyl group at 2.20 ppm in the proton NMR spectra and a corresponding upfield shift in the aromatic and aliphatic protons. The poly(anhydride-ester) (**I**) was synthesized using a melt-condensation polymerization performed at 200°C for 3 h under vacuum (2 mm Hg) in a side-armed test tube. The polymerization temperature chosen was based on previous work performed in our laboratory to optimize the polymerization conditions for *ortho*-substituted aromatic polyanhydrides [13]. The reaction mixture was flushed with dry nitrogen and the polymer isolated by precipitation. The poly(anhydride-esters) (**I**) are soluble at room temperature in tetrahydrofuran, dimethylformamide, and methylene chloride yet insoluble in water, methanol, and petroleum ether.

The thermal properties of poly(anhydride-ester) (I) were determined using DSC and TGA. The glass transition temperature (T_g) of the polymer is 23.5°C and therefore, at room temperature, is a rubbery elastic that flows at body temperature (37°C). By TGA, two decomposition transitions were observed at 397 and 441°C corresponding to thermal decomposition of the aliphatic chains followed by thermal decomposition of the aromatic rings.

3.2. In vitro degradation

In this work, degradation was measured by the appearance of salicylate (II) in the degradation media using HPLC. Degradation of poly(anhydride-ester) (I) films at 37°C under acidic (pH 3.5), neutral (pH 7.0) and basic (pH 10) conditions were performed over 90 days. At pH 3.5, degradation was insignificant as determined by visual analysis and the negligible presence of salicylate (II) in the degradation media as determined by HPLC. As expected, the anhydride and esters bonds of polymer I are relatively stable to acidic media. Even after 90 days, the polymers maintained their initial appearance, i.e., no observable changes in color, shape or size were noted. At pH 7, salicylic acid (II) was rapidly released over several days; 50% of the total content was observed by day 20. Polymer degradation was complete by 90 days (Fig. 1a).

The degradation profile reflects the relative rates of bond hydrolysis—upon cleavage of the aromatic anhydride bonds, the newly generated carboxylic acids acid-catalyze hydrolysis of the remaining ester bonds. At pH 10, there is a lag time of ~10 h, then degradation continues over a 38 h time period (Fig. 1b) as compared to pH 3.5 and 7, in which degradation occurred over much longer time periods. Base-catalyzed hydrolysis of the ester and anhydride bonds of the polymer is much more rapid under basic conditions as anticipated.

The degradation media were evaluated at all pHs and all time points to monitor pH changes during the course of polymer degradation. In media buffered to pH 3.5 and 7, no change in pH of the degradation media was detected.

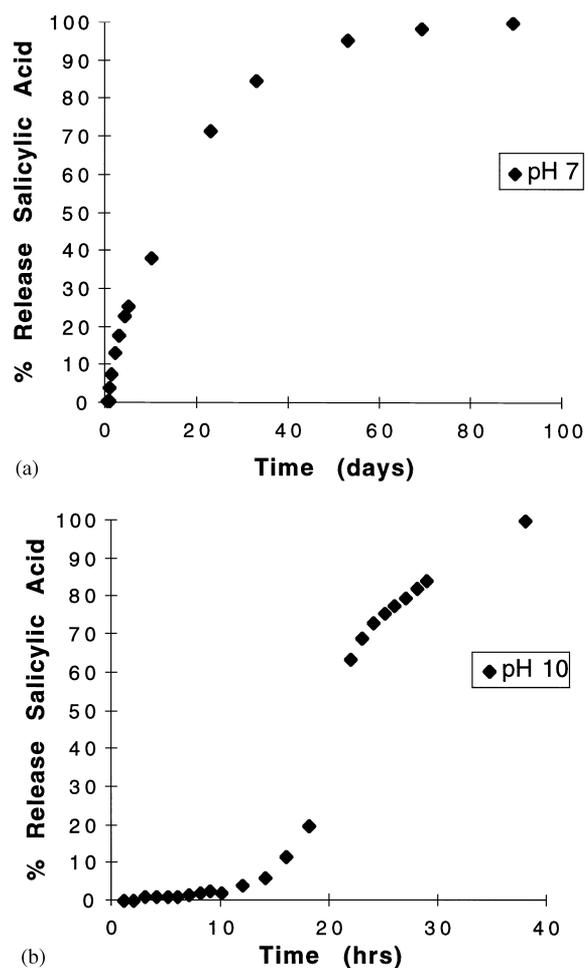
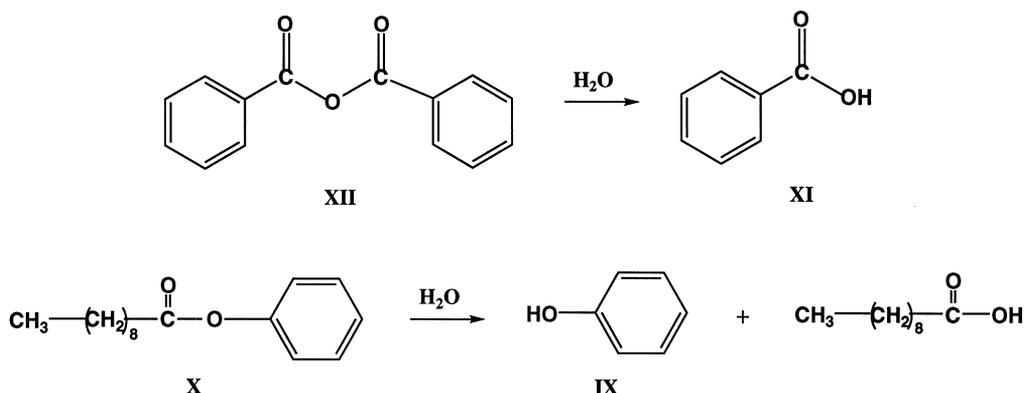


Fig. 1. In vitro degradation at (a) pH = 7 and (b) pH = 10.

ted. In media buffered to pH 10, the pH temporarily decreased to pH 9 at time points of 1 h and 2 h, which was caused by the rapid, initial release of salicylic acid (II). The degradation profiles indicate that this polymer may be an appropriate delivery system for releasing salicylic acid (II) into the lower intestine where pH is typically more basic [14].



Scheme 3. Hydrolysis of model compounds: benzoic anhydride (XII) into benzoic acid (XI), and phenyl decanoate (X) into phenol (IX).

To substantiate our observations described above, we compared the relative rates of hydrolysis of two model compounds, benzoic anhydride (**XII**) and phenyl decanoate (**X**) (Scheme 3).

The hydrolysis of benzoic anhydride (**XII**) and phenyl decanoate (**X**) into benzoic acid (**XI**) and phenol (**IX**), respectively, was monitored by HPLC over a 7 day time period in media buffered to pH 3.5, 7 and 10. At all pHs, quantitatively more benzoic acid (**XI**) was detected relative to phenol (**IX**). Concentrations of phenol were nearly negligible over this time period. This model study mirrors our results of polymer **I** degradation: anhydride bond hydrolysis is more rapid than ester bond hydrolysis, and hydrolysis is more rapid in basic media.

4. Conclusion

Poly(anhydride-esters) were successfully synthesized by incorporating a bioactive molecule, salicylic acid (**II**), into the polymer backbone. Hydrolytic degradation is pH-dependent and may be further controlled by inclusion of excipients. Because salicylic acid is released upon hydrolysis, this polymeric prodrug may show potential for a variety of applications [15] ranging from the treatment of inflammatory bowel disease [16] to periodontal prosthetics [17,18]. Given the pH-dependent degradation, we believe these polymers will be significant in treating gastrointestinal disease where it is important to release the salicylic acid derivative at the desired site—the lower intestine (basic conditions)—rather than in the stomach (acidic conditions) [16–21].

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