# NATURAL BIOACTIVE-BASED POLYANHYDRIDES FOR CONTROLLED RELEASE APPLICATIONS

by

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ABSTRACT OF THE DISSERTATION

Natural Bioactive-based Polyanhydrides for Controlled Release Applications

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Hydrolytically degradable polyanhydrides are of interest for a variety of controlled release applications because of their surface-eroding behavior and tunable degradation rate based on polymer chemical composition. Rather than physical admixtures, bioactives were chemically incorporated either directly into the polymer backbone or as pendant groups *via* hydrolytically degradable linkages.

A series of poly(anhydride-esters) containing iodinated salicylates were synthesized *via* both melt-condensation and solution polymerization to generate X-ray opaque polymers. It was found that physical and mechanical properties were affected by polymerization technique, and thermal properties such as glass transition temperature were dependent on the amount of iodine in the polymer.

The degradation rate of salicylic acid (SA)-based polyanhydrides was manipulated to release SA over prolonged periods of time (i.e., months) and over relatively short periods of time (i.e., days). First, a series of copolymers based on a SA-

based diacid and highly aromatic comonomers 1,6-bis(*o*-carboxyphenoxy)hexane (*o*-CPH) and 1,6-bis(*p*-carboxyphenoxy)hexane (*p*-CPH) were developed. By changing the molar ratios of SA-based diacid to *o*-CPH or *p*-CPH, the thermal and mechanical properties of the resulting copolymers varied, and the degradation rate was decreased. Alternately, two methods were used to form fast-degrading polymers: changing the structure of the diacid's "linker" molecule and synthesizing a copolymer containing a more hydrophilic comonomer. These polymers completely degraded in one week or less.

Mono-functional antimicrobials were chemically incorporated as pendant groups *via* ester linkages to a polyanhydride backbone based on ethylenediaminetetraacetic acid, resulting in a completely bioactive polymer. The polymers degraded in less than 1 week, and some displayed the ability to completely prevent *Salmonella* biofilm formation.

Lastly, polymers based on antimicrobial and antioxidant preservatives (i.e., hydroxycinnamates) were synthesized and found to release the preservatives over a prolonged period of time (> 1 month). Polymer degradation products exhibited antioxidant activity, and experiments indicated that free bioactives are responsible for antimicrobial activity. Furthermore, the polymers contain double bonds that can be crosslinked to form hydrophobic networks.

# PREFACE

*“Don’t let your dreams be dreams...”*

Jack Johnson

# DEDICATION

*To my family (especially my parents Dave and Michelle and my sister Dayna) and my fiancé, Ryan, for always believing in me, giving me inspiration, constant support and unwavering love.*

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# ABBREVIATIONS, SYMBOLS AND UNITS

| | |
|---|---|
| $\delta$ | chemical shift |
| $\lambda$ | wavelength |
| $\lambda_{max}$ | wavelength of maximum absorbance |
| $\mu$L | microliter |
| $\mu$m | micrometer |
| ° | degree |
| °C | degree Celsius |
| $Abs_t$ | absorbance after a period of time |
| $Abs_{t0}$ | initial absorbance |
| ASTM | American Society for Testing and Materials |
| b | broad |
| C | carbon |
| $CaH_2$ | calcium hydride |
| $CH_2Cl_2$ | methylene chloride, dichloromethane |
| $CHCl_3$ | chloroform |
| cm | centimeter |
| $cm^{-1}$ | wavenumber |
| $^{13}C$ NMR | carbon nuclear magnetic resonance |
| $CO_2$ | carbon dioxide |
| Cp | heat capacity |
| d | doublet; day |
| Da | dalton |
| DMA | dynamic mechanical analysis |
| DMSO | dimethylsulfoxide |
| DMSO-$d_6$ | deuterated dimethylsulfoxide |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| DSC | differential scanning calorimetry |
| EAFUS | everything added to foods in the United States |
| $EC_{50}$ | half maximal effective concentration |
| $Et_3N$ | triethylamine |
| FT IR | fourier transform infrared spectroscopy |
| g | gram |
| GPC | gel permeation chromatography |
| GRAS | generally regarded as safe |
| h | hour |
| H | hydrogen |
| HCl | hydrochloric acid |
| Hg | mercury |
| $^1H$ NMR | proton nuclear magnetic resonance |
| $H_2O$ | water |
| IR | infrared |
| kPa | kilopascal |
| kV | kilovolt |
| L | liter |
| LC | liquid chromatography |
| m | multiplet |
| *m*- | meta |
| M | molar |
| mA | milliampere |
| Me | methyl |
| MeOH | methanol |
| mg | milligram |
| mg/mL | milligram per milliliter |
| MHz | megahertz |
| min | minute |
| mL | milliliter |
| mm | millimeter |
| mmHg | millimeter of mercury |
| mmol | millimole |
| mN | millinewton |
| mN/min | Millinewton per minute |
| ms | millisecond |
| $M_w$ | weight-average molecular weight |
| N | nitrogen |
| NaCl | sodium chloride |
| NaOH | sodium hydroxide |
| $NEt_3$ | triethylamine |
| $NEt_3 \cdot HCl$ | triethylamine |

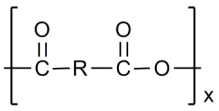
| | |
|---|---|
| EDTA | ethylenediaminetetraacetic acid |
| FDA | Food and Drug Administration |
| *o*- | ortho |
| O | oxygen |
| *o*-CPH | 1,6-bis(*o*-carboxyphenoxy)hexane |
| OMe | methoxy |
| *p*- | para |
| PAE | poly(anhydride-ester) |
| *p*-CPH | 1,6-bis(*p*-carboxyphenoxy)hexane |
| PBS | phosphate buffer solution |
| PDI | polydispersity index |
| PEG | poly(ethylene glycol) |
| PLGA | poly(lactide-*co*-glycolide) |
| psi | pounds *per* square inch |
| PTFE | polytetrafluoroethylene |
| py | pyridine |
| rpm | revolutions per minute |
| RT | room temperature |
| s | Singlet; strong |
| SA | salicylic acid |
| SAA | 1,6-bis(*o*-carboxyphenoxy)hexanoate |
| $SOCl_2$ | thionyl chloride |
| t | triplet |
| $T_d$ | thermal decomposition temperature |
| $T_g$ | glass transition temperature |
| THF | tetrahydrofuran |
| TGA | thermal gravimetric analysis |
| $T_m$ | melting temperature |
| UV | ultraviolet, ultraviolet spectroscopy |
| UV/Vis | ultraviolet/visible |
| vs | very strong |
| $W_1$ | initial weight |
| $W_2$ | final weight |
| wt | weight |
| wt % | weight percent |
| | hydrochloride |
| nm | nanometer |
| NMR | nuclear magnetic resonance |
| NSAID | non-steroidal anti-inflammatory drug |

# 1. INTRODUCTION

In the past several decades, research regarding synthetic biodegradable polymers has surged. Biodegradable polymers have been studied for a wide range of applications including delivery of therapeutics,[1-3] tissue engineering,[4-6] biomedical devices,[2-4,7,8] prodrugs,[1,9,10] packaging,[7] detergents,[7,11] and agriculture.[7,12] One class of biodegradable polymers, namely polyanhydrides, is particularly promising for controlled delivery applications based on the polymers' surface-eroding behavior, biodegradation into non-cytotoxic degradation products, and tailored degradation rate based on polymer composition.[7,13-15]

## 1.1. Polyanhydrides

Polyanhydrides are a unique class of polymers derived from diacid monomers connected through hydrolytically unstable anhydride bonds (**Figure 1.1**).[16-18]



**1**

**Figure 1.1.** Representative structure of polyanhydrides (**1**).

Since research by Langer *et. al.* in the 1980s, biodegradable polyanhydrides have been studied extensively for controlled release applications as drug delivery systems and medical devices.[19-27] Although polyanhydrides are intensively studied now, they were discovered many years prior with the first reported synthesis by Bucher and Slade in

1909.[28] In the 1930s, Hill and Carothers synthesized polyanhydrides from aliphatic diacid monomers.[29-31] Later, Conix and Yoda made modified polyanhydrides based on aromatic and heterocyclic diacid monomers, respectively.[32-41] Although polymer properties were somewhat improved, polyanhydrides were deemed inadequate because of their susceptibility to hydrolysis with attempts to use them for textile applications.

Langer was the first to use polyanhydrides because of their instability to hydrolysis.[42] Polyanhydrides are of interest because the hydrolytic degradation can be easily controlled based on polymer composition.[9,15,43] Furthermore, polyanhydrides have been found to be biocompatible with little to no evidence of inflammatory reactions.[9,15,44] One example of a polyanhydride in an FDA-approved device is poly[sebacic acid-co-1,3-bis(*p*-carboxyphenoxy)propane], which is used to deliver the chemotherapy drug carmustine for treatment of brain cancer.[45] Polyanhydrides have been used to deliver both physically admixed bioactive agents,[45-49] and more recently, bioactives that have been chemically incorporated into the polymer.[9,43,50]

## 1.2. Polyanhydride Synthesis

The two most widely used polymerization methods to synthesize polyanhydrides are melt-condensation and solution polymerization.[51-53] Each synthetic approach has its advantages and limitations as outlined below.

### 1.2.1. Melt-condensation Polymerization

Melt-condensation polymerization is the most widely used method for synthesizing polyanhydrides as it is straight-forward and reproducible on scales of tens of

grams.[29-31,51,53] The method requires the dicarboxylic acid monomer (**2**) to first be activated with acetic anhydride (**3**) to form a mixed anhydride prepolymer (**4**), which is then polymerized at high temperature (i.e., 120-200 °C) under vacuum to remove the condensation by-product, acetic anhydride (**Figure 1.2**).

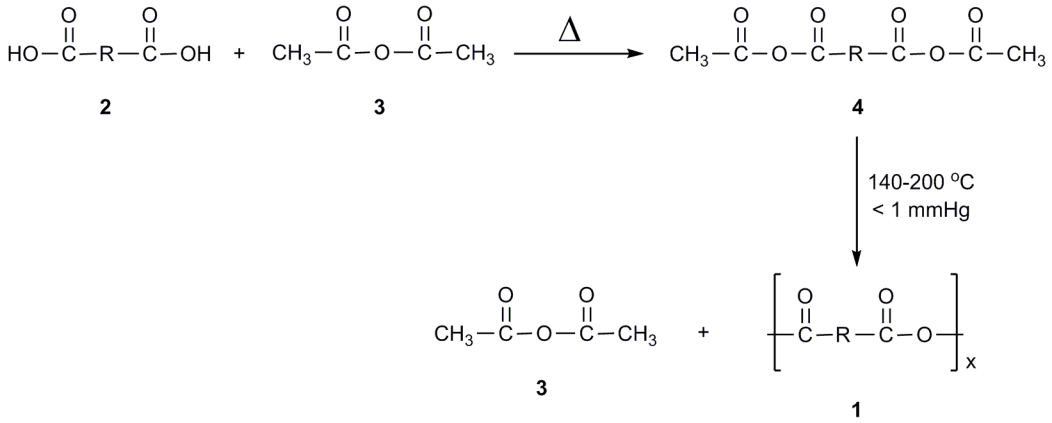



**Figure 1.2.** Synthetic scheme for the synthesis of polyanhydrides (**1**) by melt-condensation polymerization.

This method results in higher molecular weights (e.g., > 30,000) than other methods, however it is limited to heat-stable monomers.[51,52,54,55] Furthermore, it has been demonstrated that high temperatures can lead to thermal rearrangements.[53,56,57]

### 1.2.2. Solution Polymerization

Low temperature solution polymerization is best utilized for heat sensitive monomers. Several methods have been employed to prepared polyanhydrides in solution

including dehydrochlorination or dehydration.[36,51,58,59] These two methods generally result in impure or low molecular weight polymers.

A one-step polymerization method was developed by Domb *et. al*. with the use of coupling agents such as phosgene (**Figure 1.3**).[58]

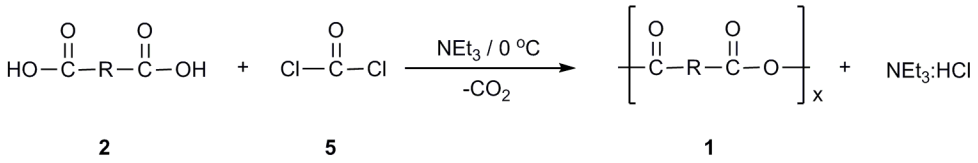



**Figure 1.3.** Synthetic scheme for the synthesis of polyanhydrides (**1**) *via* solution polymerization using phosgene (**5**) as a coupling reagent.

This method is a one-pot synthesis, where preparation of a prepolymer is not required.[58] Domb *et. al.* also used diphosgene, which is a liquid, as compared with phosgene (**5**) gas. Triphosgene has also been utilized for the preparation of polyanhydrides and is easier to handle as a solid.[53,60]

Limitations on this polymerization technique include only obtaining relatively low molecular weight polymers (i.e., 5,000-10,000) and the requirement of strict stoichiometric control over starting materials.[51,53,55]

### 1.3. Hydrolysis of Polyanhydrides

Polymer biodegradation involves two sequential processes, degradation and erosion.[61] Degradation can be defined as the process of polymer chain cleavage or the cleavage of the anhydride bonds in particular for polyanhydrides.[61] Erosion is described

by Gopferich *et. al.* as "the sum of all processes that cean lead to loss of mass from a polyanhydride matrix irrespectible of its geometry".[61] Polyanhydrides are known to be predominantly surface-eroding (heterogeneous erosion), meaning water penetration into the bulk of the polymer is very limited, allowing the erosion to remain mostly at the polymer-water interface (**Figure 1.4.a**).[5,61] In this way, bioactives incorporated into such a polymeric matrix are released in controlled, constant rate depending on the material geometry and surface area.[13,61-63]

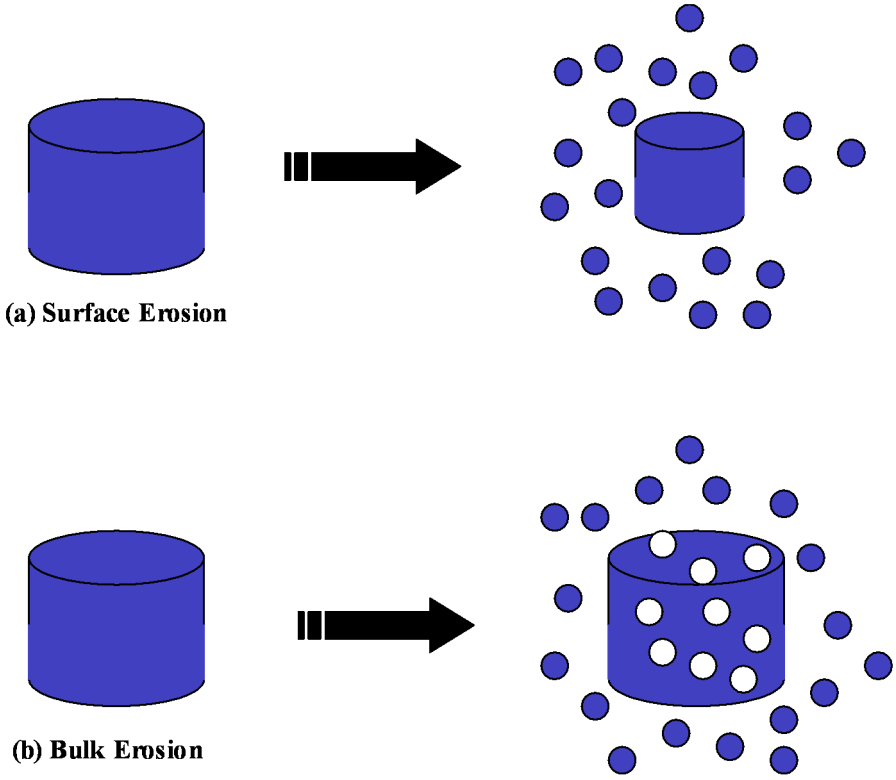



**Figure 1.4.** Delivery of bioactives from (a) surface-eroding and (b) bulk-eroding biodegradable polymers.

Alternately, other biodegradable polymers degrade *via* a bulk erosion mechanism (homogeneous erosion), such as polyesters (**Figure 1.4.b**). Polymers that are bulk-eroding typically degrade throughout the entire polymer matrix at a non-linear rate.[61-63] Polyanhydrides and their surface-eroding behavior are therefore more desirable as controlled release systems because of the predictability of their release profiles and rates.

## 1.4. Poly(anhydride-esters)

### 1.4.1. Bioactive-based Poly(anhydride-esters)

Polyanhydrides have been extensively studied for their ability to deliver therapeutics in a controlled, sustained manner.[19-21,23,55,64-66] Most of these systems, however, are physical admixtures of the bioactive molecules into the polyanhydride matrix.[45-49] More recently, our laboratory has discovered ways to chemically incorporate bioactive molecules into the backbones of polyanhydrides by the introduction of ester and amide linkages.[9,43,50,67-71] The earliest example was a poly(anhydride-ester) (**6**) composed of salicylic acid (**7**) and sebacic acid (**8**) (**Figure 1.5**).[9]

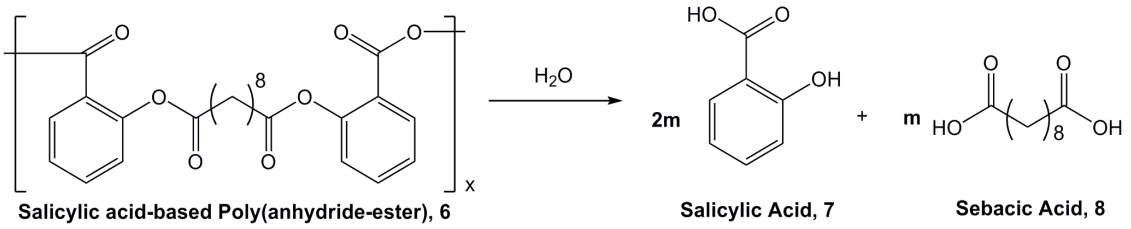



**Figure 1.5.** Hydrolytic degradation of salicylic acid-based poly(anhydride-ester), **6**.

This polymer has a high weight percentage of salicylic acid (62 %) chemically incorporated.[9,43] It was also found that by manipulating the chemical structure of the "linker" molecule connecting the two salicylic acid molecules together (e.g., sebacic acid, **8**, in polymer **6**), the release of salicylic acid could be tailored from weeks to months.[43] These polymers are non-cytotoxic and predominantly surface-eroding with a controlled, sustained release of salicylic acid upon hydrolysis of the anhydride and ester bonds.[9,43,72,73]

More recently, other bioactive molecules besides salicylates have been chemically incorporated into polyanhydrides such as non-steroidal anti-inflammatory drugs (NSAIDs),[50] antiseptics,[69,71] antioxidants,[69] and antibiotics.[69] The applicability of these bioactive-based polymers in biomedical and drug delivery fields is promising.

### 1.4.2. Applications of Poly(anhydride-esters)

As discussed above, bioactive-based polyanhydrides may be useful for a variety of applications. Salicylate-based poly(anhydride-esters) in particular have been found to be useful in a broad range of applications from bone growth/regeneration[44,74] to prevention of bacterial growth.[69,75] Furthermure, poly(anhydride-esters) can easily be manipulated into different geometries including films, fibers, disks, and microspheres.[43,76,77]

## 1.5. Research Projects

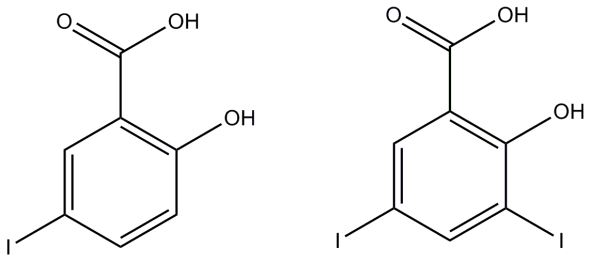
Based upon previous work on drug-based polyanhydrides from our laboratory (see **Section 1.4.1** and **Section 1.4.2**), several research projects were designed. Salicylate-based polyanhydrides were previously deemed useful for biomaterials applications (e.g.,

as coronary stents)[78] however these polymers are not radiopaque. In Chapter 2, iodinated salicylate derivatives were chemically incorporated into polymeric backbones to result in X-ray opaque polymers for biomaterials and coating applications. In Chapter 3, new polyanhydride copolymers with enhanced thermal and mechanical properties and prolonged release of salicylic acid were designed to be used as bone binders based on preliminary studies utilizing salicylate-based polymers for bone regeneration.[73,74] Conversely, in Chapter 4, new polyanhydride and polyanhydride copolymers were designed and synthesized for applications requiring a quick, yet controlled release of salicylic acid for wound and personal care applications. Previous work in our laboratory proved that salicylic acid-based polyanhydrides also prevent biofilm formation on medical device bacteria.[75] In Chapter 5, the development of novel polyanhydride composed of naturally occurring antimicrobials were developed for use in food safety and personal care applications. Finally, a new class of polyanhydrides was developed based on antioxidant and antimicrobial hydroxycinnamate derivatives. These polymers may be useful in controlled release applications for personal care and cosmetics.

### 1.5.1. Iodinated Salicylate-based Poly(anhydride-esters) as Radiopaque Biomaterials

Salicylate-based polymers release the chemically incorporated salicylic acid upon hydrolytic degradation in a controlled, sustained manner and have been deemed useful for a variety of biomaterials applications.[9,43,69,70,73-76,78] These polymers have also been proven to promote endothelialization when used as coronary stents.[78] The limitations of these polymers are that they cannot be visualized under a clinical X-ray machine, so the

surgeon cannot monitor the biomaterial placement during or post-surgery. It has been demonstrated that radiopacity can be imparted through the introduction of elements of high atomic mass (high electronic density), such as iodine.[79,80] Based on this premise and established chemistry, iodinated salicylates (**9** and **10** in **Figure 1.6**) were chemically incorporated into polymeric backbones.




**5-Iodosalicylic Acid, 9** **3,5-Diiodosalicylic Acid, 10**

**Figure 1.6.** Structures of iodinated salicylates **9** and **10**.

Two methods of polymerization were utilized (i.e., melt-condensation and low-temperature solution polymerization) to compare resulting polymer properties. Results indicated that iodinated salicylate-based poly(anhydride-esters) had enhanced thermal and mechnical properties when compared to salicylic acid-based poly(anhydride-esters). Furthermore, these polymers were highly X-ray opaque with little to no evidence of cytotoxicity on mouse fibroblasts at low concentrations.

### 1.5.2. Prolonged Release of Salicylic Acid from Poly(anhydride-ester) Copolymers

Preliminary studies utilizing salicylate-based poly(anhydride-esters) demonstrated their applicability for bone regeneration applications.[44,73,74] These polymers were previously studied as implants for cranio-facial defects, however their usefulness as bone binders was not yet studied. To be used for this and similar applications, a prolonged release of salicylic acid is necessary. Furthermore, improvements on thermal and mechanical properties would also be useful. Based on this, salicylic acid-based copolymers with other aromatic polyanhydrides, namely *o*- and *p*-(carboxyphenoxy)hexane were designed and synthesized. The resulting polymers display enhanced thermal/mechanical properties and prolonged degradation rates.

### 1.5.3. Fast-degrading Salicylate-based Poly(anhydride-esters)

Although salicylate-based poly(anhydride-esters) are promising for biomaterials applications with release rates from weeks to months or years, their ability to be utilized for other applications requiring much faster release (i.e., from hours to days) such as wound care and personal care was not explored. Therefore, novel salicylate-based polyanhydrides and polyanhydride copolymers were designed and synthesized. Polymer properties and *in vitro* hydrolytic degradation rates when formulated into microparticles were evaluated. Studies indicated that the release rate can easily be tailored to release the chemically incorporated salicylic acid from one day to one week.

### 1.5.4. Natural Antimicrobial-based Polyanhydrides

While studying salicylate-based polymers for biomedical applications, it was noted that these polymers have the ability to prevent biofilm formation.[75] Such polymers would be useful not only for medical devices but also for personal care and food safety applications. However, such applications may not require the release of a NSAID such as salicylic acid. It may be more desirable to release other natural bioactive molecules that could be used in conjunction with foods or personal care/cosmetics products. Based on this assumption, novel polyanhydrides were designed and synthesized based on naturally occurring antimicrobials derived from plants and spices (**11-13** in **Figure 1.7**).

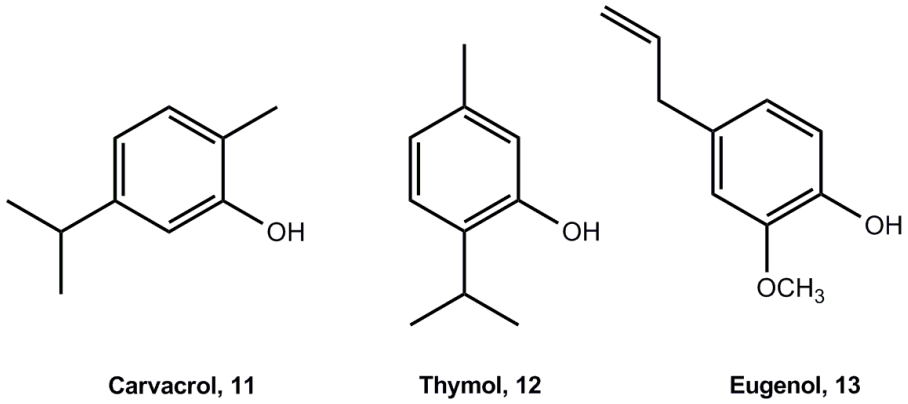



**Figure 1.7.** Structures of naturally-derived antimicrobials (**11-13**).

These bioactives were chemically incorporated *via* ester linkages to a polyanhydride backbone derived of ethylenediaminetetraacetic acid (EDTA). The resulting bioactive-based polyanhydrides were proven to have mild to strong ability to prevent biofilm formation for the food bacteria, *Salmonella enterica*.

### 1.5.5. Photocrosslinkable, Preservative-based Poly(anhydride-esters)

Other antimicrobial phytochemicals were chosen as they are commonly used as preservatives for food and personal care products and based upon their antimicrobial and antioxidant properties (**Figure 1.8**).

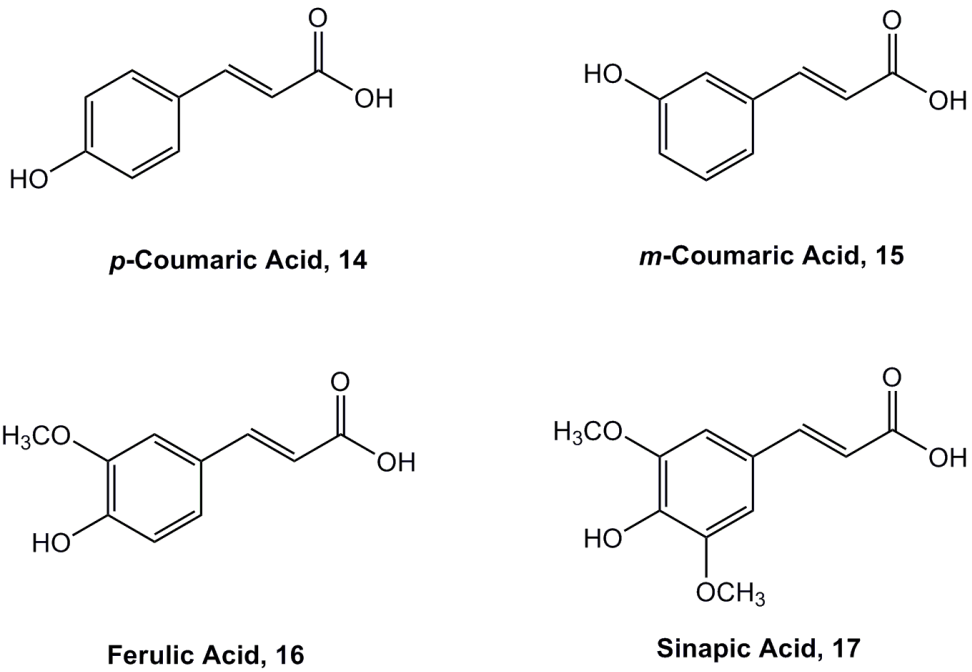



**Figure 1.8.** Structures of naturally-derived preservatives (**14-17**).

Polymer properties and release rates were monitored, as well as their ability to act as antioxidants and antimicrobials. The polymers had little antioxidant effect themselves, however, their degradation products were good antioxidants.

## 1.6. Summary

A wide range of bioactive-based polyanhydrides were designed and synthesized for controlled release applications to be used in a variety of applications ranging from medical devices and drug delivery to personal care and food safety. The degradation rate of bioactive-based polyanhydrides was tailored to release chemically incorporated bioactives from days-to-weeks or from weeks-to-months depending on the desired application. The resulting polymer properties were highly dependent on the polymer composition as well as the polymerization method, either melt-condensation or solution polymerization. Additionally, new classes of polyanhydrides were designed and synthesized based on naturally derived compounds such as antimicrobials and antioxidants, which expands the potential uses for bioactive-based polyanhydrides.

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# 2. IODINATED SALICYLATE-BASED POLY(ANHYDRIDE-ESTERS) AS RADIOPAQUE BIOMATERIALS

## 2.1. Introduction

Biodegradable polymers are desirable for a variety of biomedical applications such as cardiovascular implants, bone fixation devices, tissue engineering scaffolds, drug delivery devices, catheters, medical adhesives and dental composite filling materials. Such polymers are cannot be detected by traditional X-ray techniques when they are implanted in the body.[1-4] Invasive and complex imaging techniques are often required to visualize polymeric biomedical implants.[4] Inorganic contrast agents are commonly used to impart radiopacity, however they are not ideal and have many disadvantages.

Biomaterials that are inherently radiopaque are highly desirable for use *in vivo* because of the ability to non-invasively monitor them during surgery and post-implantation. In this chapter, the design of X-ray opaque, biodegradable poly(anhydride-esters) will be described. The goal of this project is to incorporate iodinated salicylates into polymeric backbones to render the resulting polymers radiopaque with adequate thermal and mechanical properties to use them as or in conjunction (i.e., as coatings) with biomaterials.

## 2.2. Background

### 2.2.1. Radiopacity

Radiopaque biomaterials are desirable for implantation, as the physician can non-invasively monitor implant placement to the precise location in the body and also locate

the biomaterial after surgery is complete.[1,3-7] Traditionally, inorganic contrast agents[4,8] are used to provide radiopacity to medical implants. However, these contrast agents have multiple disadvantages including degradation when physically admixed, uneven distribution of the radiopacifying agent, and negative effects on mechanical properties of the biomaterial. leaching and limited coloring options as most inorganic contrast agents generate dark-colored materials.[9-11] All these issues motivate the development of radiopaque biomaterials such that X-ray opacity is an intrinsic property of the implant.[3-5,12-19]

Conventional polymers used for medical implants are organic (i.e., contain the elements C, H, O and N), which makes them transparent to X-rays.[4] The introduction of high atomic mass elements, such as iodine, into the polymer affords radiopacity due to the increased electron density and the specific gravity of the polymer in comparison with surrounding tissue.[4,8]

In an early report by Davy, *et. al*.,[7] iodinated aromatic methacrylate monomers were prepared *via* the esterification of hydroxyl-containing methacrylate esters with 2,3,5-triiodobenzoic acid and also by nucleophilic addition of 2,3,5-triiodobenzoic acid to 2,3-epoxypropyl methacrylate. The authors proposed that the monomers could be polymerized or copolymerized with other methacrylate monomers to form X-ray opaque materials to be used in denture base acrylics or orthopedic bone cements.[7] In these systems, the iodinated aromatic molecules are introduced into the polymer as a side group *via* ester linkages. Upon *in vitro* degradation, the radiopacity is reduced: the radiopaque iodinated aromatic compound is cleaved and excreted, leaving the non-radiopaque polymethyacrylate chain.

In a related example, Kohn, *et. al.*,[2] disclosed iodinated and/or brominated derivatives of aromatic dihydroxy monomers that are polymerized to form radiopaque polymers as additives for polymeric biomaterials, drug delivery devices or medical implants. These systems covalently bind the iodinated portion into the main chain of the polymer, yielding polymers with mechanical properties comparable to the parent polymers.[2] As these systems are based on polycarbonates, they are slow degrading relative to polyanhydrides.

The polymers described in the examples above are either non-degradable polymethacrylates and poly(alkene oxides), or slow degrading polycarbonates. We synthesized fully biodegradable iodinated polymers in which a radiopaque derivative of salicylic acid is chemically incorporated into the backbone of the polymer, not attached as a pendant group. With this approach, the number of iodine atoms in the monomer and resulting polymer is well defined compared to post-polymerization modification approaches.

### 2.2.2. Salicylic Acid-based Poly(anhydride-esters) as Biomaterials

Our laboratory has previously described methods to synthesize poly(anhydride-esters) comprised of salicylic acid.[20-22] These polymers degrade completely upon hydrolysis (**Figure 1**) to release salicylic acid (**1a**) and the biocompatible "linker" molecule, which connects the two units of salicylic acid together. As outlined in **Figure 2.1**, the drug is chemically incorporated into the polymeric backbone and not attached as a side group,[23,24] allowing high drug loading levels.

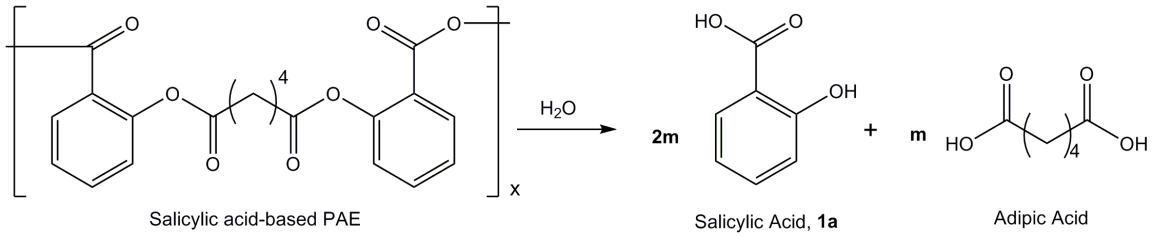


**Figure 2.1.** Hydrolytic degradation of salicylate-based poly(anhydride-ester) into salicylic acid (**1a**).

These polymers have been proven effective *in vitro*[25,26] and *in vivo*[27,28] in various biomedical applications. These polymers have great processing capabilities and can be formulated into microspheres,[29] disks,[22] fibers[30], films, coatings and cardiac stents.[31] For cardiac stents and other biomaterials applications, it is desirable for the polymer to be X-ray opaque, so the surgeon can monitor the placement of the material and its location after implantation.[1,3,10,11] Salicylate-based poly(anhydride-esters) are not radiopaque and may not have adequate mechanical properties for biomaterials applications. Therefore, we designed new polymers that may be more suitable for these applications.

The polymers described in this chapter are derived from iodinated salicylates (**Figure 2.2**). Based upon previous work,[25] we observed that salicylate derivatives with higher melting temperatures ($T_m$) yielded polymers with correspondingly higher glass transition temperatures ($T_g$). We hypothesized that poly(anhydride-esters) derived from iodinated salicylates would not only demonstrate radiopacity but would also have enhanced mechanical properties due to the higher melting points of the iodinated derivatives. The $T_m$ of salicylic acid is 159°C, compared with 189-191°C and 220-230°C for 5-iodosalicylic acid and 3,5-diiodosalicylic acid, respectively. Hence, iodinated

salicylic acid-based poly(anhydride-esters) were synthesized using previously described methods[22,32-34] with the goal of producing polymers with both X-ray opacity and enhanced mechanical properties.

### 2.2.3. Polymer Synthesis: Melt-Condensation vs. Solution Polymerization

Two methods of polymerization were compared: melt-condensation[21] and low-temperature solution polymerization[32,33] (**Figure 2.2**). In previous studies, polymer properties, especially molecular weight, can vary depending on which polymerization method is used.[32] Melt-condensation polymerization of dicarboxylic acid precursors is known to give polyanhydrides with moderate molecular weights (e.g., 10,000-30,000 Da), whereas solution polymerization typically results in lower molecular weight polymers (5,000-10,000 Da).[35,36]

To elucidate how polymer properties can differ using solution polymerization versus melt-condensation polymerization, each polymer was made using both methods. The effect of polymerization method on polymer radiopacity, thermal and mechanical properties was evaluated. Differences in polymer properties were examined by studying polymer chemical composition as a result of each polymerization method.

## 2.3. Results and Discussion

### 2.3.1. Synthesis of Iodinated Salicylic Acid-based Poly(anhydride-esters)

Iodinated salicylic acid-based poly(anhydride-esters) were successfully prepared *via* both melt-condensation and low temperature solution polymerization methods. Regardless of the polymerization methods, the first step is to prepare the diacid (**2; Figure 2.2**).

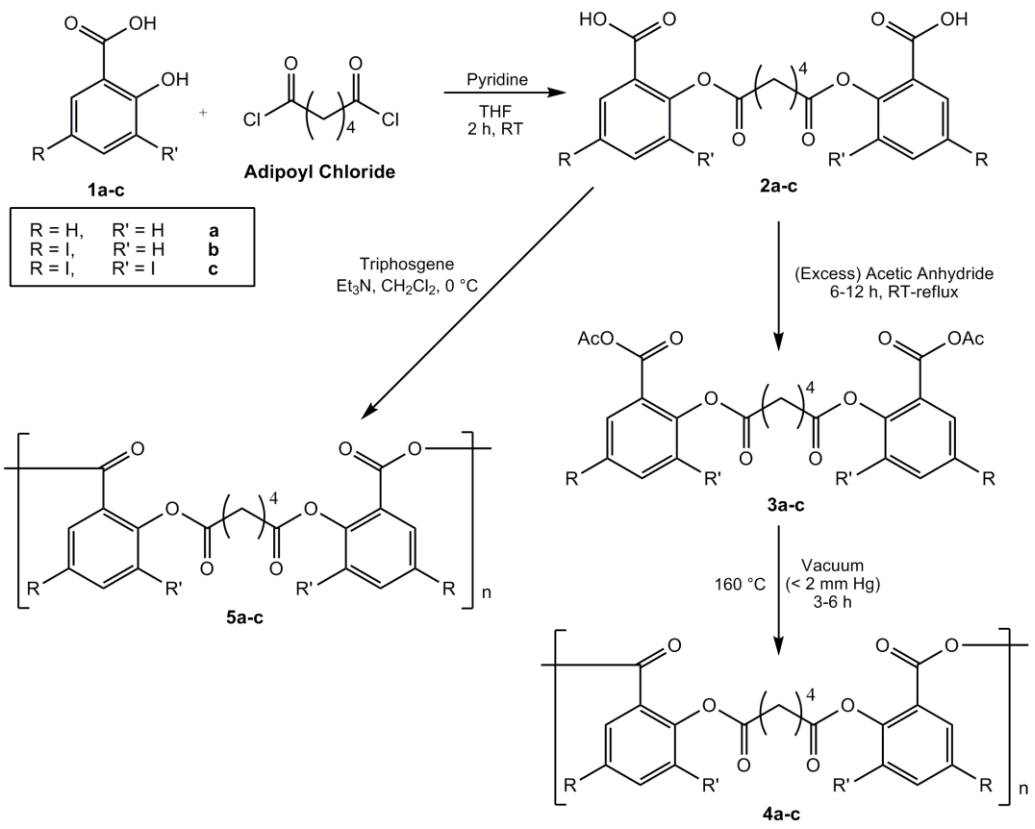


**Figure 2.2.** Outline for synthesis of salicylate-based poly(anhydride-esters) to prepare polymers by melt-condensation and solution polymerization methods.

This compound was made by directly coupling the salicylate derivative (**1**) to adipoyl chloride in an appropriate solvent (THF) and base (pyridine) at room temperature.[21,22] The pyridine deprotonates the salicylate (**1**) and also acts as a catalyst to form an acyl pyridinium ion, which reacts with the free phenolate of the salicylate to form the diacid (**2**). The carboxylic acid group on the salicylate derivative (**1**) does not need to be protected as the acyl pyridinium ion reacts faster with alcohols than acyl chlorides.[22,37,38] The products (**2**) obtained using this method are pure as determined by NMR, FTIR,

melting point and elemental analysis. Further purification was not necessary based on the large solubility differences between the reaction byproducts, starting materials and the diacid (**2**) formed.[22,39] Yields for both the 5-iodosalicylic acid-based diacid (**2b**) and the 3,5-diiodosalicylic acid-based diacid (**2c**) were quantitative as observed for the salicylic acid-based diacid (**2a**). The diacids (**2**) were used to prepare the poly(anhydride-esters) *via* melt-condensation and solution polymerization methods (**Figure 2.2**).

#### 2.3.1.a. Melt-condensation Polymerization

For melt-condensation polymerization procedures, the diacids (**2**) were activated using an excess of acetic anhydride to form the polymer precursor or monomer (**3**), which is then polymerized at elevated temperatures (i.e., 160 $^{o}$C) under vacuum to remove the melt-condensation byproduct, acetic anhydride. The resulting polymer was precipitated from methylene chloride into an excess of diethyl ether.

#### 2.3.1.b. Solution Polymerization

For solution polymerization, the diacids (**2**) were directly reacted in the presence of base (e.g., triethylamine), appropriate solvent (e.g., methylene chloride) and a coupling reagent (triphosgene) to give the resulting polymers (**5**). This one-step synthesis is mild (*i. e*, performed at 0 ˚C), however it requires strict stoichiometric control for efficient polymerization.[32,33]

### 2.3.2. Comparison of Polymer Composition

#### 2.3.2.a. NMR Spectroscopy

NMR spectroscopy was utilized to determine any changes in chemical composition of the polymer as result of polymerization method. The $^{1}$H-NMR (**Figure**

**2.3**) and $^{13}$C-NMR (**Figure 2.4**) of the melt (**4**) and solution-made polymers (**5**) are compared using 5-iodosalicylate derivatives as a representative example. The peaks for the NMR spectra are labeled with respect to the original diacid (**2**). In the $^{1}$H-NMR spectra of the solution-based polymers (**5b**), a slight broadening of peaks was observed compared to the diacid (**2b, Figure 2.3**), however the structure of the polymer is confirmed by NMR analysis.

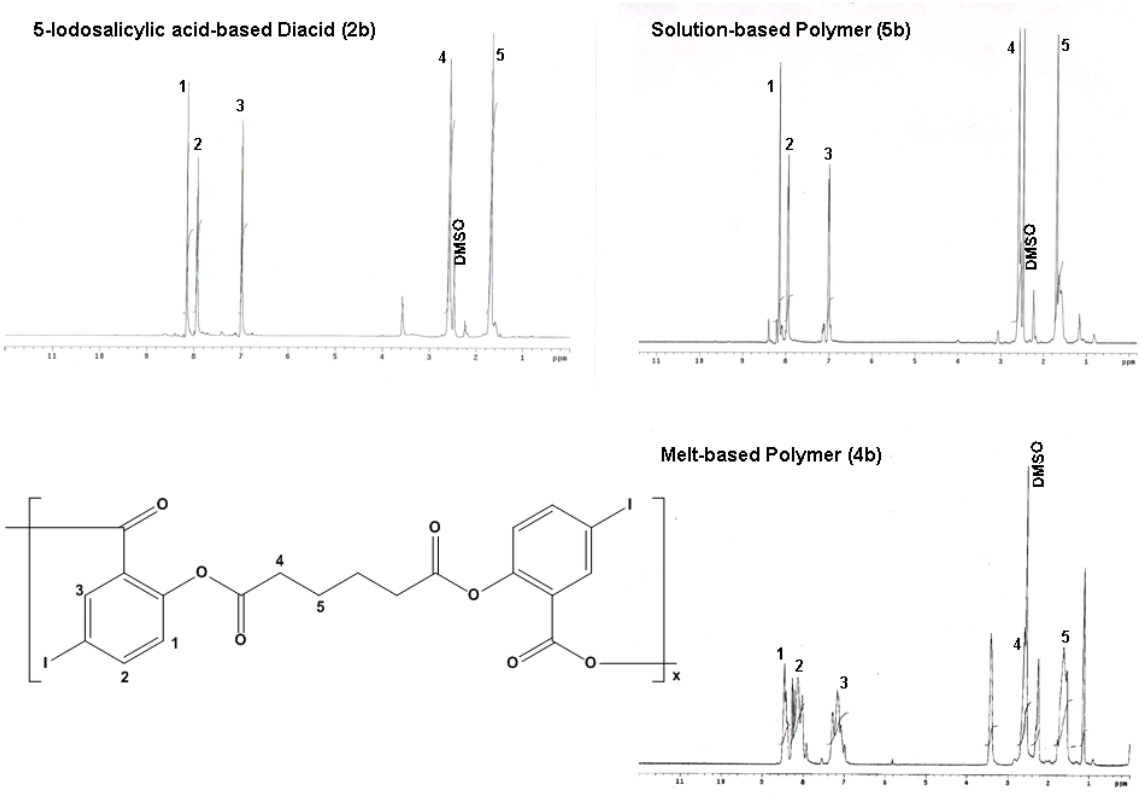


**Figure 2.3.** Representative $^{1}$H-NMR spectra: diacid **2b**, polymer **4b** and polymer **5b**. Protons correlating to diacid **2b** are indicated in the spectra.

By contrast, the peaks were much broadened for the melt-made polymers (**4b**, **Figure 2.3**). Peak broadening for melt-based polymers is attributed to different structures of the

polymer chains occurring from thermal rearrangement during the melt-condensation process.[32]

In the $^{13}$C-NMR spectra, polymers made *via* melt-condensation (**4**) have more carbon peaks than the solution-based polymers (**5b, Figure 2.4**), indicating that melt-made polymers may have a more complex composition than previously anticipated.[32]

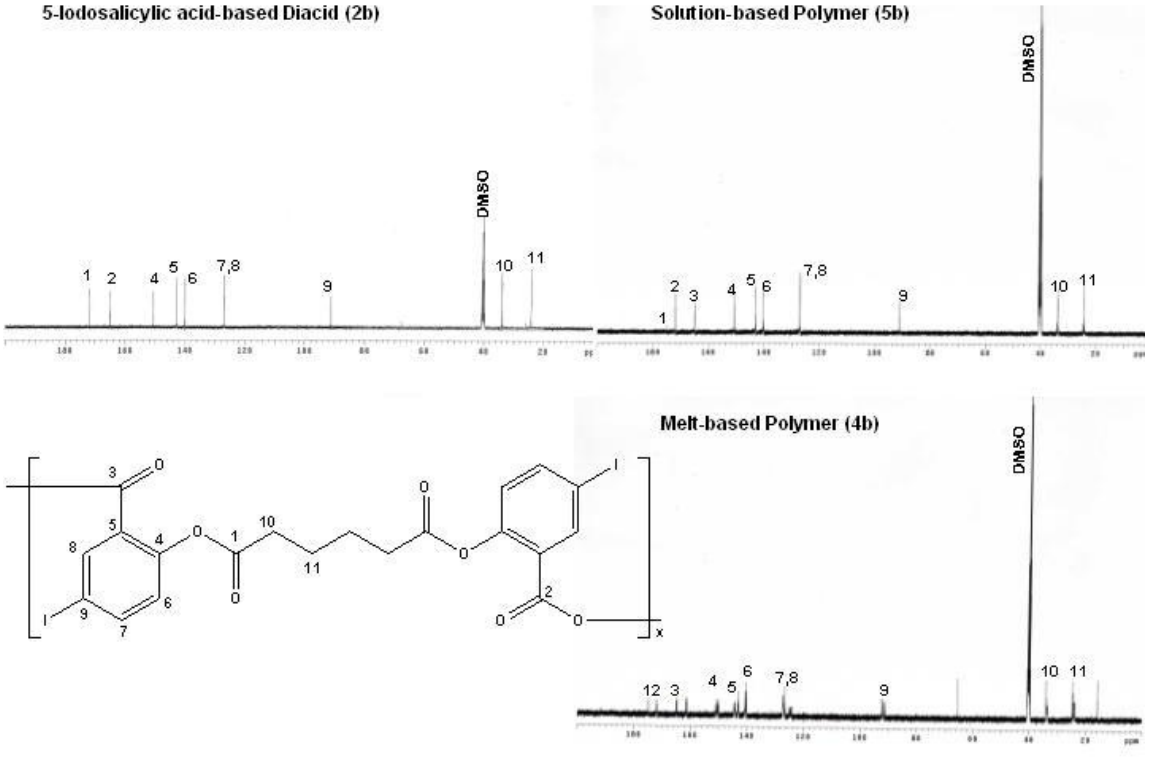


**Figure 2.4.** Representative $^{13}$C-NMR spectra: diacid **2b**, polymer **4b** and polymer **5b**. Carbons correlating to diacid **2b** are indicated in the spectra.

The $^{13}$C-NMR spectra of the solution-made polymers (**5b**) show 11 distinct carbon peaks, clearly correlating to a well-defined polymer composition. In contrast, the $^{13}$C-NMR

spectra of the melt-condensation polymers (**4b**) display > 30 carbon peaks; only the expected carbon peaks are labeled in the spectra for **4b**. The increase in number of carbon peaks likely result from thermal rearrangement.[32]

#### 2.3.2.b. FTIR Spectroscopy

The FT-IR spectra of the solution-made polymers (**5**) displayed sharp and narrow transmission bands, again indicating a well-defined polymer composition. In contrast, the FT-IR spectra of the melt-made polymers (**4**) showed broad transmission bands, with increased proportion of ester to anhydride transmission bands. This data suggests that multiple aryl-aryl anhydride and aryl-aryl ester bonds are formed in the melt-condensation polymerization process. Previous spectroscopic analyses on salicylate-based polymers in our laboratory demonstrated that rearrangements occur during the process of melt-condensation in which a thermodynamically stable salicylate ester is formed *via* a salicylate-anhydride rearrangement.[32] This rearrangement results in a mixed polyanhydride/polyester that appears to be typical for melt-condensation polymerization of salicylate-based polyanhydrides. The spectral characteristics attributed to thermal rearrangements are not observed during solution polymerization as energy in the form of heat is the driving force. The compositional differences between melt-made (**4**) solution-made (**5**) polymers explain the differences noted between the polymer properties.

### 2.3.3. Melt vs. Solution-made Polymer Properties

Key properties for the melt (**4**) and solution-made (**5**) polymers are summarized in **Table 2.1**.

| Drug | Polymer | $M_w$ | PDI | $T_g$ (°C) | $T_d$ (°C) | Young's Modulus (kPa) | X-Ray Opacity |
|---|---|---|---|---|---|---|---|
| Salicylic Acid, **1a** | **4a** | 31,800 | 1.8 | 46 | 290 | 1500 | 1 |
| | **5a** | 10,000 | 1.1 | 23 | 240 | 330 | - |
| 5-Iodosalicylic Acid, **1b** | **4b** | 33,000 | 1.5 | 52 | 260 | 2400 | 3 |
| | **5b** | 8,000 | 1.5 | 59 | 260 | 1470 | 3 |
| 3,5-Diiodosalicylic Acid, **1c** | **4c** | 7,700 | 1.7 | 78 | 270 | 3700 | 6 |
| | **5c** | 4,000 | 1.4 | 68 | 220 | 190 | 5 |

**Table 2.1.** Summary of polymer properties, comparing salicylate-based polymers prepared by melt-condensation (**4**) and solution polymerization (**5**) methods.

#### 2.3.3.a. Molecular Weight

Molecular weights for melt-condensation polymers (**4**) were typically higher than for solution-made polymers (**5**). Solution polymerization may yield lower molecular weight polymers due to the strict stoichiometric control needed for efficient conversion.[32,33,36,39]

#### 2.3.3.b. Thermal Properties

Glass transition temperatures ($T_g$) varied with the number of iodine atoms per salicylic acid molecule; $T_g$ values increased with increased numbers of iodine atoms (**Table 2.1**). For example, polymer **4a** contains no iodine and displays a glass transition temperature of 46 °C, whereas the polymers based on 5-iodosalicylic acid (**4b**) and 3,5-diiodosalicylic acid (**4c**) had higher glass transition temperatures (52 °C and 78 °C, respectively). Decomposition temperatures varied between 220-290 °C.

### 2.3.3.c. Young's Modulus

When observing the Young's modulus of polymers obtained from melt-condensation polymerization methods (**4**), increasing the number of iodine atoms increased the Young's modulus. In general, the Young's modulus of melt-condensation polymerization products (**4**) were higher than the corresponding solution polymerization products (**5**).

### 2.3.3.d. X-Ray Opacity

To evaluate radiopacity, polymers were pressed into circular disks (13 mm diameter x 1 mm thickness) and analyzed *via* X-ray. A representative X-ray image is shown in **Figure 2.5** and comparative results in **Table 2.1**.

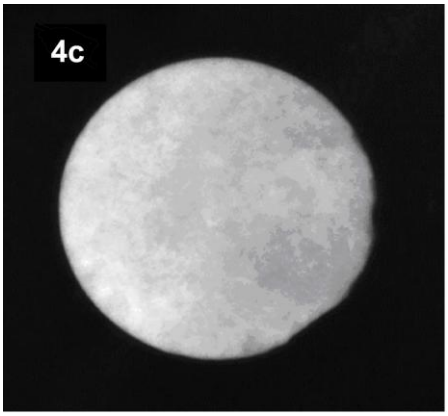


**Figure 2.5.** Representative X-ray radiograph of highly iodinated salicylate-based poly(anhydride-ester) (**4c**) disk.

Iodinated salicylate-based polymer disks of **4b-c** and **5b-c** can be readily observed using standard clinical X-ray techniques, unlike polymers derived from **1a**.

The data reported in **Table 2.1** ranks the radiopacity of the polymers from 1 to 6,[40] with 6 being most visible and 1 being invisible. Polymer disks containing no iodine (**4a**) were not visible, whereas the 3,5-diiodosalicylic acid-based polymer made by melt-condensation (**4c**) was most visible. Generally, the solution polymerization products (**5b-c**) were less X-ray visible compared to the corresponding melt-condensation polymers (**4b-c**). Moreover, the iodinated salicylic acid-based polymers **4b-c** and **5b-c** were significantly more X-ray opaque compared to bones and tissue in the hand (data not shown). The differences noted between melt (**4b-c**) and solution-made (**5b-c**) polymers in X-ray analysis may be due to differences in polymer composition due to thermal rearrangements taking place during the melt-condensation polymerization process.

**2.3.3.e. Qualitative Solubility**

Determining polymer solubility in common organic solvents is important for polymer processing. The qualitative solubility of the polymers (**4** and **5**) in selected organic solvents is shown in **Table 2.2**. Overall, salicylate-based polymers **4a** and **5a** had excellent solubility in organic solvents, and the poly(anhydride-esters) became less soluble with more highly iodinated versions of salicylic acid being incorporated into the polymer. The polymer composed of 5-iodosalicylic acid (**4b**and **5b**) had good solubility, however the polymer comprised of 3,5-diiodosaliyclic acid (**4c** and **5c**) was comparatively less soluble.

| Polymer | THF | $CH_2Cl_2$ | Acetone |
|---|---|---|---|
| **4a** | ++ | ++ | ++ |
| **5a** | ++ | ++ | ++ |
| **4b** | ++ | ++ | ++ |
| **5b** | ++ | ++ | + |
| **4c** | ++ | ++ | - |
| **5c** | + | + | - |

Symbols : ++ = soluble ; + = slightly soluble ; - = insoluble

**Table 2.2.** Qualitative solubilities of iodinated salicylate-based poly(anhydride-esters), **4** and **5.**

## 2.4. Cell Compatibility

*The cytotoxicity studies were performed by MinJung Song, a Ph.D. candidate in Biomedical Engineering (Rutgers University, Piscataway, NJ) supervised by Professor Uhrich.*

As a first step for testing biocompatibility of the polymers, preliminary cytotoxicity experiments were performed using L929 mouse fibroblasts to examine cellular response using two methods: culturing cells in polymer-containing media (0.1 and 0.01 mg/mL) and culturing cells directly on polymer-coated glass coverslips. Both studies were performed over a three-day time period, in which cell proliferation and morphology were measured. The chosen concentrations of polymer in media (0.01 and 0.1 mg/mL) were based on standard cytotoxicity protocols in our laboratory.[25] Briefly,

iodinated salicylate-based poly(anhydride-esters) prepared by both polymerization methods were found to be compatible with cells at low concentratations of polymer in media (0.01 mg/mL). Cells in media containing the salicylic acid-based polymer (**4a**) and the 5-iodosalicylic acid-based polymers (**4b**, **5b**) at higher concentrations (0.1 mg/mL) exhibited a positive growth profile over the 3 days, and cell numbers were within the standard deviation of the control. In contrast, cells in the presence of the 3,5-diiodosalicylic acid-based polymers (**4c**, **5c**) exhibited significantly low cell numbers and did not show positive growth profiles at higher concentrations of polymer in media (0.1 mg/mL). For polymer-coated surfaces, cell proliferation on salicylic acid-based polymer (**5a**) and control surfaces show a normal growth profile on days 2 and 3, whereas cells on the iodinated polymers (**4b-c, 5b-c**) displayed negative growth profiles. Furthermore, only cells on salicylic acid-based polymer **5a** and the control surfaces displayed normal satellite morphology.

## 2.5. Polymer Degradation

### 2.5.1. Degradation in Media

Based upon the cytotoxicity results, we hypothesized that the polymer-coated surfaces may be releasing a much higher dose of free drug (**1**) than the levels we evaluated in the media cytotoxicity studies. The effect of the high polymer concentration was investigated by degrading the polymer surfaces and determining the concentration of free drug (**1**) released into the media during the time period of the cytotoxicity assay. In **Table 2.3**, the maximum theoretical amount of free drug, **1**, released from each polymer is presented.

| Drug (1) | Polymer | Coated Coverslip (mg) | 0.1 mg/mL Media (mg) | 0.01 mg/mL Media (mg) |
|---|---|---|---|---|
| Salicylic Acid, **1a** | **4a/5a** | 5.0 | 0.10 | 0.010 |
| 5-Iodosalicylic Acid, **1b** | **4b/5b** | 7.0 | 0.14 | 0.014 |
| 3,5-Diiodosalicylic Acid, **1c** | **4c/5c** | 7.9 | 0.16 | 0.016 |

**Table 2.3.** Maximum release of free drug (**1**) upon complete hydrolysis**.**

The maximum amount of salicylate (**1**) released from the corresponding polymers, **4** or **5**, is much larger for the polymer-coated coverslips than in the polymer-containing media. Thus, much larger local concentration of free drug (**1**) in media is possible with the coated coverslips.

The amount of free drug (**1**) actually released in media was analyzed by UV/vis spectrophotometry, and the results are presented in **Figure 2.6**. Over 3 days, the cumulative release of salicylic acid (**1a**) from coated coverslips with polymers **4a** and **5a** were 7.8 and 4.2 mg/mL, respectively (~ 7 % of total 1a content). The cumulative release of the iodinated derivatives of salicylic acid (**1b-c**) after 3 days was much larger, ~ 35 % released from each polymer, **4b-c** and **5b-c**. After 3 days, the concentration of salicylate (**1**) for each polymer is as follows: **4a** is 0.39 mg/mL , **4b** is 2.5 mg/mL, **4c** is 2.6 mg/mL, **5a** is 0.21 mg/mL, **5b** is 2.5 mg/mL, and **5c** is 2.8 mg/mL.

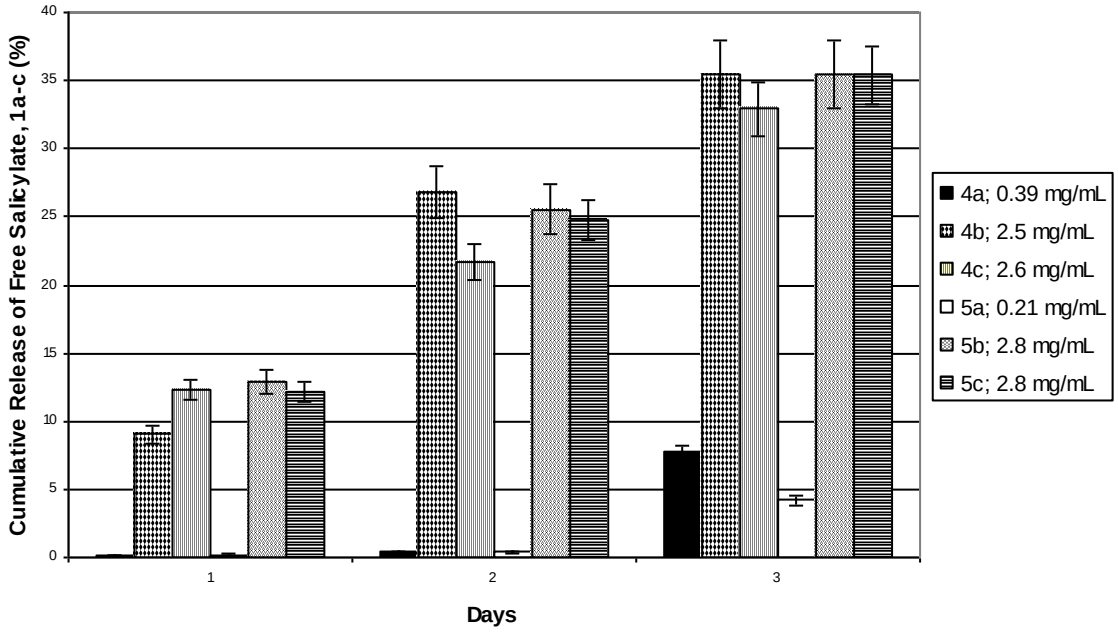


**Figure 2.6**. Cumulative release of free drug (**1**) from polymers **4** and **5** with final concentration of **1** in degradation media.

Over three days, larger concentrations of iodinated salicylates (**1b-c**) from polymers **4b-c** and **5b-c** were detected in cell media as compared with salicylic acid (**1a**) release from polymers **4a** and **5a**. This increase in amount of iodinated salicylate (**1b-c**) compared to salicylate (**1a**) detected in the degradation media after 3 days can be attributed to the consistently lower molecular weights of the iodinated polymers (**4b-c** and **5b-c**). As these polymers are more difficult to polymerize, lower molecular weights are obtained as compared with the salicylate-based polymers (**4a** and **5a**). A lower molecular weight corresponds to less anhydride and ester bonds to hydrolyze and a resulting shorter overall degradation time. A similar correlation between polymer molecular weight and release of bioactive has been noted in the literature.[41-43] Although after 3 days degradation in

PBS media, an increased amount of **1b-c** as compared with **1a** was observed and the overall degradation rate may be similar; as noted in the literature, initial polymer molecular weight is independent of degradation rate for most polyanhydrides.[42] For the polymer degradation in strong base, all of the polymers (**4a-c** and **5a-**c) completely degraded within the same time period, namely ~1 d.

The increased concentrations of **1a** in media may have an adverse effect on the cell growth and proliferation, with fibroblasts grown on polymer-coated glass coverslips. At lower concentrations of polymer (**4** or **5**) and corresponding free salicylate (**1**) in cell media, as seen in cytotoxicity studies in polymer-containing media, favorable cell attachment and proliferation was observed.[44]

### 2.5.2. Degradation in Strong Base

As outlined in **Table 2.1**, different properties were obtained for melt (**4**) versus solution-made (**5**) polymers, yet a design goal for these polymers is to completely degrade into the salicylates. A complete hydrolytic degradation of the polymers was performed in highly basic conditions (0.1 N NaOH) to measure the concentrations of the final polymer degradation products. The amount of salicylate (**1a**) or iodinated salicylate (**1b-c**) in each polymer was calculated before and after complete hydrolytic degradation: the amount of **1** released upon base hydrolysis was quantitative relative to the concentration of 1 in the polymers (**4** and **5**). Clearly, the final degradation products (i.e., salicylates **1**) are independent of the polymer polymerization method (melt or solution): regardless of polymerization method, the polymer degrades and ultimately releases the expected amount of salicylate (**1**).

## 2.6. Summary

Poly(anhydride-esters) based on iodinated versions of salicylic acid were synthesized *via* both melt-condensation and solution polymerization techniques to generate radiopaque biomaterials. The poly(anhydride-esters) from iodinated salicylates were highly X-ray opaque compared to poly(anhydride-esters) from salicylic acid. Molecular weight and Young's modulus of polymers prepared by melt-condensation were typically two-to-three times higher than polymers prepared by solution methods. The glass transition temperatures of the polymers were dependent on the iodine concentration; polymers containing more iodine had higher glass transition temperatures. Cytotoxicity studies using mouse fibroblasts indicated that iodinated salicylate-based poly(anhydride-esters) prepared by both polymerization methods are biocompatible with cells at low polymer concentrations (0.01 mg/mL). Based upon these results, we anticipate that iodinated poly(anhydride-esters) will be useful as radiopaque coatings or implants.

## 2.7. Experimental

### 2.7.1. Materials

All solvents and reagents were purchased from Fisher Scientific (Pittsburgh, PA), and all other fine chemicals were purchased from Sigma-Aldrich (Milwaukee, WI).

### 2.7.2. Methods

#### 2.7.2.a. Proton Nuclear Magnetic Resonance ($^{1}$H NMR) Spectroscopy

Proton and carbon nuclear magnetic resonance ($^{1}$H- and $^{13}$C-NMR) spectra of the products were obtained using a Varian 200 MHz, 300 MHz, or 400 MHz

spectrophotometer. The chosen deuterated solvent was dimethyl sulfoxide-$d_6$, which was also used as the internal reference.

**2.7.2.b. Infrared (IR) Spectroscopy**

Fourier-transform infrared (IR) absorption spectra were recorded using a Thermo Nicolet/Avatar 360 FT-IR spectrometer by solvent-casting samples with acetone or methylene chloride onto sodium chloride plates.

**2.7.2.c. Melting Point Determination**

Melting points were determined using a Model 1002D Manual Mel-temp apparatus (Barnstead/Thermolyne, Dubuque, Iowa).

**2.7.2.d. Gel Permeation Chromatography (GPC)**

Molecular weights ($M_w$) and polydispersity indices (PDI) were determined using gel permeation chromatography (GPC) with polystyrene standards (Polymer Source Inc., Dorval, Canada) on a Jordi DVB mixed-bed GPC column (7.8 x 300 mm, Alltech, Deerfield, IL). The Perkin-Elmer LC system was equipped with a Series 200 refractive index detector, a Series 200 pump, and ISS 200 autosampler. A Dell OptiPlex GX110 computer with Perkin-Elmer TurboChrom 4 software was used for collection and processing of the data and for the automation of the GPC analyses using a Perkin-Elmer Nelson 900 Series Interface and Perkin-Elmer Nelson 600 Series Link. The polymer samples (~5 mg/mL) were dissolved in methylene chloride and filtered using 0.45 μm pore size poly(tetrafluoroethylene) (PTFE) syringe filters (Nalge Nunc International, Rochester, NY) and placed in sample vials for autoinjection.

### 2.7.2.e. Differential Scanning Calorimetry (DSC)

Thermal analyses were measured using a Perkin-Elmer system consisting of a Pyris 1 differential scanning calorimeter (DSC) or Thermal Advantage system consisting of a differential scanning calorimeter (DSC) Q200. A Dell Optiplex GX110 computer equipped with Perkin-Elmer Pyris software or IBM ThinkCentre computer equipped with Thermal Advantage Universal Analysis software were used for data collection and processing. The glass transition temperature ($T_g$) was determined on samples (5-10 mg) under nitrogen gas heating from –10 °C to 200 °C at a heating rate of 10 °C/min and cooling to –10 °C at a rate of 10 °C/min with a minimum of two heating/cooling cycles. The $T_g$ was calculated as half $C_p$ extrapolated.

### 2.7.2.f. Thermal Gravimetric Analysis (TGA)

Decomposition temperature ($T_d$) was measured using a Perkin-Elmer system consisting of a thermogravimetric analyzer (TGA) with TAC 7/DX instrument controller or Thermal Advantage system consisting of a thermogravimetric analyzer (TGA) Q50. A Dell Optiplex GX110 computer equipped with Perkin-Elmer Pyris software or IBM ThinkCentre computer equipped with Thermal Advantage Universal Analysis software were used for data collection and processing. For TGA, samples (5-10 mg) were heated under nitrogen gas from 25 °C to 400 °C at a rate of 10 °C/min; the decomposition temperature ($T_d$) was calculated as the onset of thermal decomposition.

### 2.7.2.g. Dynamic Mechanical Analysis (DMA)

Mechanical properties were measured using a Perkin-Elmer system consisting of a Pyris 1 dynamic mechanical analyzer (DMA) with TAC 7/DX instrument controllers. A Dell Optiplex GX110 computer equipped with Perkin-Elmer Pyris software was used

for data collection and processing. Young's modulus was determined on pressed disks (see below) by DMA using the slope of the linear portion of the static stress versus strain curve. The DMA method applied constant pressure from 0 mN to 8000 mN at a rate of 500 mN/min at 22 °C.

**2.7.2.h. Elemental Analysis**

Elemental analyses were provided by QTI (Whitehouse, NJ).

**2.7.2.i. Polymer Disk Preparation**

Polymer disks were prepared from powdered samples (~160 mg) using Carver model M bench-top hydraulic press (Carver Inc., Wabash, IN). Pressure of 10,000 psi was applied for 5 min at room temperature to generate disks (13 mm diameter and 1 mm thickness) using a stainless steel mold.

**2.5.2.j. Contact Angle Measurement**

Static contact angles were measured by dropping deionized water onto pressed polymer disks using a Ramé-Hart Standard Goniometer Model Number 250-00 (Mountain Lakes, NJ) outfitted with a Dell Dimension 3000 computer with DROPimage Advanced software.

**2.7.2.k. X-ray Analysis**

Radiopacity of polymer disks was measured using a clinical X-ray machine according to Method B of the ASTM F 640-79 Standard Test Methods for Radiopacity of Plastics for Medical Use.[40] In brief, polymer disks were placed on the stage of the C-arm and blindly ranked from 1 to 6, where 6 is the darkest (i.e., most X-ray opaque) and compared to a standard aluminum-step wedge. The C-arm setup consisted of the X-ray

instrument equipped with a 2.5 mm aluminum filtration set at 70 kV with a 10-20 mA current for 15 ms.

#### 2.7.2.l. Qualitative Solubility

Polymer solubility in common organic solvents was determined by mixing powdered polymer samples (10 mg) with the appropriate solvent (1 mL) and observing the clarity or turbidity of the solution after mixing with the naked eye.

### 2.7.3. Poly(anhydride-ester) Precursor: Diacid

Poly[1,6-bis(*o*-carboxyphenoxy)-hexanoate] (**2a**) was prepared using previously described methods.[22] All other diacids were prepared using the procedure outlined in Figure 2. The salicylate (**1**; 1.4 g, 10 mmol) was dissolved in a solution of tetrahydrofuran (40 mL) and pyridine (1.7 mL, 20 mmol). Adipoyl chloride (0.80 mL, 5.0 mmol) dissolved in tetrahydrofuran (10 mL) was added drop-wise to the reaction mixture at room temperature using a syringe. The reaction was stirred for 2 – 4 h and quenched by pouring over water (~600 mL) and acidifying to pH 2 with concentrated hydrochloric acid. The diacid (**2**) was filtered, washed with deionized water (3 x 200 mL) and dried under vacuum at room temperature for 24 h. The diacid was recrystallized from either acetone/hexanes or diethyl ether/hexanes.

**1,6-bis(5-iodo-1,2-carboxyphenoxy)-hexanoate (2b).** Yield: 97 % (white powder). $^{1}$H-NMR (DMSO-$d_6$): 8.20 (s, 2H, ArH), 7.98 (d, 2H, ArH), 7.05 (d, 2H, ArH), 2.63 (t, 4H, $CH_2$), 1.74 (m, 4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): 171.9, 165.7, 150.8, 142.9, 140.6, 126.9, 126.7, 91.7, 33.9, 24.1. IR (NaCl, $cm^{-1}$): 1749 (C=O, ester), 1705 (C=O, COOH), 3579 (OH, COOH). Anal. Calcd: C, 37.69%; H, 2.53%; I, 39.82%; O. Found:

C, 38.31%; H, 2.50%; I, 39.99%. $T_m$: 210-212 °C.

**1,6-bis(3,5-diiodo-1,2-carboxyphenoxy)-hexanoate (2c).** Yield: 98 % (white powder). $^1$H-NMR (DMSO-$d_6$): 8.43 (s, 2H, ArH), 8.15 (s, 2H, ArH), 2.64 (t, 4H, $CH_2$), 1.76 (m, 4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): 170.9, 164.3, 150.8, 150.5, 140.4, 127.5, 97.5, 92.8, 33.9, 24.1. IR (NaCl, $cm^{-1}$): 1773 (C=O, ester), 1697 (C=O, COOH), 3583 (OH, COOH). Anal. Calcd: C, 27.01%; H, 1.59%; I, 57.09%. Found: C, 27.50%; H, 1.52%; I, 56.85%. $T_m$: 202-205 °C.

### 2.7.4. Acetylated Monomer

The diacid (**2a**) was activated by acetylation to yield **3a** as previously outlined.[22,25] All activated monomers (**3**) were prepared following a similar procedure. The diacid (**2**; 2 g) was added to an excess of acetic anhydride (100 mL) and stirred either at room temperature (for **2a**) or heated to reflux temperature (for **2b-c**) until a clear, homogeneous solution was observed (~2-12 h). The excess acetic anhydride was removed using a rotary evaporator (Buchi Model R-205 equipped with a V-800 vacuum controller, B-490 heating bath, and V-500 vacuum pump) to afford the acetylated compound (**3**), which was washed with diethyl ether (3 x 10 mL).

**1,6-bis(5-iodo-1,2-carboxyphenoxy)-hexanoate Monomer (3b).** Yield: quantitative (pale yellow oil). $^1$H-NMR (DMSO-$d_6$): 8.22 (s, 2H, ArH), 8.01 (d, 2H, ArH), 7.05 (d, 2H, ArH), 2.65 (t, 4H, $CH_2$), 2.06 (s, 6H, $CH_3$), 1.76 (m, 4H, $CH_2$). IR (NaCl, $cm^{-1}$): 1814 (C=O, anhydride), 1766 (C=O, ester). $T_d$: 293 °C.

**1,6-bis(3,5-diiodo-1,2-carboxyphenoxy)-hexanoate Monomer (3c).** Yield: quantitative (pale orange oil). $^1$H-NMR (DMSO-$d_6$): 8.44 (s, 2H, ArH), 8.18 (s, 2H,

ArH), 2.66 (t, 4H, $CH_2$), 2.30 (s, 6H, $CH_3$), 1.72 (m, 4H, $CH_2$). IR (NaCl, $cm^{-1}$): 1817 (C=O, anhydride), 1771 (C=O, ester). $T_d$: 268 °C.

### 2.7.5. Poly(anhydride-ester) Synthesis

#### 2.7.5.a. Melt-Condensation Polymerization

The activated monomer (**3a**) undergoes melt condensation polymerization to yield **4a** as previously outlined.[22] Polymers (**4**) were prepared following the same procedure. The acetylated compound (**3**; 2 g) was placed in a double-necked round-bottom flask equipped with overhead stirrer (T-line Laboratory Stirrer, Model 104, Talboys Engineering, Thorofare, NJ) and heated to 160 °C using a temperature controller (Cole-Parmer, Vernon Hills, Illinois) in a silicone oil bath under vacuum (< 2 mm Hg) until the viscosity of the melt remained constant or solidified (~ 3-6 h). The monomer was vigorously stirred at ~100 rpm/min using the overhead stirrer during the polymerization. When complete, the polymer was cooled to room temperature and isolated by precipitation from methylene chloride into a 20-fold excess of diethyl ether.

**Melt-Condensation Poly[1,6-bis(5-iodo-1,2-carboxyphenoxy)-hexanoate] (4b).** Yield: 62.7 % after fractionation (beige powder). $^1$H-NMR (DMSO-$d_6$): 8.22 (b, 2H, ArH), 8.01 (b, 2H, ArH), 7.05 (b, 2H, ArH), 2.65 (b, 4H, $CH_2$), 1.76 (b, 4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): 175.0, 174.9, 172.0, 171.87, 165.0, 164.8, 164.7, 161.7, 161.4, 161.3, 151.0, 150.9, 150.6, 150.5, 150.3, 150.1, 144.6, 144.2, 144.0, 143.2, 142.9, 140.7, 140.5, 140.2, 138.8, 127.3, 127.0, 126.9, 126.7, 124.4, 124.1, 120.6, 92.2, 91.9, 91.5, 91.2, 65.6, 34.1, 34.0, 33.9, 33.8, 33.7, 33.6, 24.7, 24.6, 24.5, 24.3, 24.1, 24.0, 23.9, 15.9. IR (NaCl, $cm^{-1}$): 1798, 1699 (C=O, anhydride), 1750 (C=O, ester). Contact angle: 72 °.

**Melt-Condensation Poly[1,6-bis(3,5-diiodo-1,2-carboxyphenoxy)-hexanoate] (4c).** Yield: 74.6 % after fractionation (pale brown powder). $^{1}$H-NMR (DMSO-$d_6$): 8.45 (s, 2H, ArH), 8.20 (s, 2H, ArH), 2.63 (t, 4H, $CH_2$), 1.68 (m, 4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): 179.7, 174.9, 170.5, 166.1, 164.3, 163.1, 159.8, 154.9, 151.7, 150.5, 148.9, 140.4, 138.7, 136.5, 132.5, 128.9, 127.5, 125.0, 122.5, 121.1, 115.8, 109.4, 104.4, 103.3, 101.5, 99.8, 97.5, 94.8, 92.8, 89.7, 35.2, 34.0, 24.7, 22.4, 18.9, 14.6. IR (NaCl, $cm^{-1}$): 1812, 1699 (C=O, anhydride), 1756 (C=O, ester). Contact angle: 79 °.

**2.7.5.b. Solution Polymerization**

The diacid (**2**) was polymerized by solution polymerization as previously outlined.[32,33] Polymers (**5**) were prepared following the same procedure.[32,33] Polymerization was performed under anhydrous conditions using nitrogen gas. The diacid (**2**; 4 g, 10 mmol) was dissolved in anhydrous methylene chloride (16 mL). Freshly distilled triethylamine (6.0 mL, 50 mmol) was added drop-wise to the reaction mixture at room temperature. The reaction was then cooled to 0 °C using an ice bath for 15 min. Triphosgene (3.4 g, 11 mmol) dissolved in anhydrous methylene chloride (15 mL) was added drop-wise to the reaction mixture at 0 °C over 1 h using a syringe. After stirring for 1.5 h, the reaction mixture was poured over diethyl ether (300 mL), the solid filtered and washed with acidified water (1 L, pH 2 with concentrated HCl). The products were dried under vacuum at room temperature.

**Solution Polymerization Poly[1,6-bis(o-carboxyphenoxy)-hexanoate] (5a).** Yield: 89.1 % after fractionation (beige powder). $^{1}$H-NMR (DMSO-$d_6$): 7.94 (d, 2H, ArH), 7.67 (t, 2H, ArH), 7.38 (t, 2H, ArH), 7.19 (d, 2H, ArH), 2.62 (t, 4H, $CH_2$), 1.77 (m, 4H, $CH_2$). $^{1}$H-NMR (DMSO-$d_6$): 172.0, 160.2, 155.6, 151.8, 132.9, 127.5, 125.3, 121.8,

33.5, 24.0. IR (NaCl, $cm^{-1}$): 1781, 1733 (C=O, anhydride), 1760 (C=O, ester). Contact angle: 38 °.

**Solution Polymerization Poly[1,6-bis(5-iodo-1,2-carboxyphenoxy)-hexanoate] (5b).** Yield: quantitative after fractionation (pale orange powder). $^{1}$H-NMR (DMSO-$d_6$): 8.16 (s, 2H, ArH), 7.96 (d, 2H, ArH), 7.02 (d, 2H, ArH), 2.60 (t, 4H, $CH_2$), 1.70 (m, 4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): 175.1, 171.9, 164.9, 150.6, 142.9, 140.1, 126.9, 126.8, 91.2, 33.8, 24.0. IR (NaCl, $cm^{-1}$): 1795, 1732 (C=O, anhydride), 1765 (C=O, ester). Contact angle: 67 °.

**Solution Polymerization Poly[1,6-bis(3,5-diiodo-1,2-carboxyphenoxy)-hexanoate] (5c).** Yield: quantitative (pale pink powder). $^{1}$H-NMR (DMSO-$d_6$): 8.43 (s, 2H, ArH), 8.18 (s, 2H, ArH), 2.67 (t, 4H, $CH_2$), 1.73 (m, 4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): 174.9, 172.0, 164.8, 150.6, 144.4, 140.4, 126.9, 126.7, 91.9, 33.9, 24.5. IR (NaCl, $cm^{-1}$): 1795, 1732 (C=O, anhydride), 1765 (C=O, ester). Contact angle: 84 °.

### 2.7.6. *In Vitro* Degradation

#### 2.7.6.a. *In Vitro* Hydrolytic Degradation in Media

Microscope glass coverslips were coated with polymer (~10 mg/coverslip) and UV-sterilized, seeded with cell media (2 mL) in a 12-well plate (Fisher Scientific, Fair Lawn, NJ) and incubated at 37 ℃ for 3 days. At predetermined time points (24, 48 and 72 h), aliquots of media were removed and analyzed using UV/vis spectrophotometry (DU520, Beckman Instruments, Fullerton, CA) to determine the amount of free drug (**1**) in the media at $\lambda_{max}$ 300 nm. The amounts were calculated with respect to calibration curves of standard solutions of each compound (**1**).

### 2.7.6.b. *In Vitro* Hydrolytic Degradation in Strong Base

Powdered polymer samples (~100 mg) were individually placed in 20 mL glass scintillation vials (Fisher Scientific, Fair Lawn, NJ), and aqueous 0.1 N NaOH solution (10 mL) added. The vials were incubated at 37 ºC with agitation to allow complete hydrolysis of the melt (**4**) and solution-made (**5**) polymers. Once degraded (~24 h), the amount of salicylate (**1a**) or iodinated salicylate (**1b-c**) released in the aqueous NaOH solution during the hydrolysis was quantitatively determined using a DU520 UV spectrophotometer (Beckman Instruments, Fullerton, CA). UV/vis measurements were taken at the maximum absorbance for **1** ($\lambda_{max}$ 300 nm). This wavelength did not overlap with the media or other polymer degradation products (i.e., adipic acid or diacids, **2**). The amount released was calculated with respect to standard solutions of each compound (**1**).

### 2.7.7. Cell Compatibility

[*Cell studies were performed by MinJung Song, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ*].

Cytotoxicity of the polymers (**4** and **5**) was tested in two different ways: culturing cells in media containing the polymers (to evaluate both the polymer and polymer degradation products) and on polymer-coated surfaces (to directly assess the polymer-cell interface). Cellular morphology and proliferation was investigated for both systems, solution and surface.

#### 2.7.7.a. Polymer-containing Media

Experimental procedures for polymer solution preparation, fibroblast culture, cell morphology evaluation and cell proliferation quantification were previously published.[44]

#### 2.7.7.b. Polymer-coated Surfaces

Polymers (**4** and **5**) were dissolved in methylene chloride at a concentration of 100 mg/mL. Two to three drops of polymer solution were used to coat glass coverslips (18 mm diameter, 0.15 mm thickness; Fisher Scientific, Pittsburgh, PA) with a spin-coater at 2000 rpm for 30 s in less than 20 % humidity (Headway Research, Inc., Garland, TX). Polymer-coated coverslips were sterilized under UV-light at 254 nm for 900 s using a Spectrolinker XL-1500 UV crosslinker (Spectronics Corp., Westbury, NY) and placed onto a 12-well tissue culture plate (Fisher Scientific, Fair Lawn, NJ). Blank coverslips (uncoated) were used as controls.

Experimental procedures for fibroblast culture, cell morphology evaluation and cell proliferation quantification were previously published.[44]

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# 3. PROLONGED RELEASE OF SALICYLIC ACID FROM POLY(ANHYDRIDE-ESTER) COPOLYMERS

## 3.1. Introduction

Salicylic acid has many therapeutic properties, as it is has an anti-inflammatory, analgesic, antiseptic, keratolytic, and antipyretic properties.[1,2] As discovered in our laboratory and described in Chapter 2, polyanhydrides derived from salicylic acid have been proven effective for various biomedical applications.[3-10] One characteristic of polyanhydrides is the ability to tailor the degradation rate of the polymer based upon its chemical composition, specifically the chemical structure of the "linker" molecule that connects two salicylic acid molecules together in each repeat unit.[5]

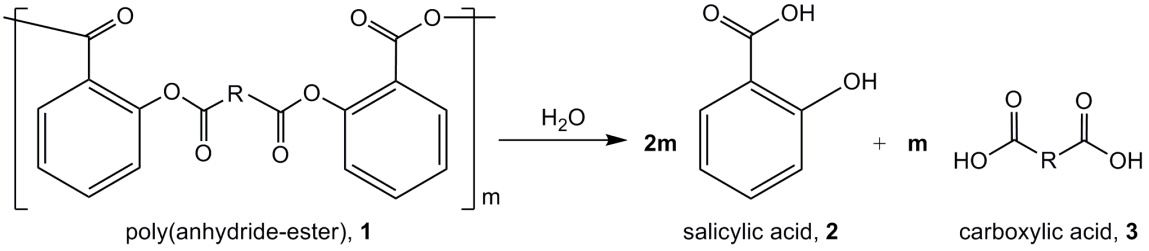



**Figure 3.1.** Hydrolysis of salicylic acid-derived poly(anhydride-ester) **1**.

The release rate of salicylic acid could be tailored from weeks to months depending on the structure of the linker.[5] A variety of different linker molecules were studied and most polymers completely degrade, releasing the chemically incorporated salicylic acid in up to 30 days.[5]

## 3.2. Background.

### 3.2.1. Applications of Slow-degrading Polyanhydrides

Salicylate-based polyanhydrides may be valuable in tissue engineering and reconstructive procedures as these polymers deliver a high load of chemically incorporated non-steroidal anti-inflammatory drugs,[4,5] inhibit bacterial biofilm formation,[7,9] and have been proven to stimulate new bone formation.[3,6,11] For use as artificial matrices tissue engineering, the polymer properties should mimic the properties of the tissue being regenerated.[12] The polymeric matrix can function as a scaffold for cells to adhere onto, proliferate and form tissue that acts similarly to the original tissue.[12]

One application of interest to salicylate-based polyanhydrides is bone regeneration, for which the polymeric matrix must be mechanically stiff to bear dynamic loads.[12-15] Based on previous results for cranio-facial cavarial defects,[11] it has been found that the degradation rate of the polymer and subsequent release of salicylic acid must be tailored to the desired time frame, > 6-8 weeks. This time frame allows salicylic acid to be released to prevent inflammation and biofilm formation, yet enable cells to proliferate on the scaffold. Most salicylate-based polyanhydrides from our laboratory degrade in less than 30 days.[4,5]

### 3.2.2. Copolymers

To tailor the degradation rate and thermal/mechanical properties of the salicylate-based polyanhydride, copolymers were made from the salicylate-based polymers, 1,6-bis(*o*-carboxyphenoxy)hexanoate (SAA), with two different highly aromatic

comonomers, namely 1,6-bis(*o*-carboxyphenoxy)hexane (*o*-CPH) and 1,6-bis(*p*-carboxyphenoxy)hexane (*p*-CPH) (**Figure 3.2)**.

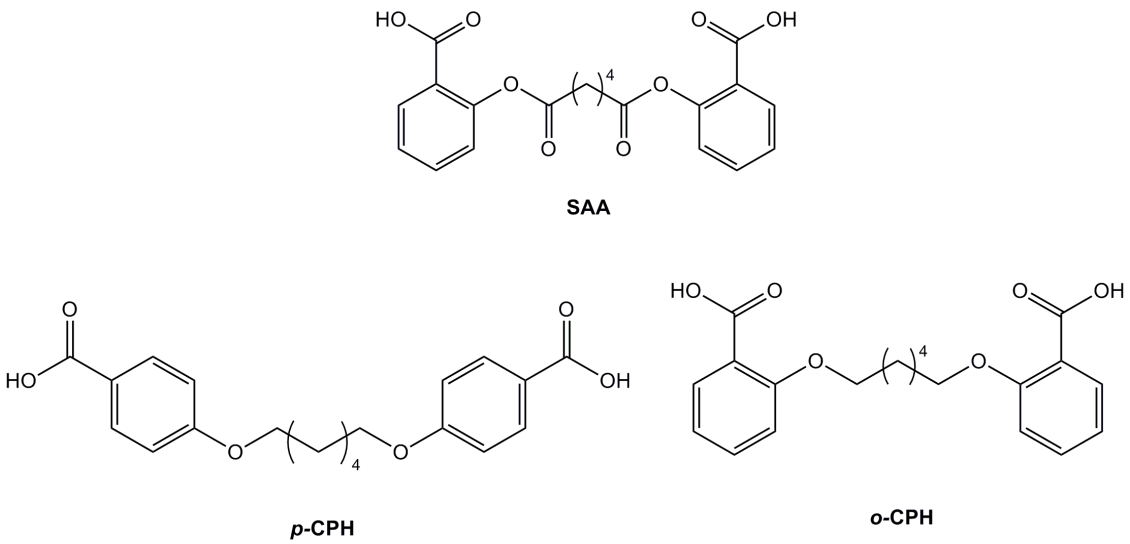


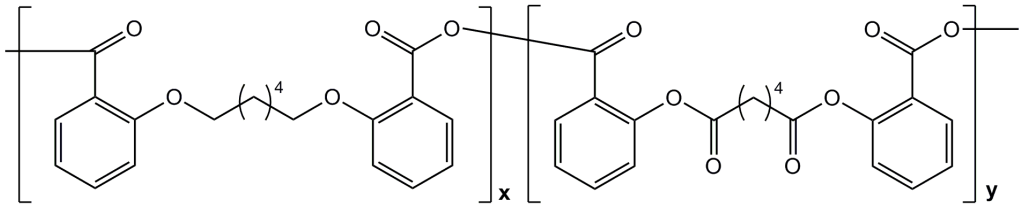

**Figure 3.2.** Chemical structures of salicylate-based polymer (SAA), *o*-CPH, and *p*-CPH.

In early reports by Conix and Yoda, the introduction of aromatic and heterocyclic monomers into polyanhydrides led to polymers with enhanced hydrolytic and thermal stabilities.[16-25] Furthermore, preliminary data from our laboratory showed that thermal properties of similar copolymers comprised of salicylate-based polymer, 1,10-bis(*o*-carboxyphenoxy)decanoate, and *p*-CPH could be tailored depending on the ratio of comonomers.[26] However, mechanical properties and degradation studies on such polymers were never evaluated, and *o*-CPH was not utilized. Therefore, a variety of different copolymer compositions of SAA with *o*-CPH or *p*-CPH were synthesized and studied.

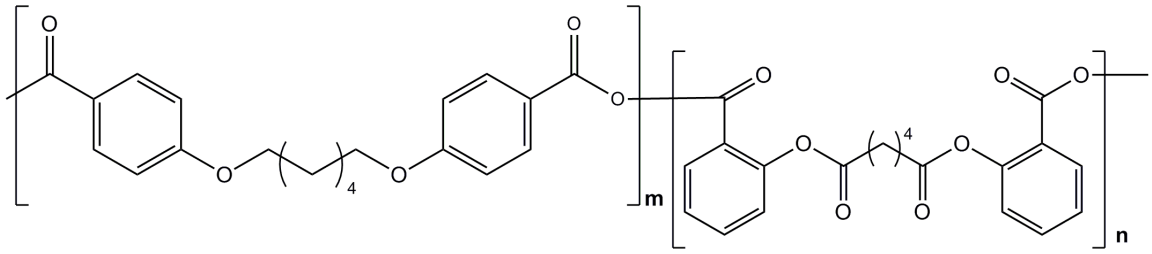
## 3.3. Results and Discussion

### 3.3.1. Synthesis of Slow-degrading Poly(anhydride-ester) Copolymers

A series of copolymers based on SAA and *o*-CPH or *p*-CPH were synthesized and the resulting polymer properties compared as a function of copolymer composition. The polymers (**4** and **5**) were synthesized by melt-condensation polymerization at high temperatures (i.e., 180 °C) and high vacuum (< 2 mm Hg), **Figure 3.3**.[4,5,27]



***o*-CPH:SAA Copolymer, 4**



***p*-CPH:SAA Copolymer, 5**

**Figure 3.3.** Structures of CPH:SAA copolymers **4** and **5**.

### 3.3.2. Properties of Salicylate-based Poly(anhydride-ester) Copolymers

The physiochemical properties of the copolymers based on SAA and *o*-CPH are given in **Table 3.1**, and for copolymers of SAA and *p*-CPH in **Table 3.2**. The copolymer compositions were calculated from $^{1}$H-NMR spectral data.

| ***o*-CPH:SAA (Theoretical)** | ***o*-CPH:SAA (Calculated)** | **$M_w$** | **PDI** | **$T_g$ (°C)** | **$T_m$ (° C)** | **$T_d$ (°C)** |
|---|---|---|---|---|---|---|
| 0:100 | 0:100 | 11,800 | 2.6 | 44 | n/a | 300 |
| 25:75 | 27:83 | 14,000 | 4.1 | 50 | n/a | 300 |
| 50:50 | 55:45 | 20,200 | 1.9 | 48 | n/a | 325 |
| 75:25 | 70:30 | 31,400 | 2.8 | 39 | n/a | 300 |
| 100:0 | 100:0 | 43,500 | 2.2 | 27 | n/a | 315 |

**Table 3.1.** Properties of *o*-CPH:SAA copolymers, **4**.

| ***p*-CPH:SAA (Theoretical)** | ***p*-CPH:SAA (Calculated)** | **$M_w$** | **PDI** | **$T_g$ (° C)** | **$T_m$ (° C)** | **$T_d$ (°C)** |
|---|---|---|---|---|---|---|
| 0:100 | 0:100 | 11,800 | 2.6 | 44 | n/a | 300 |
| 25:75 | 21:79 | 15,800 | 2.9 | 62 | n/a | 315 |
| 50:50 | 52:48 | 20,700 | 2.0 | 88 | 128 | 315 |
| 72:25 | 72:28 | 8,400 | 1.3 | 90 | 121 | 325 |
| 100:0 | 100:0 | 23,800 | 2.4 | 115 | 132 | 360 |

**Table 3.2.** Properties of *p*-CPH:SAA copolymers, **5**.

### 3.3.2.a. Molecular Weight

The molecular weights obtained for all copolymers were typical for polyanhydrides synthesized by melt-condensation, ranging from ~10,000 to.~40,000.[4,5,26,27] For the copolymers based on *o*-CPH and SAA (**4**), the molecular weight increased with increasing *o*-CPH content.

### 3.3.2.b. Thermal Properties

Thermal properties varied depending on the copolymer composition. For copolymers based on *o*-CPH and SAA (**4**, **Table 3.1**), the glass transition temperature ($T_g$) decreased with increasing *o*-CPH content. For example, a $T_g$ of 50 °C was obtained

for the 25:75 *o*-CPH: SAA copolymer and 39 °C for the 75:25 *o*-CPH:SAA copolymer. All copolymers containing *o*-CPH had no indication of a melting temperature, indicating they are amorphous.

For copolymers based on *p*-CPH and SAA (**5**, **Table 3.2**), a different trend in thermal properties was observed. As content of *p*-CPH to SAA increased, so did the $T_g$. Furthermore, a melting transition ($T_m$) was noted with increasing *p*-CPH content, beginning with the 50:50 composition. With a high *p*-CPH content (> 50 %), the copolymers obtained displayed semi-crystalline properties with both glass transition and melting temperatures.

### 3.3.2.c. Mechanical Properties

Dynamic mechanical analysis was utilized to study the mechanical properties of the copolymers (**Table 3.3**).

| Polymer | Young's Modulus (kPa) |
|---|---|
| SAA | 26.8 |
| 25/75 *o*-CPH/SAA | 31.0 |
| 50/50 *o*-CPH/SAA | 62.7 |
| 75/25 *o*-CPH/SAA | 43.5 |
| *o*-CPH | 69.2 |
| 25/75 *p*-CPH/SAA | 49.9 |
| 50/50 *p*-CPH/SAA | 65.9 |
| 75/25 *p*-CPH/SAA | 138.2 |
| *p*-CPH | 125.3 |

**Table 3.3.** Evaluation of mechanical properties of SAA:CPH copolymers (**4** and **5**) by dynamic mechanical analysis.

The Young's modulus (i.e., stiffness) of the copolymers increased with incorporation of CPH as comonomer. For *p*-CPH copolymers (**5**), the Young's modulus values increased as glass transition and the *p*-CPH content increased. Therefore, introducing a highly

aromatic comonomer results in more robust thermal and mechanical properties of the resulting copolymers.

### 3.3.3. *In Vitro* Hydrolytic Degradation

The release of salicylic acid from the copolymers (**4** and **5**) was studied for ~1 month on 50:50 copolymers to obtain preliminary data on their release profiles. (**Figure 3.4**).

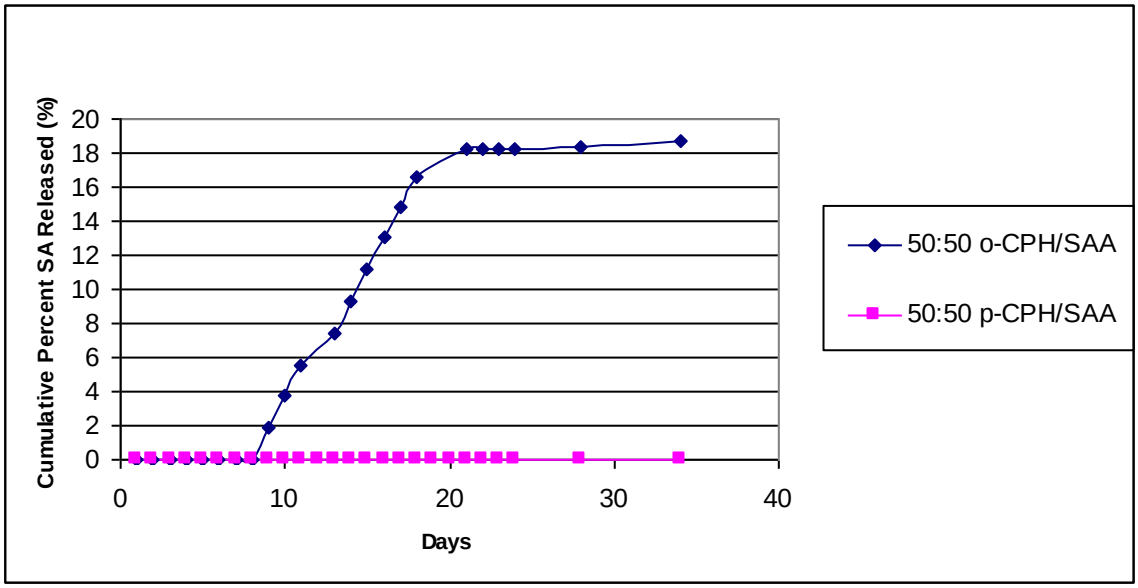



**Figure 3.4.** Cumulative release of salicylic acid from selected CPH/SAA copolymers (**4** and **5**).

The copolymer derived from 50:50 *p*-CPH:SAA (**5**) was very slow degrading, releasing less than 1 % after 34 days. For the copolymer containing 50:50 *o*-CPH:SAA (**4**), ~20 % of the incorporated salicylic acid released in 34 days. For comparison, the SAA polymer

alone degrades in < 7 days.[5] Therefore, incorporating the CPH comonomer into the polymer decreases the release rate, making these polymers more suitable for tissue engineering applications.

### 3.4. Summary

A series of biodegradable polyanhydrides derived from a salicylate-based poly(anhydride-ester) (SAA) and *o*-CPH and *p*-CPH were synthesized by melt-condensation polymerization. When copolymerizing SAA and CPH monomers at different molar ratios, different thermal and mechanical properties were observed. For copolymers containing SAA and *o*-CPH (**4**), as the content of *o*-CPH increased, the $T_g$ decreased, with no presence of a $T_m$, indicating all copolymers in the series were amorphous. Alternately, for copolymers containing SAA and *p*-CPH (**5**), the $T_g$ increased as the *p*-CPH content increased. These copolymers were semi-crystalline with a $T_m$ present when *p*-CPH content was greater than 50 %.

Also notable, the mechanical properties of the copolymers were enhanced with copolymerization with CPH, increasing the Young's modulus (i.e., stiffness) of the resulting copolymers (**4** and **5**). The degradation rate and subsequent release can also be altered by chemically incorporating CPH into a copolymer with SAA. Therefore, the degradation rate can be tailored for a specific application requiring a longer release, such as tissue engineering applications.

## 3.5. Experimental

### 3.5.1. Materials

All chemicals, reagents and solvents were purchased from Aldrich (Milwaukee, WI) and used as received.

#### 3.5.1.a. Salicylate-based Diacid

Salicylate-based diacid was synthesized using previously described methods.[4,5]

### 3.5.2. Methods

#### 3.5.2.a. Proton Nuclear Magnetic Resonance ($^1$H NMR) Spectroscopy

Proton nuclear magnetic resonance ($^1$H-NMR) spectra were recorded on a Varian 300 or 400 MHz spectrophotometer. Samples (5-10 mg) were dissolved in deuterated solvent (DMSO-$d_6$), which was also the internal reference.

#### 3.5.2.b. Infrared (IR) Spectroscopy

Infrared (IR) spectra were measured on a Thermo Nicolet/Avatar 360 FT IR spectrometer, by solvent-casting samples from acetone or methylene chloride onto NaCl plates.

#### 3.5.2.c. Gel Permeation Chromatography (GPC)

Weight-averaged molecular weights ($M_w$) of polymers were determined using a Perkin-Elmer LC system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 advanced sample processor. A Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software was utilized for data collection and automation of the system,. The connection from the LC system to the computer was made using a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Polymers

(**4** and **5**) were dissolved in methylene chloride (10 mg/mL) and filtered through 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe filters (Whatman, Clifton, NJ) prior to elution through a Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm) (Alltech Associates, Deerfield, IL). Molecular weights were calculated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

### 3.5.2.d. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was used to determine glass transition temperature ($T_g$) with a Perkin-Elmer system consisting of a Pyris 1 differential scanning calorimeter (DSC). A Dell Optiplex GX110 computer equipped with Perkin-Elmer Pyris software was used for data collection and processing. For glass transition temperatures, samples (~5 mg) were heated under dry nitrogen gas at heating and cooling rates of 10 $^{o}$C/min with a two-cycle minimum. Glass transition temperatures were calculated as half $C_p$ extrapolated.

### 3.5.2.e. Thermal Gravimetric Analysis (TGA)

Decomposition temperature ($T_d$) was measured using a Perkin-Elmer system consisting of a thermogravimetric analyzer (TGA) with TAC 7/DX instrument controller. A Dell Optiplex GX110 computer equipped with Perkin-Elmer Pyris software was used for data collection and processing. Samples (5-10 mg) were heated under nitrogen gas from 25 °C to 400 °C at a rate of 10 °C/min; the decomposition temperature ($T_d$) was calculated as the onset of thermal decomposition.

### 3.5.2.f. Dynamic Mechanical Analysis (DMA)

Mechanical properties (i.e., Young's modulus) were measured using a Perkin-Elmer system consisting of a Pyris 1 dynamic mechanical analyzer (DMA) with TAC

7/DX instrument controllers. A Dell Optiplex GX110 computer equipped with Perkin-Elmer Pyris software was used for data collection and processing. Young's modulus was determined on pressed disks (see below) with DMA using the slope of the linear portion of the static stress versus strain curve. The DMA method applied constant pressure from 0 mN to 8000 mN at a rate of 500 mN/min at 22 °C.

#### 3.5.2.g Polymer Disk Preparation

Polymer disks were prepared from powdered polymer samples (~160 mg) using Carver model M bench-top hydraulic press (Carver Inc., Wabash, IN). Pressure of 10,000 psi was applied for 5 min at room temperature to generate disks (13 mm diameter and 1 mm thickness) using a stainless steel mold.

#### 3.5.2.h. UV/Vis Spectrophotometry

Salicylate release from polymers (**4** and **5**) was monitored using a DU 520 UV/vis spectrophotometer (Beckman Instruments, Fullerton, CA) at $\lambda_{max}$ 303 nm for SA.

### 3.5.3. Polymer Precursor: Diacid

#### 3.5.3.a. *o*-Carboxyphenoxy hexane (*o*-CPH) Diacid

1,6-Bis(*o*-carboxyphenoxy)hexane diacid (*o*-CPH) was synthesized using previously described methods with small modifications.[28]

In brief, sodium hydride (57 mmol) was added to dimethylformamide (150 mL) to give a white suspension. Methyl salicylate (57 mmol) was added to give a pale yellow suspension. Then, 1,6-dibromohexane (28 mmol) was added, and the reaction was refluxed overnight. After refluxing, the reaction was poured onto water (~750 mL) and acidified to pH 2 using concentrated hydrochloric acid to give a white suspension. The

suspension was filtered to give an off-white solid (*o*-CPH methyl ester). The methyl ester was then mixed with a methanol:water solution (10:1, 100 mL), and sodium hydroxide was added until the pH was ~12. This reaction mixture was refluxed for 2 h, and subsequently poured onto water (~750 mL) and acidified to pH 2 using concentrated hydrochloric acid. The solid obtained is filtered off, washed with water (3 x 150 mL) and dried under vacuum at room temperature. Typical yields were ~90 %.

**3.5.3.b. *p*-Carboxyphenoxy hexane (*p*-CPH) Diacid**

1,6-Bis(*p*-carboxyphenoxy)hexane diacid (*p*-CPH) was synthesized using techniques similar to those previously described with some minor modifications.[26-28] The same procedure as described above for *o*-CPH was utilized using methyl-4-hydroxybenzoate instead of methyl salicylate. Typical yields were ~95 %.

**3.5.3.c. Salicylate-based Poly(anhydride-ester) Copolymers**

Different copolymers of the salicylate-based diacid and either *o*-CPH or *p*-CPH diacid were synthesized using melt-condensation polymerization.[4,5,27] The molar ratios of salicylate-based diacid to *o*-CPH or *p*-CPH ranged from 0:1, 1:3, 1:1, 3:1, and 1:0. A typical procedure is as follows: the monomers (salicylate-based diacid and *o*-CPH or *p*-CPH) were refluxed in excess acetic anhydride in separate flasks. Appropriate molar amounts of each diacid were then placed in a two-necked round-bottom flask equipped with an overhead stirrer (T-line Laboratory Stirrer, Talboys Engineering). The reaction mixture was heated to 180 °C using a temperature-controlled silicone oil bath (Cole Parmer) under high vacuum (< 2 mm Hg) for 4-6 h.

**SAA:*o*-CPH Poly(anhydride-ester) (4).** $^1$H NMR (DMSO-$d_6$): $\delta$ = 8.13 (b, 2H, Ar-H), 7.97 (b, 4H, Ar-H), 7.72 (b, 2H, Ar-H), 7.43 (b, 2H, Ar-H), 7.09 (b, 2H, Ar-H),

6.97 (b, 4H, Ar-H), 4.04 (4H, $CH_2$), 3.30 (4H, $CH_2$), 2.59 (4H, $CH_2$), 1.74 (4H, $CH_2$), 1.50 (4H, $CH_2$). IR (NaCl): 1 780, 1 720 (vs, C=O, anhydride), 1 750 (vs, C=O, ester), 1 250 $cm^{-1}$ (s, C-O-C, ether).

**SAA:*p*-CPH Poly(anhydride-ester) (5).** $^1$H NMR (DMSO-$d_6$): δ = 8.18 (b, 2H, Ar-H), 7.98 (b, 4H, Ar-H), 7.83 (b, 2H, Ar-H), 7.42 (b, 2H, Ar-H), 7.06 (b, 2H, Ar-H), 6.98 (b, 4H, Ar-H), 4.05 (4H, $CH_2$), 3.31 (4H, $CH_2$), 2.61 (4H, $CH_2$), 1.73 (4H, $CH_2$), 1.45 (4H, $CH_2$). IR (NaCl): 1 780, 1 720 (vs, C=O, anhydride), 1 740 (vs, C=O, ester), 1 250 $cm^{-1}$ (s, C-O-C, ether).

### 3.5.4. *In Vitro* Hydrolytic Degradation

#### 3.5.4.a. Sample Preparation

Polymer disks (**Section 3.5.2.g**) were prepared as described above and placed in 20 mL glass scintillation vials (Wheaton, Fisher, Fair Lawn, NJ) with 20 mL phosphate buffer solution (PBS) and incubated at 37 °C with agitation at 60 rpm in a controlled environment incubator-shaker (New Brunswick Scientific Co., Edison, NJ).

#### 3.5.4.b. Degradation Media Preparation

Degradation media used was phosphate buffer saline (PBS) containing 0.1 M potassium hydrogen phosphate and 0.1 M potassium dihydrogen phosphate. The pH was adjusted to 7.4 using either 1 N hydrochloric acid or 1 M sodium hydroxide solutions. Measurements for pH were obtained using an Accumet® AR15 pH meter (Fisher Scientific, Fair Lawn, NJ).

### 3.5.4.c. Free Salicylate Release

Polymer disks were incubated in PBS, and at daily time intervals, the media was removed, replaced with fresh media and analyzed by UV spectrophotometry (Beckman Instruments DU520 UV/vis spectrophotometer, Fullerton, CA). UV measurements were taken at the wavelength of maximum absorbance for salicylic acid (2) ($\lambda_{max}$ 313 nm), which did not overlap with any other degradation products. Data was calculated against a calibration curve made from standard solutions of salicylic acid in PBS ($1 \times 10^{-3}$, $2.5 \times 10^{-3}$, $5 \times 10^{-3}$, $1 \times 10^{-2}$ mg/mL). Degradation was performed in triplicate.

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# 4. FAST-DEGRADING SALICYLATE-BASED POLY(ANHYDRIDE-ESTERS)

## 4.1. Introduction

Salicylic acid is anti-inflammatory, antiseptic, analgesic, antipyretic and keratolytic, and thus desirable for formulations in many different areas outside of biomaterials.[1,2] Relative to sustained drug delivery systems, faster degrading polymers are desirable for wound care, personal care, oral care, cosmetic and food applications; these applications require rapid but controlled release of the bioactive.[3-5] For example, a controlled, high release rate of bioactive is desirable for applications such as wound care in which a high initial release is necessary within 12 h-3 d[6-9] or for encapsulating flavors and aromas in the food industry or fragrances in the personal care/cosmetics industries, with release in the range of 6-12 h.[6,10] As such, we designed salicylate-based polyanhydrides to release a significant amount of the salicylic acid in a short time period (hours to days). This effect was achieved by altering the chemical composition of the linker molecule or introducing a second, more hydrophilic monomer to make poly(anhydride-ester) copolymers.

## 4.2. Background: Manipulation of Bioactive Release from Poly(anhydride-esters)

### 4.2.1. Linker Structure

Bioactive molecules can be chemically incorporated into poly(anhydride-esters), directly into the backbone of the polymer (e.g., **1a, Figure 4.1**).[11-13]

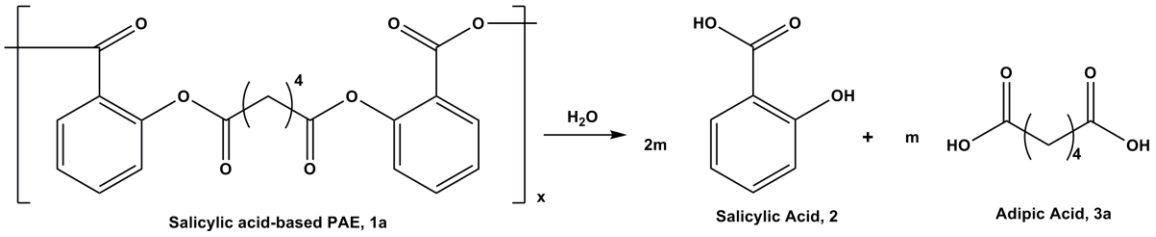
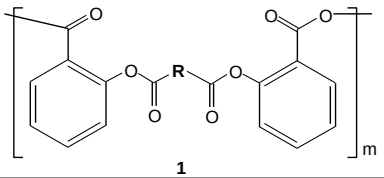



**Figure 4.1.** Hydrolysis of salicylic acid-based poly(anhydride-ester), **1a**, to release the bioactive compound (**2**) and the biocompatible linker molecule (**3a**).

Upon hydrolytic degradation, the labile anhydride and ester bonds are cleaved releasing the bioactive component (e.g., salicylic acid or SA, **2**) and the water-soluble biocompatible "linker" molecule (e.g., adipic acid, **3a**). Previously in our laboratory, a series of salicylate-based poly(anhydride-esters) with different linker molecules were synthesized (**Table 4.1**).[14]



**1**

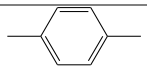
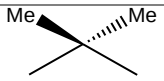
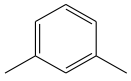
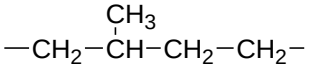
| **R** | | |
|---|---|---|
| **Linear Aliphatic** | **Aromatic** | **Branched Aliphatic** |
| $(CH_2)_3$ |  |  |
| $(CH_2)_4$ | | |
| $(CH_2)_5$ |  |  |
| $(CH_2)_6$ | | |
| $(CH_2)_7$ | |  |
| $(CH_2)_8$ | | |

**Table 4.1.** "Linker" structures of **R** in polymer **1**.[14]

It was found that the resulting polymer properties and hydrolytic degradation rates in physiological conditions (pH 7.4, 37 ˚C) were dependent on the chemical structure of the linker molecule.[14] Similar drug release profiles were noted for each polymer, however, the release rates were dependent on the linker molecule. Polymers containing linear, aliphatic linkers degraded the fastest (6-16 d), followed by polymers with aromatic linkers (up to 20 d), and lastly, the polymers containing the branched aliphatic linkers only partially degraded after 30 days of incubation.[14] Among the polymers containing the linear linkers, the shorter carbon chains corresponded to faster release of salicylic acid.

Based on these results, the linker molecules chosen for faster degrading polymers are based on short, aliphatic chains or short, aliphatic chains containing heteroatoms (i.e., oxygen). The linkers in this chapter are also known to be biocompatible or have GRAS (generally regarded as safe) status,[15] thus, the degradation products are already known to be safe. For example, adipic acid is a GRAS (generally regarded as safe) and EAFUS (everything added to food in the United States) compound,[15] glutaric acid is naturally occurring in sugar beets,[1] and diglycolic acid is used in many biocompatible polymer formulations.[16-18]

### 4.2.2. Copolymers

Another way to control polyanhydride properties and resulting degradation rate is to generate copolymers.[19,20] As discussed in Chapter 3, copolymers of salicylate-based poly(anhydride-esters) (e.g., **1a**) with highly aromatic poly(1,6-bis(*p*-carboxyphenoxy)hexane (*p*-CPH) or poly(1,6-bis(*o*-carboxyphenoxy)hexane (*o*-CPH)

increased the portion of CPH to **1a** increased the thermal and mechanical properties, but decreased the drug loading and release rate of the incorporated SA.

Alternately, using hydrophilic comonomers containing heteroatoms (e.g, diglycolic acid) with **1a** should increase the degradation rate of the resulting copolymer. A variety of copolymers containing hydrophilic comonomers, such as poly(ethylene glycol) (PEG), have been utilized for drug delivery and other biomedical applications.[21] For example, copolymers comprised of poly(lactic acid) and hydrophilic PEG have been synthesized with increased water absorption.[22-24] Copolymerization of sebacic acid or trimellitylimidoglycine with PEG segments resulted in copolymers that completely degraded within 3 to 12 days.[25]

## 4.3. Results and Discussion

### 4.3.1. Synthesis of Fast-degrading Poly(anhydride-esters)

#### 4.3.1.a. Fast-degrading Poly(anhydride-esters)

Diacids (**5**) were synthesized by direct coupling of salicylic acid (**2**) with a diacyl chloride (**4**) in the presence of base (i.e., pyridine) in THF. Polymers **1** were synthesized as previously described using solution polymerization techniques (**Figure 4.2**) with the use of triphosgene as the coupling reagent in the presence of triethylamine.[26-28]

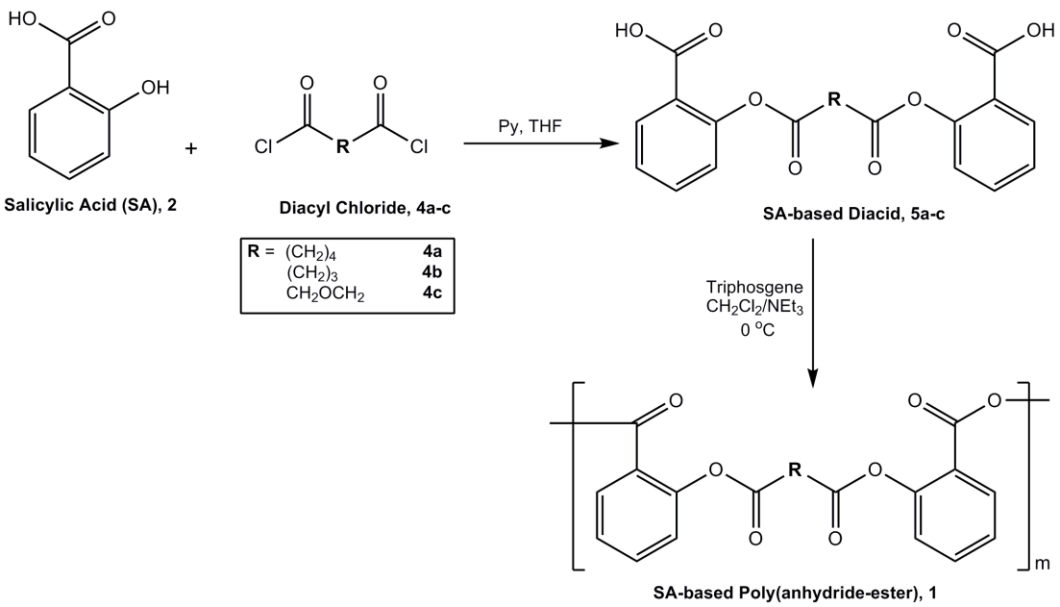



**Figure 4.2.** Synthesis salicylic acid-based poly(anhydride-esters), **1.**

Polymers were purified by isolation from methylene chloride into diethyl ether and subsequent washing with acidic water.

#### 4.3.1.b. Fast-degrading Poly(anhydride-ester) Copolymers

The copolymer (**6**) was synthesized by polymerizing a 60:40 molar ratio of SA-based diacid (**5a**) with diglycolic acid (**3c**) using similar solution polymerization techniques as described above and elsewhere (**Figure 4.3**).[26-28] This molar ratio was chosen to have the same weight percent of diglycolic acid in the polymer backbone as in polymer **1c**, as shown in **Table 4.2**.

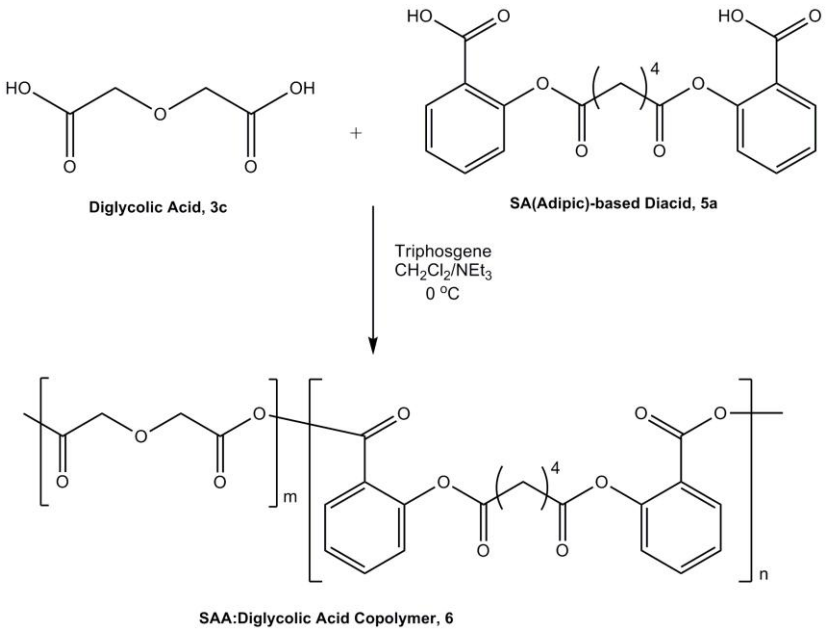



**Figure 4.3.** Synthesis of salicylate-based copolymer, **6**, from diglycolic acid (**3c**) and salicylate-based diacid, **5a**.

### 4.3.2. Properties of Fast-degrading Salicylate-based Poly(anhydride-esters) and Copolymers

Polymer properties are summarized in **Table 4.2**. The high drug loading (by weight) of salicylic acid for the polymers varied from 61.2 for polymer **6** up to 74.2 % for polymer **1b**.

| Polymer | Wt % SA | $M_w$ | PDI | $T_g$ (°C) | $T_d$ (°C) | Contact Angle (°) |
|---|---|---|---|---|---|---|
| **1a** | 71.5 | 27,800 | 1.0 | 40 | 283 | 83.1 |
| **1b** | 74.2 | 18,100 | 1.3 | 38 | 295 | 85.2 |
| **1c** | 73.8 | 14,500 | 1.5 | 35 | 326 | 49.5 |
| **6** | 61.2 | 21,700 | 1.1 | 33 | 269 | 65.4 |

**Table 4.2.** Selected properties of polymers **1** and **6**.

#### 4.3.2.a. Molecular Weight

Polymer properties such as molecular weight, ranging from ~15,000 to 28,000, are typical for most polyanhydrides made by solution polymerization methods.[26-28] Isolating polymers by precipitation removes most small oligomers and residual monomer, resulting in polymers with low polydispersity indices.[4,26,28,29]

#### 4.3.2.b. Thermal Properties

A single glass transition temperature was noted for polymer **6**, indicating no phase separation. Similarly, no melting temperatures were observed for the polymers **1**, demonstrating that all polymers are amorphous, with glass transition temperatures near physiological temperature.

### 4.3.3. *In Vitro* Hydrolytic Degradation

#### 4.3.3.a. Polymer Hydrophobicity

Polymer degradation is influenced by multiple factors, including water permeation into the polymer matrix and water-solubility of the degradation products.[11,12,19,30-32] The contact angles for all polymers were measured (**Table 4.2**). By introducing diglycolic acid (**3c**) into the polymeric backbone either as a "linker" molecule (**1c**) or as a co-monomer (**6**), the resulting poly(anhydride-ester) becomes more hydrophilic. Polymers **1b** and **1a** have three and four methylenes, respectively, and display similar contact angles. When diglycolic acid (**3c**) is incorporated as the linker, the polymer (**1c**) becomes much more hydrophilic (49.5 °). The copolymer (**6**) comprised of diacid **5a** and diglycolic acid (**3c**) had an intermediate contact angle (65.4 °) between that of **1a** (83.1 °) and **1c** (49.5 °).

#### 4.3.3.b. *In Vitro* Degradation Products

Diacid solubility was determined using UV/vis spectrophotometry. The results are summarized in **Table 4.3**. Comparing diacids (**5**), **5c** with the oxygen-containing linker was most soluble in PBS, correlating with contact angle measurements, which indicated the resulting polymer (**1c**) was most hydrophilic. The co-monomer in polymer **6**, diglycolic acid (**3c**), however, was overall the most soluble in PBS.

| Diacid | Maximum Solubility (mg/mL) |
|---|---|
| **3c** | 16.0 |
| **5a** | 4.38 |
| **5b** | 7.68 |
| **5c** | 8.12 |

**Table 4.3.** Maximum solubilities of diacids **3c** and **5** in PBS.

#### 4.3.3.c. Polymer Degradation and Free Salicylate Release

SA release studies were conducted on ground-up powdered polymer samples in PBS at physiological conditions to determine how much SA (**2**) is released over a 2-day period. Again, 48 h is the critical time period for wound care and other applications that require a controlled, yet high release rate, and is thus the chosen end point for the degradation study. The results of the release study are shown in **Figure 4.4**.

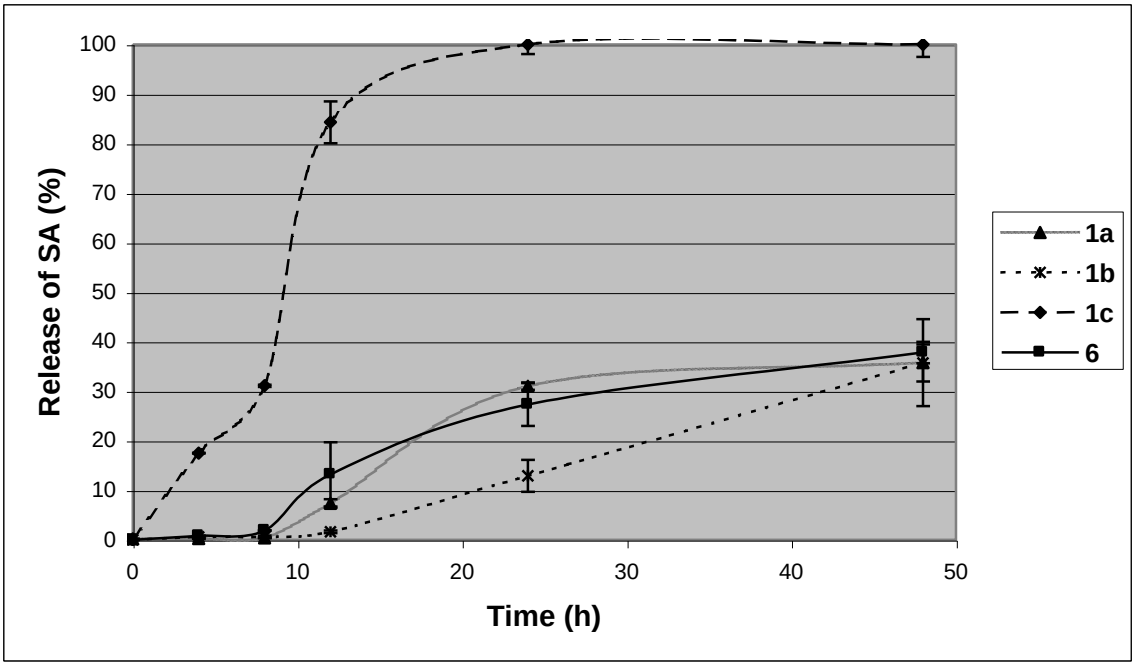



**Figure 4.4.** Cumulative release of salicylic acid, **2**, from salicylate-based poly(anhydride-esters), **1** and **6**.

By 24 h, 100 % of the SA is released from polymers **1c**. After 48 h, ~35 % of SA is released from polymers **1a** and **1b**, whereas 38 % is released from polymer **6**. In less than one week, all polymers were completely degraded. When diglycolic acid (**3c**) was incorporated into the polymer as a linker, the SA release was faster compared with copolymer **6** at the same weight SA percent.

## 4.4. Summary

Fast-degrading, bioactive-based polyanhydrides (**1**) were synthesized by altering the structure of linker molecule to incorporate small aliphatic chains (i.e., adipic and glutaric acid) or more hydrophilic, oxygen-containing aliphatics (i.e., diglycolic acid).

For comparison, a copolymer (**6**) of diglycolic acid was made with a salicylate-based diacid containing a short, aliphatic linker. Both methods rendered polyanhydrides that released a significant amount of the chemically incorporated SA and completely degraded within one week. The controlled, sustained release over the first few days may be beneficial for wound, personal/oral care, cosmetic and food applications.

Based on results from preliminary experiments using polymers **1** and **6**, we recommend these polymers in fast-acting applications. For specific applications such as wound care, which require a significant to complete release of bioactive in the early stages of healing (12-24 h), polymer **1c** is most suitable. For other applications that require a slightly longer release, specifically within ~1 week, polymers **1a-b** and **6** are more appropriate.

## 4.5. Experimental

### 4.5.1. Materials

Diethyl ether was obtained from Fisher (Fair Lawn, NJ). All other chemicals and reagents were purchased from Aldrich (Milwaukee, WI) and used as received.

### 4.5.2. Methods

#### 4.5.2.a. Proton Nuclear Magnetic Resonance ($^1$H NMR) Spectroscopy

Proton nuclear magnetic resonance ($^1$H-NMR) spectra were recorded on a Varian 500 MHz spectrophotometer. Samples (5-10 mg) were dissolved in an appropriate deuterated solvent (DMSO-$d_6$), which was also the internal reference.

#### 4.5.2.b. Infrared (IR) Spectroscopy

Infrared (IR) spectra were measured on a Thermo Nicolet/Avatar 360 FT IR spectrometer, by solvent-casting samples from acetone onto NaCl plates.

#### 4.5.2.c. Gel Permeation Chromatography (GPC)

A Perkin-Elmer LC system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 advanced sample processor was used to determine weight-averaged molecular weights ($M_w$) and polydispersity indices (PDI). For data collection and automation of the system, a Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software was utilized. The connection from the LC system to the computer was achieved via a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Polymers (**1a-c**, **6**) were dissolved in methylene chloride (10 mg/mL) and filtered through 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe filters (Whatman, Clifton, NJ) before elution onto the Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm) (Alltech Associates, Deerfield, IL). Molecular weights were calculated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

#### 4.5.2.d. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was used to determine glass transition ($T_g$) and melting point ($T_m$) temperatures using a TA DSC Q200 outfitted with a Dell Dimension 3000 computer running TA Universal Analysis 2000 software was used for data collection and processing. Melting points of diacids (**5**) were obtained using a heating rate of 10 °C/min heating from 25 °C to 200 °C. For glass transition temperatures, samples (~5 mg) were heated under dry nitrogen gas at heating and cooling rates of 10

ºC/min with a two-cycle minimum. Glass transition temperatures were calculated as half $C_p$ extrapolated.

#### 4.5.2.e. Thermal Gravimetric Analysis (TGA)

Thermogravimetric analysis for decomposition temperatures ($T_d$) was performed on a Perkin-Elmer TGA7 analyzer with TAC7/DX controller equipped with a Dell OptiPlex GX110 computer running Perkin-Elmer Pyris software. Samples (~5 mg) were heated under dry nitrogen gas at a heating rate of 10 ºC/min from 25 ºC to 400 ºC. Decomposition temperatures were defined as the onset of decomposition.

#### 4.5.2.f. Mass Spectrometry

Negative electrospray ionization mass spectrometry (ESI-MS) was performed using a Finnigan LCQDUO LC/MS/MS spectrometer.

#### 4.5.2.g Contact Angle Measurements

Static contact angles were measured by dropping deionized water onto polymer films made by solvent-casting polymers in methylene chloride onto glass coverslips using a Ramé-Hart Standard Goniometer Model Number 250-00 (Mountain Lakes, NJ) outfitted with a Dell Dimension 3000 computer with DROPimage Advanced software. The measurements taken were in duplicate with an average of five readings recorded within 30 seconds of depositing the water droplet onto the polymer film.

#### 4.5.2.h. UV/Vis Spectrophotometry

Maximum solubility of diacids (**5**) was determined by monitoring absorbance of saturated solutions of each diacid (**5**) in PBS using UV/vis spectrophotometry ($\lambda$ = 235 nm for **5a-c**; $\lambda$ = 215 nm for **3c**). The absorbance was compared to standard solutions of each diacid (between 0.001 and 0.01 mg/mL).

Salicylate (**2**) release from polymers (**1**, **6**) was also monitored using a DU 520 UV/vis spectrophotometer (Beckman Instruments, Fullerton, CA) at $\lambda_{max}$ 303 nm for SA.

### 4.5.3. Polymer Precursor: Diacid (5)

Diacids, **5**, were synthesized using previously described techniques.[11,12] The characterization of **5a-b** has already been published, however, **5c** is a new compound and its properties are outlined below. In brief, salicylic acid (**2**; 32 mmol) was dissolved in 100 mL THF and pyridine (64 mmol). Diglycolyl chloride (**4c**; 16 mmol) was dissolved in 15 mL THF and added drop-wise to the stirring reaction mixture to give a suspension. The reaction was allowed to stir at room temperature overnight and subsequently quenched by pouring over water (~700 mL) and adding HCl while stirring until pH ~2. The solid formed (**5c**) was filtered off, washed with water (1 L) and allowed to dry under vacuum at room temperature.

**Salicylic Acid (Diglycolic) Diacid (5c).** Yield: 70.8 % (beige powder). $^1$H NMR (DMSO-$d_6$): $\delta$ = 7.93 (d, 2H, Ar-H), 7.65 (t, 2H, Ar-H), 7.40 (t, 2H, Ar-H), 7.24 (d, 2H, Ar-H), 4.55 (s, 4H, $CH_2$). IR (NaCl): 3 600-3 300 (s, OH, COOH), 1 780 (vs, C=O, ester), 1 757 (vs, C=O, COOH), 1 209 cm$^{-1}$ (s, C-O-C, ether). $(C_{18}H_{14}O_9)_n$ $(374.3)_n$: ESI-MS m/z 373 *z*-1; $T_m$: 182 ºC.

### 4.5.4. Polymer Synthesis

Polymer **1a** was synthesized by solution polymerization as previously described.[27,28] New polymers (**1b**, **1c** and **6**) were also synthesized using solution polymerization methods.[26-28]

#### 4.5.4.a. Salicylate-based Poly(anhydride-ester)

In general, the diacid (**5**; 20 mmol) was dissolved in 20 w/v % anhydrous methylene chloride. Triethylamine (88 mmol) was then added, and the reaction mixture cooled to 0 °C with an ice/water bath. Triphosgene (22 mmol) dissolved in anhydrous methylene chloride was added drop-wise using a syringe pump or addition funnel at a rate of 14 mL/h. The reaction was allowed to stir at 0 °C for ~2 h. Then, the reaction mixture is poured over diethyl ether (~400 mL), washed with acidic water (~1L, pH 2 using concentrated HCl). The solid obtained (**1** or **6**) was dried under vacuum at room temperature.

**Salicylic Acid (Diglycolic) Polymer (1c).** Yield: 74.1 % (off-white powder). $^{1}$H NMR (DMSO-$d_6$): δ = 8.18 (b, 2H, Ar-H), 7.81 (b, 2H, Ar-H), 7.54 (b, 2H, Ar-H), 7.39 (b, 2H, Ar-H), 4.51 (4H, $CH_2$). IR (NaCl): 1 786, 1 727 (vs, C=O, anhydride), 1 744 (vs, C=O, ester), 1 210 cm$^{-1}$ (s, C-O-C, ether).

#### 4.5.4.b. Salicylate-based Poly(anhydride-ester) Copolymer (6)

The molar ratio of 5a to diglycolic acid (3c) incorporated was 60:40. Yield: 50.2 % (beige powder). $^{1}$H NMR (DMSO-$d_6$): δ = 8.08 (b, 2H, Ar-H), 7.78 (b, 2H, Ar-H), 7.47 (b, 2H, Ar-H), 7.31 (b, 2H, Ar-H), 3.32 (b, 4H, $CH_2$), 3.07 (b, 2H, $CH_2$), 1.56 (b, 2H, $CH_2$). IR (NaCl, cm$^{-1}$): 1 790, 1 728 (vs, C=O, anhydride), 1 763 (vs, C=O, ester), 1 208 (s, C-O-C, ether). Composition (by NMR): 61:39.

### 4.5.5. *In Vitro* Hydrolytic Degradation

The release rate of SA was evaluated from hydrolytic degradation of polymers **1** and **6.**

#### 4.5.5.a. Sample Preparation

Polymers were ground with mortar and pestle to particle sizes of ~ 75-300 μm. Particle size was determined using standard testing sieves (Aldrich, Milwaukee, WI).

#### 4.5.5.b. Degradation Media Preparation

Degradation media used was phosphate buffer saline (PBS) containing 0.1 M potassium hydrogen phosphate and 0.1 M potassium dihydrogen phosphate. The pH was adjusted to 7.4 using either 1 N hydrochloric acid or 1 M sodium hydroxide solutions. All pH measurements were performed using an Accumet® AR15 pH meter (Fisher Scientific, Fair Lawn, NJ).

#### 4.5.5.c. Free Salicylate Release

Ground-up polymer samples (~20 mg) were incubated at 37 °C with agitation at 60 rpm in 10 mL PBS (pH 7.4) in 20 mL Wheaton glass scintillation vials (Fisher, Fair Lawn, NJ) using a controlled environment incubator-shaker (New Brunswick Scientific Co., Edison, NJ). At regular time intervals, the media was analyzed by UV spectrophotometry (Beckman Instruments DU520 UV/vis spectrophotometer, Fullerton, CA). UV measurements were taken at the wavelength of maximum absorbance for salicylic acid (2) ($\lambda_{max}$ 303 nm), which did not overlap with other polymer degradation products (i.e., diacid, 5). Data was calculated against a calibration curve made from standard solutions of salicylic acid in PBS ($1 \times 10^{-3}$, $2.5 \times 10^{-3}$, $5 \times 10^{-3}$, $1 \times 10^{-2}$ mg/mL). Degradation was performed in triplicate.

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# 5. NATURAL ANTIMICROBIAL-BASED POLYANHYDRIDES

## 5.1. Introduction

Bacterial contamination is a serious issue for many different industries including food, personal care and medical devices. The development of communities of microorganisms, or biofilms, is similar for each industry and leads to serious infection, food spoilage and other complications.[1-3] To reduce or inhibit the occurrence of infection due to biofilms, antimicrobials can be utilized.

Slow, controlled release of bioactive compounds that can suppress microbial growth would be extremely useful not only in foods, but in medical and personal care fields. This effect can be achieved with the use of antimicrobial-based, biodegradable polymers. As discussed in Chapters 2 and 4, biodegradable salicylate-based poly(anhydride-esters)[4,5] (**1**) that degrade *in vitro* and *in vivo* to release the active component, salicylic acid (SA, **2**), and appropriate dicarboxylic acid "linker" molecule (e.g., adipic acid, **3**) have been studied for medical applications including inflammation control,[6,7] bone growth[8,9] and as cardiac stents[10] (**Figure 5.1**).

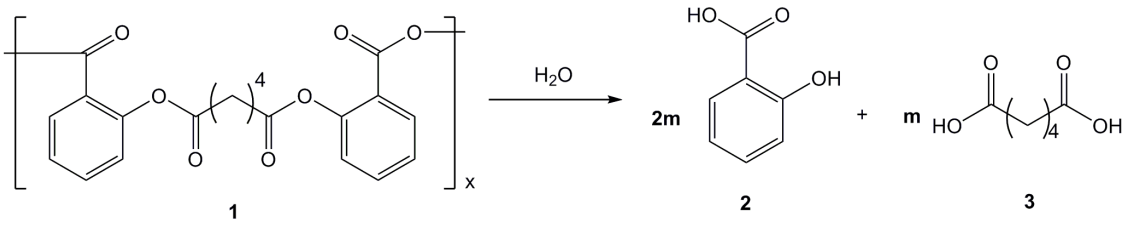


**Figure 5.1.** Hydrolytic degradation of salicylate-based polymer (**1**) into salicylic acid (**2**).

More recently, polymer **1** has been studied to test its ability to inhibit biofilm formation.[6,11] Controlled, sustained release of the mild antimicrobial, salicylic acid,[12] from polymer **1** during biodegradation prevents colonization by medical device bacteria *Pseudomonas aeruginosa*[6] and food-borne bacteria *Salmonella enterica* serovar Typhimurium[11].

For food applications, it would be desirable to utilize antimicrobials that are derived from nature or already used in combination with foods, as safety of food additives and preservatives is a major concern.[13-16] On that basis, we designed and synthesized bioactive-based polymers containing natural antimicrobials derived from plants and spice extracts.[12,17-19] The natural antimicrobials were chemically incorporated into a novel polyanhydride backbone as pendant groups *via* ester linkages to obtain polymers with antimicrobial activity.

## 5.2. Background

### 5.2.1. Microbial Biofilms

Biofilms can be found in nature (e.g., on rocks by streams and oceans), on medical devices or on foods. They are composed of microorganisms embedded within an extracellular polymeric matrix largely composed of proteins, nucleic acids and other complex molecules.[20] These bacterial communities attach to a surface with a high level of organization, the individual microorganisms communicate and coordinate their interactions and resulting behaviors.[1,20] Biofilms are extremely resistant to different environmental stresses and the use of antibiotics, which makes their eradication

difficult.[1,2] Instead, preventative methods to inhibit their formation altogether may be a better mode of defense.

#### 5.2.1.a. Biofilms in Medical Devices

Medical device-related and chronic bacterial infections were the impetus for the study of early biofilm microbiology.[2,21,22] Biofilms can be found on surfaces of devices (e.g., catheters, stents, pacemakers, contact lenses, etc) or on tissues (e.g., lungs in cystic fibrosis patients).[2] Because they are impenetrable by traditional antibiotics, even when levels up to 1,000 times the amount that can kill planktonic cells are used, methods to prevent their formation are of critical.[1,2]

#### 5.2.1.b. Biofilms in Food

The mechanisms for biofilm formation are similar whether they form on medical devices or on foods. Formation of biofilms in food can arise from various sources: raw materials, ingredients, personnel, processing equipment or overall working conditions.[3] Prevention of biofilm formation is imperative for food safety to control food spoilage and foodborne illness. In 2002, it was estimated that as many as 30 % of people in industrialized countries suffer from food-borne illness.[23] Furthermore, the financial expenses associated with food spoilage are steep, costing the food industry millions of dollars per year.[1,24]

The difference between medical device and food biofilms is the type of microorganisms (e.g., *Pseudomonas aeruginosa* in medical devices versus *Salmonella enterica* in foods) forming the biofilms. *S. enterica* is one of the leading causes of food-borne illness, associated with foods of animal origin, such as eggs and raw meat, as well

as fruits and vegetables and is a major target for current biofilm prevention techniques.[25,26]

### 5.2.2. Natural-based Bioactives

For microbial contamination and biofilm prevention in the food industry, it is desirable to incorporate naturally occurring antimicrobials. Safety is a primary concern with an increase in food-borne illness cases being reported, and the food industry and consumers in particular show increased interest for more natural, safer preservatives and antimicrobials as opposed to those deemed "synthetic" or "artificial".[13-16] The assumption is that antimicrobials synthesized industrially are hazardous to human health, however, consumers do not realize that some of these "synthetic" antimicrobials are naturally found in plant and animal tissues as defense mechanisms against invading microorganisms.[17]

The chosen natural antimicrobials to be incorporated into polymers (**Figure 5.2**) are essential oils derived from plant and spice extracts such as thyme, cloves, cinnamon and bay leaf.

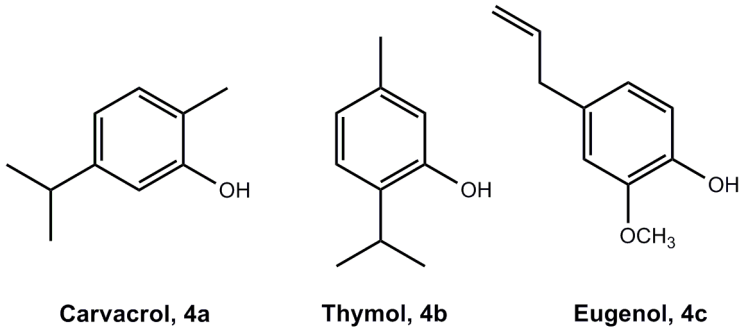


**Figure 5.2.** Natural antimicrobials incorporated into polyanhydrides (**4**).

These phenolic compounds have antimicrobial activity against both Gram-negative and Gram-positive bacteria.[12] The compounds chosen are known to have FDA GRAS status (Generally Regarded as Safe), and the use of such natural, food-based antimicrobials would be advantageous for food safety and other applications for prolonging shelf-life of products and controlling microbial contamination.[16,18]

**5.2.2.a. Thymol (4a)**

Thymol is a constituent in the essential oils of thyme, oregano, savory and sage and inhibitory against over 25 genera of bacteria including *Staphylococcus aureus* and *Salmonella typhimurium*.[17] Thymol is known to have bactericidal and antifungal activity.[12,17] It is used as a preservative, topical antiseptic and anthelmintic.[12]

**5.2.2.b. Carvacrol (4b)**

Carvacrol is found in the oil of oregano, thyme, marjoram and summer savory and often used as a disinfectant and anthelmintic.[12] Like thymol, carvacrol has potent antimicrobial activity against both Gram negative and Gram positive bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[18,19]

**5.2.2.c. Eugenol (4c)**

Eugenol is a natural antimicrobial and a major component in clove oil and allspice. It has been proven effective against a variety of microorganisms including *Salmonella typhimurium* and *Staphylococcus aureus*.[17] Eugenol is used in perfumery, as an insect attractant and as a dental analgesic.[12]

### 5.2.3. Bioactive-based Polyanhydrides

#### 5.2.3.a. Bioactives as Pendants

Relative to the bifunctional salicylate-based polyanhydrides (**1**, **Figure 5.1**)**,** the chosen bioactives (**4**) have only one reactive functional group (i.e., hydroxyl). Therefore, an alternate synthetic approach was needed to accommodate a single reactive group. Based on previous work in our laboratory, the bioactive is attached to the repeat unit prior to polymerization[27] by ring-opening of a symmetric dianhydride. We demonstrated that monofunctional phenolic compounds can be chemically incorporated into a polymer as pendant groups *via* ester linkages (**Figure 5.3)**.[28]

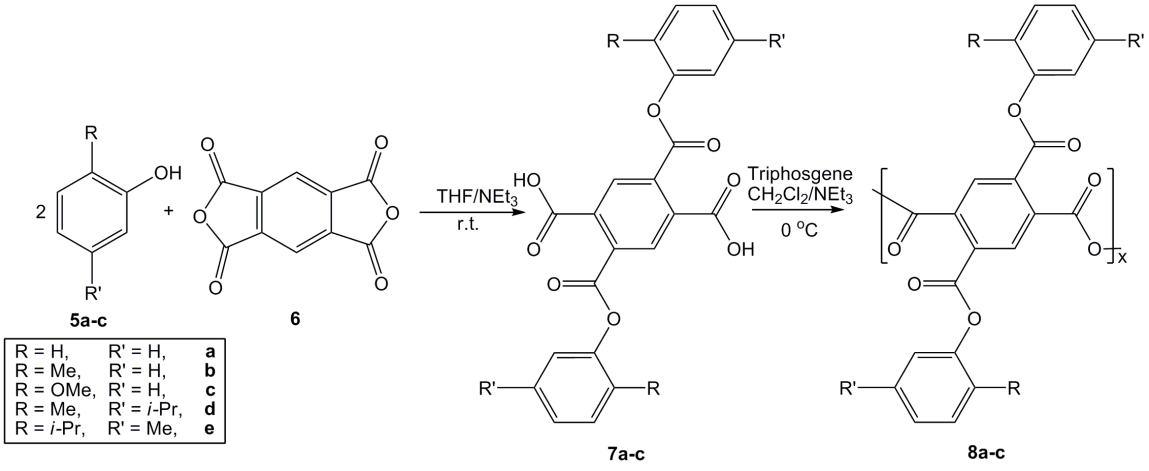


**Figure 5.3.** Incorporation of mono-functional phenolic derivatives (**5**) into polyanhydrides (**8**).

In general, a symmetrical dianhydride (**6**) undergoes ring-opening with a free phenol (**5**) in the presence of triethylamine to yield the polymer precursor (**7**). Compound **7** is

subsequently polymerized under mild conditions using triphosgene as a coupling agent to yield the resulting polyanhydride (**8**) with phenol (**5**) as a pendant group.[28]

In this chapter, an alternate symmetric dianhydride, ethylaminediaminetetraacetic dianhydride (**9**, **Figure 5.4**), was chosen, as it is bioactive and biocompatible.[29-33] In addition, polymer degradation in this case releases two compounds: the natural antimicrobial (**4**) and ethylaminediaminetetraacetic acid (EDTA, **12**).

## 5.3. Results and Discussion

### 5.3.1. Synthesis of Antimicrobial-based Polyanhydrides

The phenolic antimicrobials (**4**) were reacted with EDTA dianhydride (**9**) to yield a symmetrical repeat unit (**10**) via a ring-opening transesterification in the presence of triethylamine (**Figure 5.4**). The diacids **10** were successfully prepared in high yields (76-95 %) with only minor purification necessary, such as washing to remove reaction byproducts and starting materials. The diacids **10** were polymerized using solution polymerization techniques,[27,34-36] using triphosgene as the coupling reagent in the presence of triethylamine at 0 °C. Polymers **11** were prepared in moderate to good yields (40-94 %). Solution polymerization was chosen instead of melt-condensation to prevent potential ring-closure and regeneration of the EDTA dianhydride, **9**.[27]

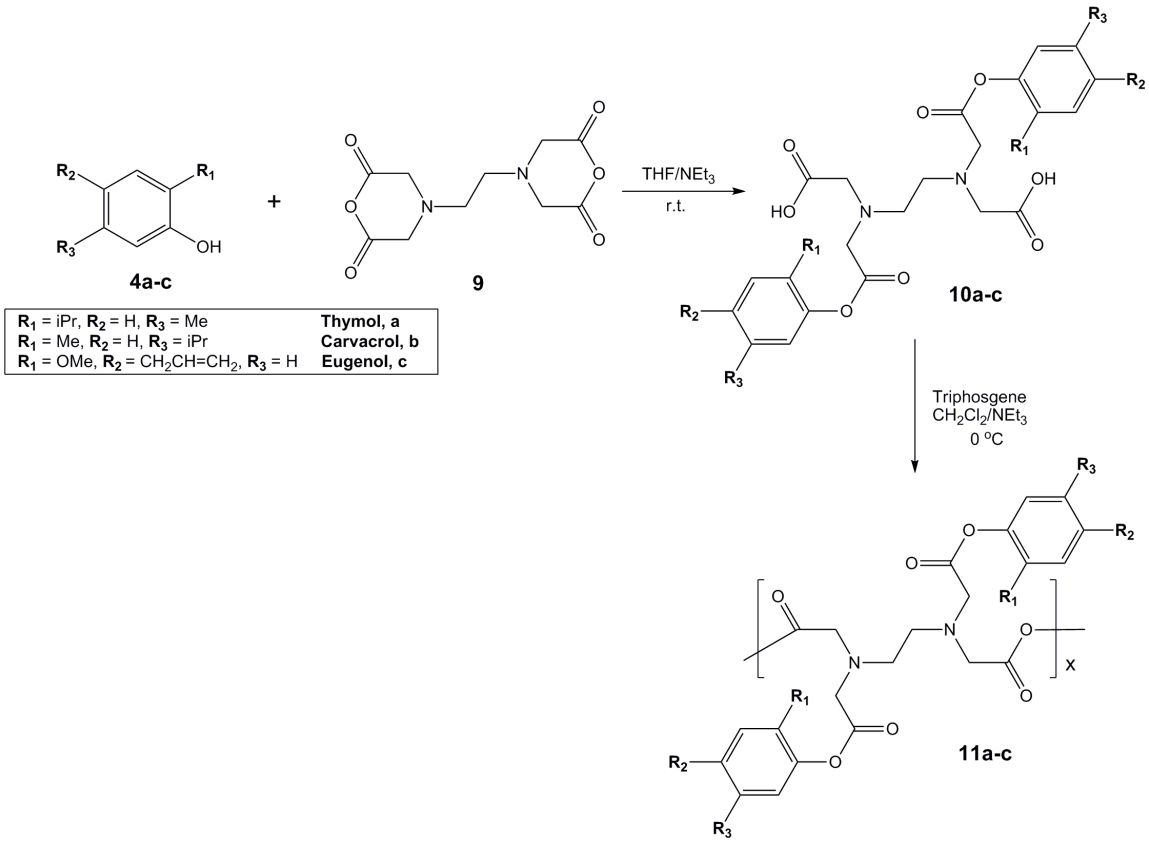


**Figure 5.4.** Synthetic route to natural antimicrobial-based poly(anhydride-esters), **11**.

### 5.3.2. Antimicrobial-based Polyanhydride Properties

Polymer properties are summarized in **Table 5.1**. According to the inherent chemical composition, polymers **11** contained 53.6 % to 55.8 % by weight of the pendant antimicrobial attached to the polyanhydride backbone through ester linkages (**Table 5.1**).

| Polymer | Antiseptic (wt %) | Molecular Weight (Da) | PDI | $T_g$ (°C) | $T_d$ (°C) | Contact Angle (°) |
|---|---|---|---|---|---|---|
| **11a** | 53.6 | 23,200 | 1.0 | 77 | 223 | 40.3 |
| **11b** | 53.6 | 19,500 | 1.1 | 65 | 221 | 36.4 |
| **11c** | 55.8 | 11,100 | 1.5 | 86 | 229 | 34.3 |

**Table 5.1.** Chemical and thermal properties of polyanhydrides, **11**, containing antimicrobials as pendant groups.

#### 5.3.2.a. Molecular Weight

The polymers (**11**) had molecular weights typical for solution polymerization techniques, ranging from 11,100 to 23,200 Da.[27,34-36] Polydispersity indices were narrow following isolation from the reaction mixture. The polyanhydride with the bulkiest antimicrobial, eugenol (**11c**), was the most difficult to polymerize and displayed the lowest molecular weight.

#### 5.3.2.b. Thermal Properties

Polymers **11** are completely amorphous with no indication of melting temperatures (up to 200 °C), exhibiting only glass transition temperatures well above body temperature (i.e., 37 °C) in the range of 65 to 86 °C.

### 5.3.3. *In Vitro* Hydrolytic Degradation

Polymer degradation was carried out on ground polymer samples (**11**) in PBS buffer solution at physiological conditions (37 °C, pH 7.4) (**Figure 5.5**). The degradation media was monitored at regular time intervals for the duration of the experiment (7 days).

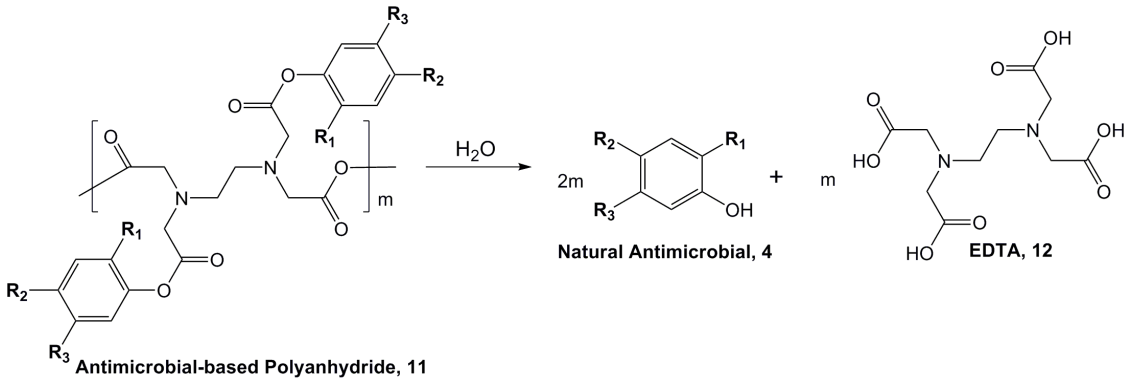


**Figure 5.5.** Antimicrobial-based polyanhydrides (**11**) degrade *in vitro* to release both the natural antimicrobial (**4**) and EDTA (**12**).

The release of the natural antimicrobial (**4**) was followed using UV/vis spectrophotometry. All polymers (**11**) exhibit similar, near zero-order release profiles, with a short lag time (**Figure 5.6**).

For all three polymers (**11**), a short > 8 h lag phase occurred before the antimicrobials **4** could be observed in the media. The carvacrol-based polyanhydride (**11b**) completely degraded in 5 days, followed by the eugenol-based polyanhydride (**11c**) at 6 days and the thymol-based polyanhydride (**11a**) by 7 days. As expected based on the

relative hydrophilicity of polymers **11** with contact angles ~35-40 °C, they exhibited relatively fast release rates.

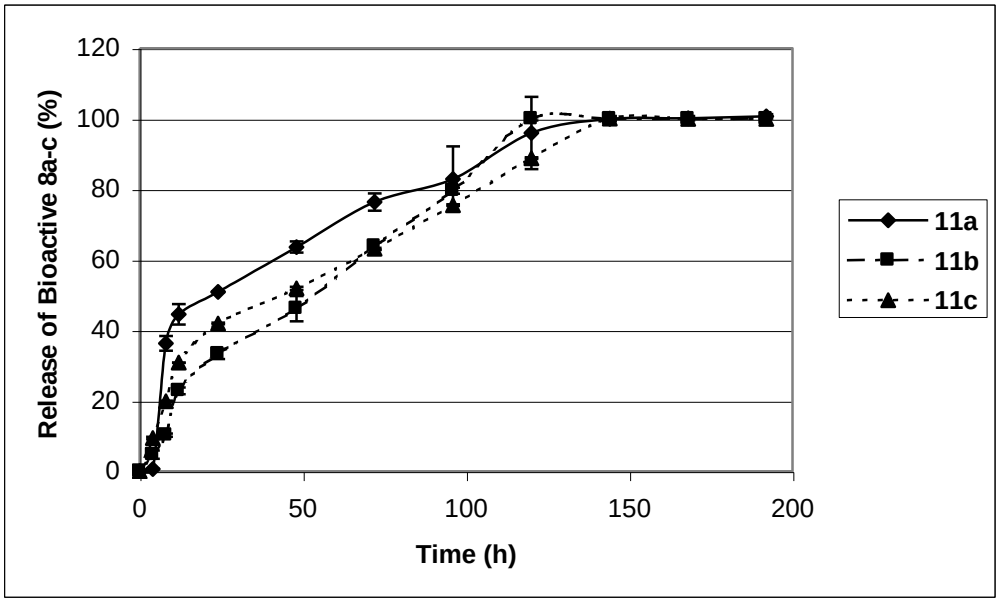


**Figure 5.6.** Release of pendant antimicrobials (**4**) from the polyanhydrides **11** as a result of *in vitro* hydrolytic degradation.

### 5.3.4. Biofilm Inhibition Assays

*[Microbiological assays were performed by Linda Rosenberg Minkow, Department of Food Science, Rutgers University, New Brunswick, NJ].*

The antimicrobials (**4**), namely thymol, carvacrol and eugenol were all released into media with or without the presence of *Salmonella*. By 40 h exposure to bacteria, the thymol-containing polyanhydride (**11a**) displayed a weak biofilm. Similarly, the eugenol-based polyanhydride (**11c**) displayed weak biofilms by 32 h. In contrast, the

polyanhydride containing carvacrol (**11b**) was found to completely prevent biofilm formation up to 48 h.

### 5.4. Summary

Polyanhydrides (**11**) were prepared containing antimicrobials (**4**) derived from plant and spice extracts. Using the synthetic approach described, the number of bioactive groups is well controlled and well defined. The polymers were prepared using solution methods and displayed molecular weights typical for this approach (11,100 to 23,200 Da).[28,34,36] Glass transition temperatures for polymers **11** were above physiological temperature (i.e., 65-86 °C), such that the polyanhydrides can be formulated into multiple geometries (films, fibers, disks, microspheres) for a variety of applications, from food safety to personal care. Polymers (**11**) completely degraded in less than 1 week, releasing the antimicrobials into the media in a controlled manner.

Polymers **11** are unique as they are completely bioactive with an EDTA-based backbone and pendant antimicrobial (**4**). These antimicrobial-based polymers (**11**) are a dual-action delivery system releasing both antimicrobials (**4**) and the chelating agent, EDTA (**12**), which synergizes with some antimicrobials.[29-31,33] Finally, polymers **11a** and **11c** showed weak antimicrobial activity, whereas polymer **11b** completely prevented *Salmonella* biofilm formation. As these polymers are relatively fast degrading, these polymers would be useful for applications requiring a quick, yet sustained release of antimicrobial compounds, such as protection of foods against biofilm formation and dermal therapies for personal care and acne treatments.

## 5.5. Experimental

### 5.5.1. Materials

All solvents and fine chemicals were obtained from Aldrich (Milwaukee, WI). Triethylamine was distilled over calcium hydride ($CaH_2$), and all other reagents were used as received.

### 5.5.2. Methods

#### 5.5.2.a. Proton Nuclear Magnetic Resonance ($^1$H NMR) Spectroscopy

Proton nuclear magnetic resonance ($^1$H-NMR) spectra of all products were recorded on a Varian 400 MHz or 500 MHz spectrophotometer. Samples (5-10 mg) were dissolved in the appropriate deuterated solvent (DMSO-$d_6$), which was also used as the internal reference.

#### 5.5.2.b. Infrared (IR) Spectroscopy

Fourier-transform infrared (IR) absorbance spectra were measured on a Thermo Nicolet/Avatar 360 FT-IR spectrometer. Samples were solvent-casted by dropping samples dissolved in acetone or methylene chloride onto sodium chloride plates.

#### 5.5.2.c. Gel Permeation Chromatography (GPC)

Weight-averaged molecular weights ($M_w$) and polydispersity indices (PDI) were determined by gel permeation chromatography (GPC) on a Perkin-Elmer liquid chromatography system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 autosampler. Automation of the samples and collection and processing of the data was done using a Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software using a Perkin-Elmer Nelson 900 Series Interface and 600

Series Link. Polymer samples were prepared for autoinjection by dissolving polymer in methylene chloride (10 mg/mL) and filtering through 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe filters (Whatman, Clifton, NJ). Samples were resolved on a Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm, Alltech Associates, Deerfield, IL) at 25 °C, with methylene chloride as eluent at a flow rate of 0.5 mL/min. Molecular weights were calibrated relative to polystyrene standards (Polymer Source Inc., Dorval, Canada).

#### 5.5.2.d. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was performed using a Thermal Advantage (TA) DSC Q200 to evaluate thermal transitions (i.e., melting points of polymer precursors and glass transition temperatures of polymers). TA Universal Analysis 2000 software was used for automation and data collection on an IBM ThinkCentre computer. Samples (5-10 mg) were heated under dry nitrogen gas from –10 °C to 200 °C at a heating rate of 10 °C/min and cooled to –10 °C at a rate of 10 °C/min with a two-cycle minimum. Glass transition temperatures were calculated as half $C_p$ extrapolated. Melting temperatures were calculated at the peak of melting.

#### 5.5.2.e. Thermal Gravimetric Analysis (TGA)

Thermogravimetric analyses (TGA) were performed on a Perkin-Elmer Pyris 1 system with TAC 7/DX instrument controller. Perkin-Elmer Pyris software running on a Dell Optiplex GX110 computer was used for automation and data collection and processing. Samples (5-10 mg) were heated under dry nitrogen gas from 25 °C to 400 °C at a heating rate of 10 °C/min. Decomposition temperatures were measured at the onset of thermal decomposition.

#### 5.5.2.f. Elemental Analysis

Elemental analyses were performed by QTI (Whitehouse, NJ).

#### 5.5.2.g Contact Angle Measurements

Static contact angles were measured by dropping deionized water onto pressed polymer disks using a Ramé-Hart Standard Goniometer Model Number 250-00 (Mountain Lakes, NJ) outfitted with a Dell Dimension 3000 computer with DROPimage Advanced software.

#### 5.5.2.h. UV/Vis Spectrophotometry

Antimicrobial (**4**) release was monitored using a DU 520 UV/vis spectrophotometer (Beckman Instruments, Fullerton, CA) at $\lambda_{max}$ 270 nm.

### 5.5.3. Antimicrobial-based Polymer Precursor: Diacid

Diacids were synthesized using a similar procedure as previously described.[27] Diacids (**10**) were prepared by reaction of ethylaminediaminetetraacetic acid (EDTA) dianhydride (**9**) with the appropriate antimicrobial (**4**) in the presence of a base (triethylamine) (Figure 2). The preparation of **10a** is provided as an example. Thymol (**4a**) (18 mmol) was dissolved in anhydrous THF (75 mL) and freshly distilled triethylamine (64 mmol). EDTA anhydride (**9**) (9 mmol) was added to the reaction mixture while stirring at room temperature to yield a suspension. The reaction stirred at room temperature under nitrogen overnight. Then, the reaction mixture was poured over water (~500 mL) and acidified to pH~2 using concentrated hydrochloric acid. The solid diacid (**10a**) that formed was isolated by vacuum filtration, washed with water (3 x 200 mL) and dried overnight under vacuum at room temperature.

**Thymol-based Diacid (10a).** Yield: 76.0 % (white powder). $^{1}$H-NMR (DMSO-$d_6$): δ 7.21 (d, 2H, Ar-H); 7.02 (d, 2H, Ar-H); 6.83 (s, 2H, Ar-H); 3.91 (s, 4H, $CH_2$); 3.54 (s, 4H, $CH_2$); 2.88 (m, 5H, $CH_2$, CH); 2.23 (s, 6H, $CH_3$); 1.08 (d, 12H, $CH_3$). IR (NaCl, $cm^{-1}$): 3400 (OH, COOH), 1761 (C=O, ester), 1712 (C=O, COOH). Anal. Calcd.: C, 64.14 %; H, 7.46 %; N, 4.70 %. Found: C, 64.73 %; H, 7.24%; N, 5.03 %. $T_m$: 113 ºC

**Carvacrol-based Diacid (10b).** Yield: 95.2 % (powder). $^{1}$H-NMR (DMSO-$d_6$): δ 7.19 (d, 2H, Ar-H); 7.04 (d, 2H, Ar-H); 6.91 (s, 2H, Ar-H); 3.92 (s, 4H, $CH_2$); 3.58 (s, 4H, $CH_2$); 2.94 (s, 4H, $CH_2$); 2.81 (m, 2H, CH); 2.13 (s, 6H, $CH_3$); 1.07 (d, 12H, $CH_3$). IR (NaCl, $cm^{-1}$): 3500 (OH, COOH), 1761 (C=O, ester), 1699 (C=O, COOH). Anal. Calcd.: C, 64.73 %; H, 7.24 %; N, 5.03 %. Found: C, C, 63.72 %; H, 7.46 %; N, 4.92 %. $T_m$: 158 ºC.

**Eugenol-based Diacid (10c).** Yield: 82.0 % (white powder). $^{1}$H-NMR (DMSO-$d_6$): δ 6.97 (d, 2H, Ar-H); 6.93 (s, 2H, Ar-H); 6.73 (d, 2H, Ar-H); 5.96 (m, 2H, CH); 5.08 (m, 4H, $CH_2$); 3.84 (s, 4H, $CH_2$); 3.72 (d, 6H, $CH_3$); 3.53 (s, 4H, $CH_2$); 3.35 (d, 4H, $CH_2$); 2.86 (s, 4H, $CH_2$). IR (NaCl, $cm^{-1}$): 3300 (OH, COOH), 1769 (C=O, ester), 1703 (C=O, COOH). Anal. Calcd.: C, 61.63 %; H, 6.21 %; N, 4.80 %. Found: C, 61.13 %; H, 6.25 %; N, 4.76 %. $T_m$: 166 ºC.

### 5.5.4. Antimicrobial-based Polyanhydride

Diacid (**10**) (5.4 mmol) was dissolved 20 % (w/v) anhydrous $CH_2Cl_2$, and freshly distilled triethylamine (24 mmol) was added. Then, triphosgene (6 mmol) dissolved in anhydrous $CH_2Cl_2$ (15 mL) was added drop-wise at 0 °C to the stirring reaction mixture over 1 h using a syringe pump to yield a suspension. After stirring for 2 h at 0 °C under

nitrogen, the reaction mixture is poured over diethyl ether (~400 mL). The solid polymer (**11**) that formed was isolated by vacuum filtration, washed with acidic water (3 x 100 mL) and dried overnight under vacuum at room temperature.

**Thymol-based Polymer (11a).** Yield: 39.3 %. $^1$H-NMR (DMSO-$d_6$): δ 7.22 (d, 2H, Ar-H); 7.03 (d, 2H, Ar-H); 6.82 (s, 2H, Ar-H); 4.21 (s, 4H, $CH_2$); 3.68 (s, 4H, $CH_2$); 2.86 (m, 5H, $CH_2$, CH); 2.24 (s, 6H, $CH_3$); 1.06 (d, 12H, $CH_3$). IR (NaCl, $cm^{-1}$): 1813, 1716 (C=O, anhydride), 1740 (C=O, ester).

**Carvacrol-based Polymer (11b).** Yield: 55.8 % (white powder). $^1$H-NMR (DMSO-$d_6$): δ 7.21 (d, 2H, Ar-H); 7.08 (d, 2H, Ar-H); 6.99 (s, 2H, Ar-H); 4.35 (s, 4H, $CH_2$); 3.99 (s, 4H, $CH_2$); 3.37 (s, 4H, $CH_2$); 2.86 (m, 2H, CH); 2.03 (s, 6H, $CH_3$); 1.18 (d, 12H, $CH_3$). IR (NaCl, $cm^{-1}$): 1763, 1716 (C=O, anhydride), 1732 (C=O, ester).

**Eugenol-based Polymer (11c).** Yield: 93.8 %. $^1$H-NMR (DMSO-$d_6$): δ 6.94 (b, 2H, Ar-H); 6.72 (b, 2H, Ar-H); 6.67 (b, 2H, Ar-H); 5.94 (b, 2H, CH); 5.06 (b, 4H, $CH_2$); 3.83 (b, 4H, $CH_2$); 3.72 (b, 6H, $CH_3$); 3.35 (s, 4H, $CH_2$); 3.24 (d, 4H, $CH_2$); 3.05 (s, 4H, $CH_2$). IR (NaCl, $cm^{-1}$): 1755, 1699 (C=O, anhydride), 1749 (C=O, ester).

### 5.5.5. *In Vitro* Hydrolytic Degradation

The release of bioactive (**4**) from polymer (**11**) was evaluated by *in vitro* degradation in phosphate buffer solution (PBS).

#### 5.5.5.a. Sample Preparation

Polymers were formulated into particles by grinding with mortar and pestle to particles of ~ 300-500 μm. Particle size was determined using standard testing sieves (Aldrich, Milwaukee, WI). Powdered polymer samples (~10-20 mg) were incubated in

10 mL PBS (pH 7.4) in 20 mL Wheaton glass scintillation vials (Fisher, Fair Lawn, NJ) using a controlled environment incubator-shaker (New Brunswick Scientific Co., Edison, NJ) at 60 rpm at 37 °C with agitation.

#### 5.5.5.b. Degradation Media Preparation

Degradation media used was phosphate buffer saline (PBS) containing 0.1 M potassium hydrogen phosphate and 0.1 M potassium dihydrogen phosphate. The pH was adjusted to 7.4 using either 1 N hydrochloric acid or 1 M sodium hydroxide solutions. All pH measurements were performed using an Accumet® AR15 pH meter (Fisher Scientific, Fair Lawn, NJ).

#### 5.5.5.c. Diacid Solubility

Maximum solubility of diacids (**10**) was determined by monitoring absorbance of saturated solutions of each diacid (**10**) in PBS using UV/vis spectrophotometry ($\lambda = 225$). The absorbance was compared to standard solutions of each diacid (0.01 and 0.30 mg/mL).

#### 5.5.5.d. Polymer Degradation

At predetermined time intervals, the media was replaced with fresh media and the spent media analyzed by UV/vis spectrophotometry. The concentration of free bioactive (**4**) in the PBS media was determined by comparison to calibration curves of standard solutions of the bioactives ($1 \times 10^{-3}$, $3 \times 10^{-3}$, $5 \times 10^{-3}$, $1 \times 10^{-2}$, $2 \times 10^{-2}$mg/mL) at their maximum wavelength of absorption ($\lambda = 270$ nm). The degradation experiments were performed in triplicate.

### 5.5.6. Biofilm Inhibition Assays

*[Microbiological assays were performed by Linda Rosenberg Minkow, Department of Food Science, Rutgers University, New Brunswick, NJ].*

Experimental procedures will be published at a later date.[37]

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# 6. PHOTOCROSSLINKABLE, PRESERVATIVE-BASED POLY(ANHYDRIDE-ESTERS)

## 6.1 Introduction

Biodegradable, preservative-based polymers are desirable for many personal care, oral care, cosmetic and food applications. As discussed in previous chapters, chemically incorporating bioactive molecules into the backbone of a polyanhydride allows for a controlled, sustained release of the bioactive upon hydrolytic degradation.[1,2] Previously in our laboratory, antioxidants, namely vanillic and syringic acid have been incorporated into polyanhydrides, however their antioxidant activity was not fully tested.[3]

Other bioactive molecules, such as hydroxycinnamates, that have both antioxidant and antimicrobial properties were chemically incorporated into polyanhydrides to result in polymers that are "dual-action". Furthermore, these preservative-based polymers contain double bonds, which can be utilized for photocrosslinking reactions that may result in networks with enhanced thermal or mechnical properties.

## 6.2 Background

### 6.2.1. Natural-based Preservatives

As discussed in **Chapter 5**, natural bioactives are advantageous for food safety and preservation for preventing microbial contamination. Because of preconceived notions by consumers that synthetic chemical antimicrobials are harmful, food preservation is moving towards more natural methods to preserve foods.[4]

The compounds described in this chapter are either phytochemicals – plant/fruit derived – or derived from spices making them desirable for the above-mentioned applications due to their inherent bioactivity. These compounds found in plants/fuits are believed to be formed due to the hosts' defensive mechanisms against antimicrobials.[4]

Hydroxycinnamates and derivatives have many potential health benefits, as they are antioxidants and known antimicrobials against both Gram negative and Gram positive bacteria and yeasts (**Figure 6.1**).[5,6]

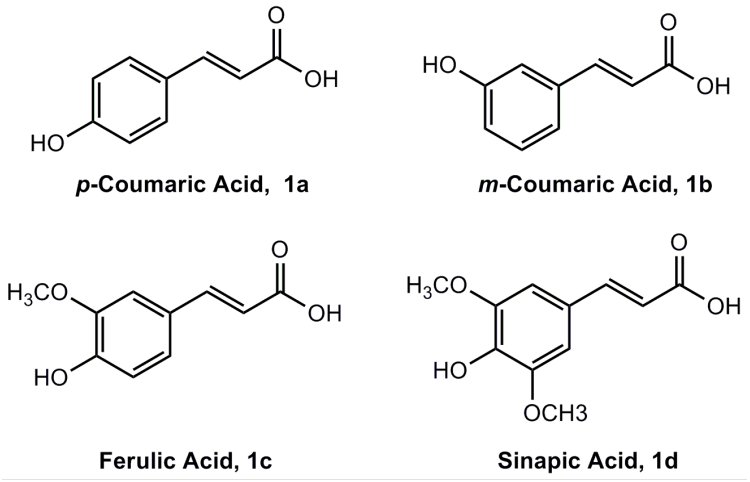


**Figure 6.1.** Chemical structures of selected hydroxycinnamates (**1**) for chemical incorporation into polymer backbones.

Such compounds are often formulated into cosmetics, skin care, personal care and food products. Some of these compounds have been also found to be anti-inflammatory, anti-thrombotic, anti-cancer, anti-tumor, and have the ability lower cholesterol indicating the potential for a variety of applications.[5-7]

*p*-Coumaric acid (**1a**) is present in olive oil and berries and is known to inhibit growth of both Gram negative and Gram positive bacteria including *Escherichia Coli* and *Staphylococcus aureus*.[4,5] *m*-Coumaric acid (**1b**) has been indicated to have neutraceutical benefits as an antioxidant and is derived from berries.[8] Ferulic acid (**1c**) is present in olive oil and is known to inhibit growth of both *Escherichia Coli* and *Staphylococcus aureus*.[4] It is widely used a food preservative, and has been approved by the FDA to be used as an antioxidant in foods.[6,9,10] Furthernore, it has been indicated to prevent oxidative stress for anti-aging remedies.[11] Sinapic acid (**1d**) is a phytochemical readily found in rapeseed and mustard seed with potential as a food preservative because of its potent antimicrobial and antioxidant activities.[12,13]

### 6.2.2. Photocrosslinked Networks

Unsaturated groups, such as the cinnamoyl group in hydroxycinnamates (**1**), have the potential to be photochemically crosslinked.[14] The cinnamoyl group is known to readily undergo a [2+2] cycloaddition.[15-18] By incorporating this photoreactive double bond into a biodegradable polymer backbone, the resulting polymer may be crosslinked to form a network with enhanced thermal or mechanical properties.[14,19,20] Other examples in literature include crosslinked networks derived from *p*-coumaric acid (**1a**) and poly(ethylene glycol) or caprolactone to form hydrogels for biomedical applications[14,21] and methacrylates with pendant cinnamoyl groups for photoresist materials.[22]

## 6.3. Results and Discussion

### 6.3.1. Synthesis of Preservative-based Poly(anhydride-esters)

An outline of the synthesis of preservative-based poly(anhydride-esters) (**3**), starting from the free bioactive (**1**) and adipoyl chloride is shown in **Figure 6.2**.

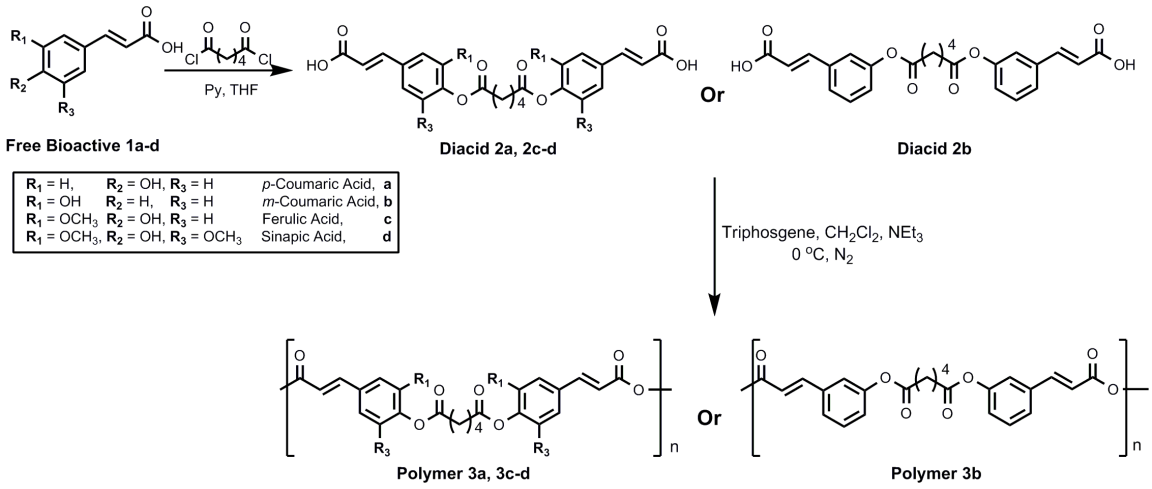


**Figure 6.2.** Synthetic scheme for preservative-based poly(anhydride-esters), **3**.

The bioactive-based diacid (**2**) was formed by coupling of free bioactive (**1**) to adipoyl chloride in THF containing pyridine.[2] The diacids **2** were obtained in relatively high yields (~80-90 %) and purity monitored by $^{1}$H- and $^{13}$C-NMR, FTIR, DSC for melting point determination and elemental analysis. The diacids (**2**) were used directly for low-temperature solution polymerization using triphosgene as the coupling agent in the presense of triethylamine to form the poly(anhydride-esters) **3**.

### 6.3.2. Preservative-based Poly(anhydride-ester) Properties

Key properties of preservative-based polymers (**3**) are summarized in **Table 6.1**.

| Polymer | Preservative (wt %) | Molecular Weight (Da) | PDI | $T_g$ (°C) | $T_d$ (°C) | Contact Angle (°) |
|---|---|---|---|---|---|---|
| **3a** | 74.9 | 26,700 | 1.7 | 57 | 302 | 81.4 |
| **3b** | 74.9 | 33,600 | 1.7 | 51 | 322 | 77.5 |
| **3c** | 77.9 | 71,400 | 1.5 | 81 | 339 | 86.5 |
| **3d** | 80.3 | 38,300 | 1.7 | 136 | 315 | 85.9 |

**Table 6.1.** Properties of preservative-based poly(anhydride-esters), **3**.

By chemically incorporating the free bioactives (**1**) into the backbone of poly(anhydride-esters) (**3**), a high loading of the preservative was obtained (up to 80 %). Polymers were found to have molecular weights between 26,700-71,400 with relatively narrow polydispersity ranging from 1.5-1.7 after isolation, which is typical for solution-made polyanhydrides.[23,24] Glass transition temperatures ($T_g$) were well above body temperature (i.e., 37 °C), and decomposition temperatures ($T_d$) ranged from 300-340 ° C.

#### 6.3.2.a. Polymer Hydrophobicity

Relative hydrophobicity of the poly(anhydride-esters) **3** was studied by measuring sessile water contact angles on pressed polymer disks (8 mm diameter x 1 mm thickness). The polymers were found to be relatively hydrophobic with contact angles ranging from 77.5-85.9 °. Relative hydrophobicity may influence the degradation rate and subsequent release of bioactives (**1**), as water penetration into the polymeric matrix is an important factor in degradation of polyanhydrides.[25,26]

### 6.3.2.b. Antioxidant Activity

To determine antioxidant activity of the free bioactives (**1**), intermediate degradation products (**2**) and polymers (**3**), samples were mixed with 2,2-diphenyl-1-picrylhydrazyl (DPPH) as the free radical source[27,28] and assayed by UV/vis spectrophotometry over 40 minutes. Relative DPPH radical reduction (%) was calculated by: $[(Abs_{t0} - Abs_t)/Abs_{t0}] \times 100$, where $Abs_{t0}$ is the initial absorbance and $Abs_t$ is the absorbance after a period of time, namely 20 or 40 min. The DPPH reduction for free bioactives **1** is shown in **Figure 6.3-6.6**.

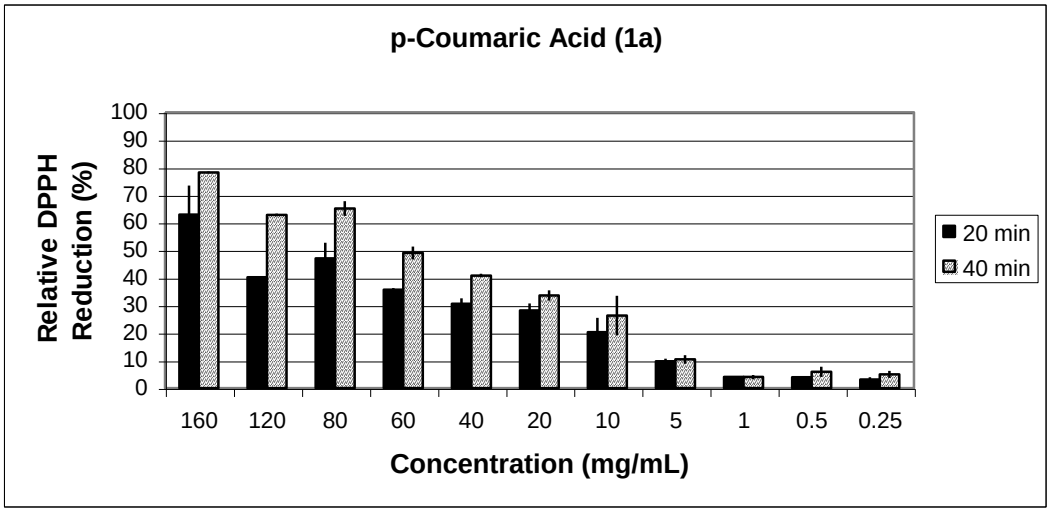


**Figure 6.3.** DPPH reduction as a function of concentration of *p*-coumaric acid, **1a**. The maximum solubility of **1a** in the solvent for the assay was 160 mg/mL.

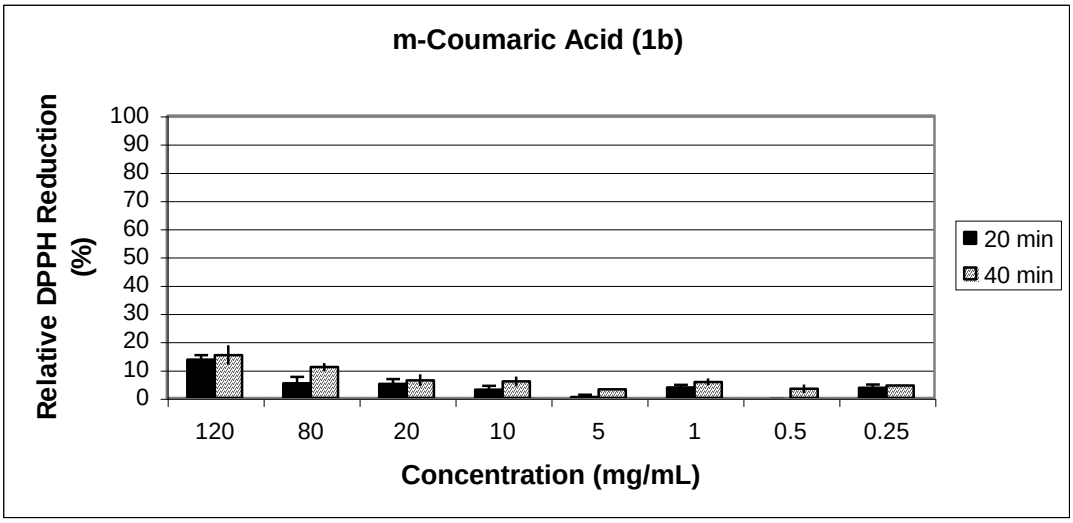


**Figure 6.4.** DPPH reduction as a function of concentration of *m*-coumaric acid, **1b**. The maximum solubility of **1b** in the solvent for the assay was 120 mg/mL.

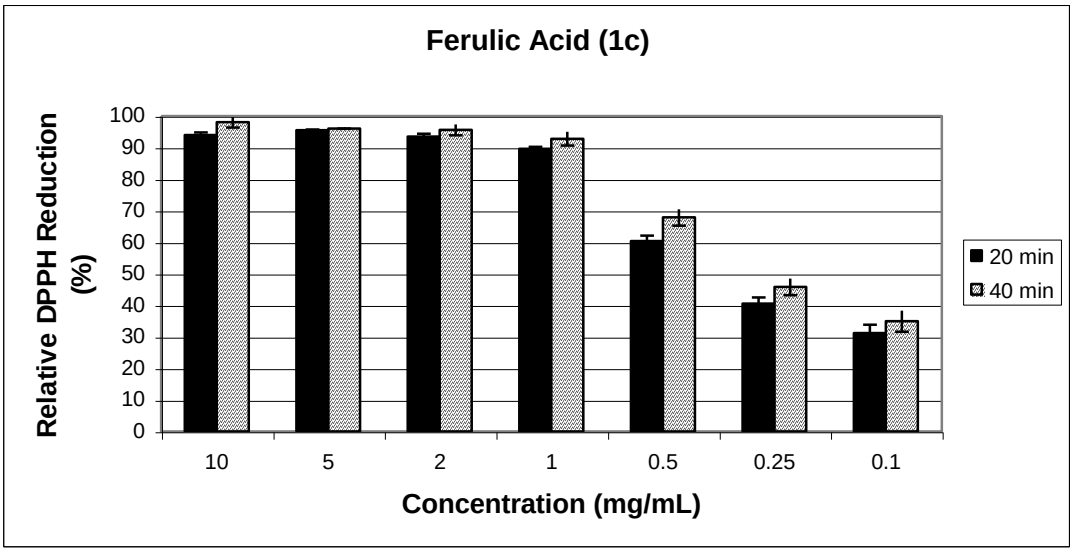


**Figure 6.5.** DPPH reduction as a function of concentration of ferulic acid, **1c**.

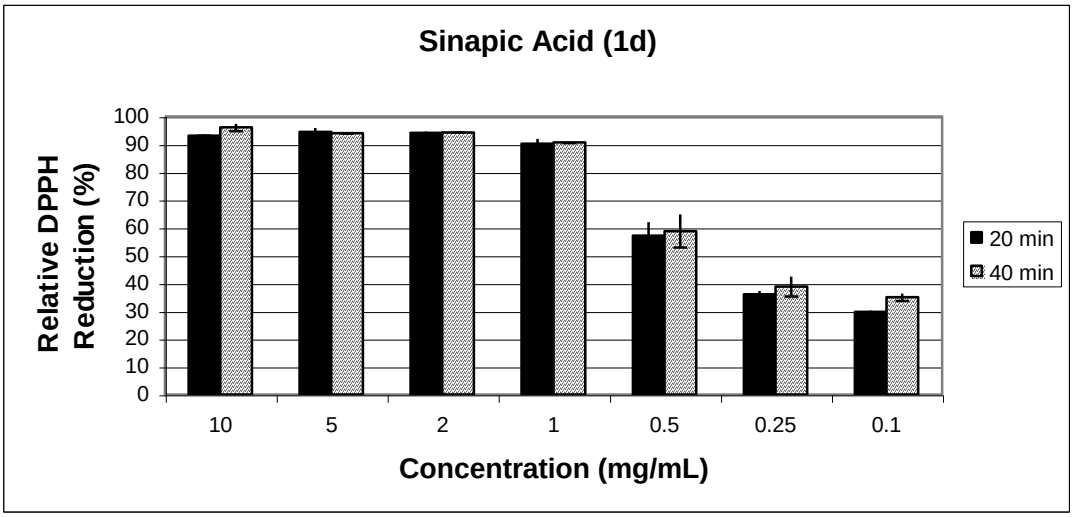


**Figure 6.6.** DPPH reduction as a function of concentration of sinapic acid, **1d**.

The effective concentration required to reduce the original concentration of the DPPH radicals by 50 % ($EC_{50}$) was calculated by plotting % DPPH reduction as a function of sample concentration. The $EC_{50}$ values for the free bioactives (**1**) are shown in **Table 6.2**.

| **Bioactive** | **Experimental $EC_{50}$ (mg/mL)** | **$EC_{50}$ in Literature[29] (mg/mL)** |
|---|---|---|
| **1a** | n/a | - |
| **1b** | n/a | - |
| **1c** | 0.30 | 0.25 |
| **1d** | 0.37 | - |

**Table 6.2.** Half maximal effective concentration ($EC_{50}$) of free bioactives **1**.

The $EC_{50}$ values for **1a-b** could not be determined due to the limited solubility of the free bioactives. In general, the order of antioxidant activity for free bioactives **1** can be summarized as: **1c** > **1d** > **1a** > **1b**.

Next, the antioxidant ability of the intermediate degradation products (i.e., diacids **2**) was analyzed. The DPPH reduction data for diacids **2** are shown in **Figures 6.7-6.9**.

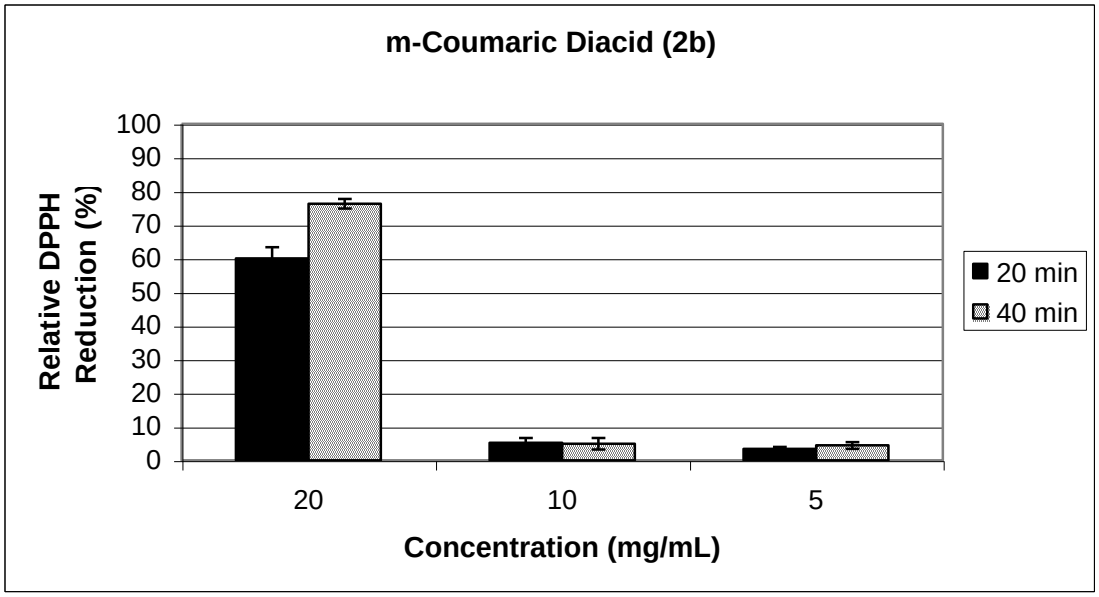


**Figure 6.7.** DPPH reduction as a function of concentration of *m*-coumaric acid-based diacid, **2b**. The maximum solubility of **2b** in the solvent for the assay was 20 mg/mL.

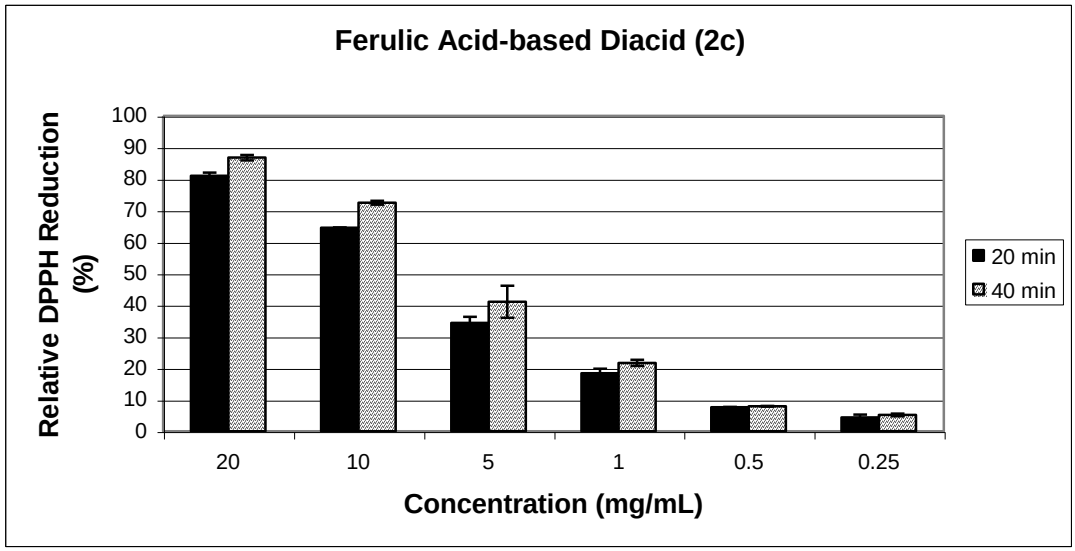


**Figure 6.8.** DPPH reduction as a function of concentration of ferulic acid-based diacid, **2c**. The maximum solubility of **2c** in the solvent for the assay was 20 mg/mL.

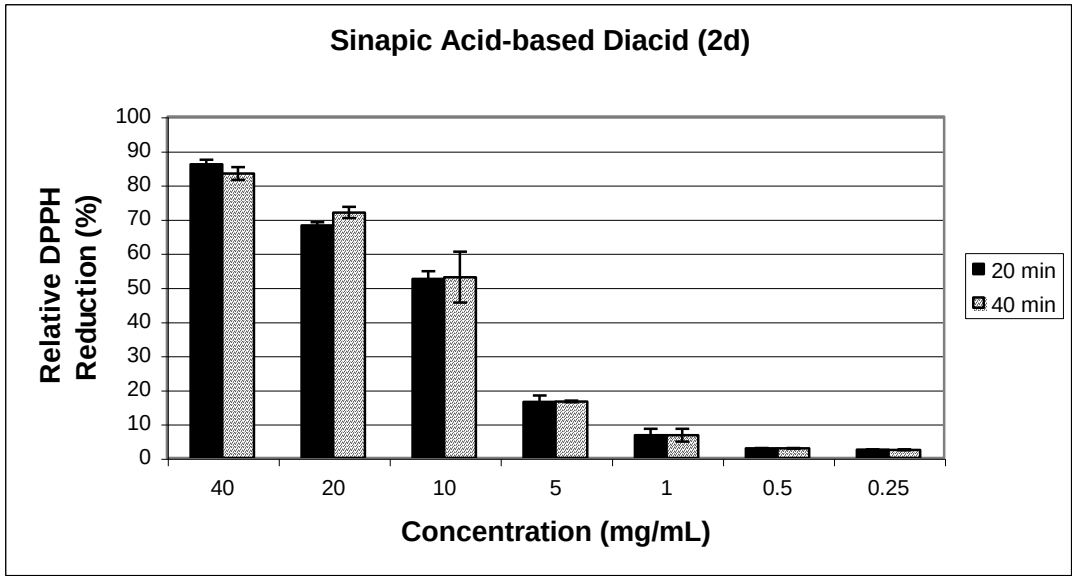


**Figure 6.9.** DPPH reduction as a function of concentration of sinapic acid-based diacid, **2d**. The maximum solubility of **2d** in the solvent for the assay was 40 mg/mL.

*p*-Coumaric acid-based diacid had no activity up to 10 mg/mL and was not soluble above that concentration. Furthermore, the $EC_{50}$ values for diacids **2** could not be determined due to the limited solubility of the diacids. In general, the order of antioxidant activity for diacids **2** can be summarized as: **2c** > **2d** > **2b** > **2a**.

Lastly, the antioxidant activity of the polymers (**3**) was studied. The maximum solubility of the polymers was 20 mg/mL and the results for this concentration are summarized in **Figure 6.10**.

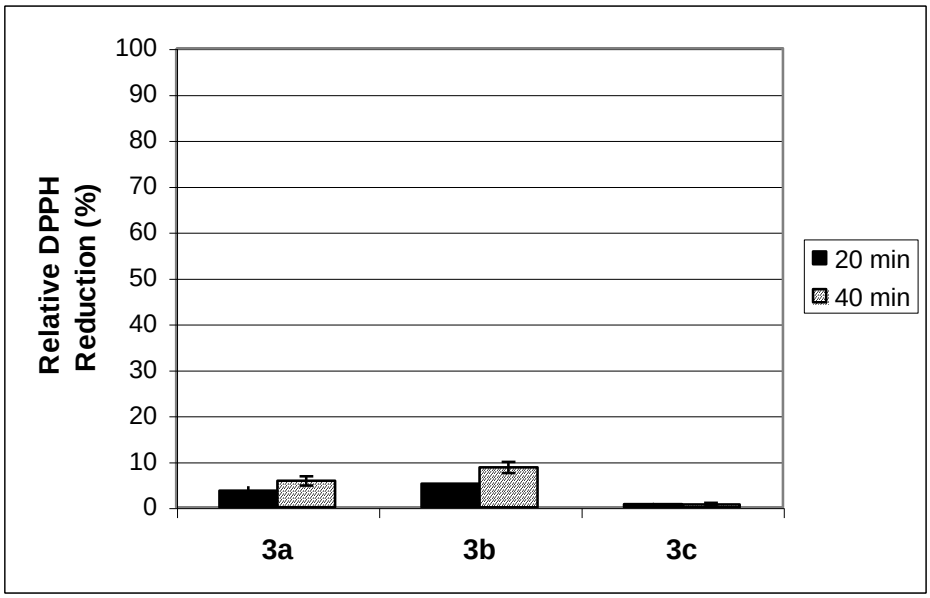


**Figure 6.10.** DPPH reduction for polymers **3**. The maximum solubility of **3** in the solvent for the assay was 20 mg/mL.

Minor activity for the polymers (**3**) at relatively high concentration (20 mg/mL) was observed with no activity noted for polymer **3d** at 20 mg/mL. From these results, we conclude that the polymer degradation products are responsible for the antioxidant

activity. As the polymer **3** hydrolyzes, the free bioactives **1** will be released resulting in the antioxidant activity.

#### 6.3.2.c. Cytotoxicity Assays

*[Cell studies were performed by Jeremy Griffin, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ].*

If used in cosmetic or personal care applicaions, assessing cytotoxicity is important. Therefore, preliminary experiments were performed using L929 mouse fibroblasts to examine cellular response by culturing cells in polymer-containing media (0.1 and 0.01 mg/mL). These polymers did not exhibit any cytotoxic effects, had positive growth profiles comparable with controls, and displayed normal satellite morphology.

#### 6.3.2.d. Biofilm Inhibition

*[Biofilm studies were performed by Allison Guinta, Department of Food Science, Rutgers University, New Brunswick, NJ].*

Polymer-coated coverslips (12 mm diameter, containing ~10 mg of polymer/coverslip) were exposed to *Salmonella enterica* over 48 h. After 24 h, polymer **3b** prevented biofilm formation, whereas bacteria incubated in the presence of polymers **3a** and **3c-d** had thin biofilms beginning to form at this time. By 48 h exposure to bacteria, the *m*-coumaric acid and ferulic acid-containing poly(anhydride-esters) (**3b** and **3c**) displayed a weak biofilm, and the *p*-coumaric acid and sinapic acid-based poly(anhydride-esters) (**3a** and **3d**) displayed full biofilms.

### 6.3.2.e. *In Vitro* Hydrolytic Degradation

In an attempt to understand the results from the biofilm inhibition assays and release rates of the polymers (**3**), *in vitro* degradation of the polymers (**3**) was done to determine the amount of free bioactives **1** released over the timecourse of the experiment. Polymer degradation was carried out on pressed polymer disks (**3**) in PBS buffer solution at physiological conditions (37 °C, pH 7.4). The degradation media was monitored daily for the duration of the experiment (30 days). The release was monitored using UV/vis spectrophotometry at 305 nm for polymers **3a-b** and 340 nm for polymers **3c-d**. The release profiles of polymers **3** are shown in **Figure 6.11**.

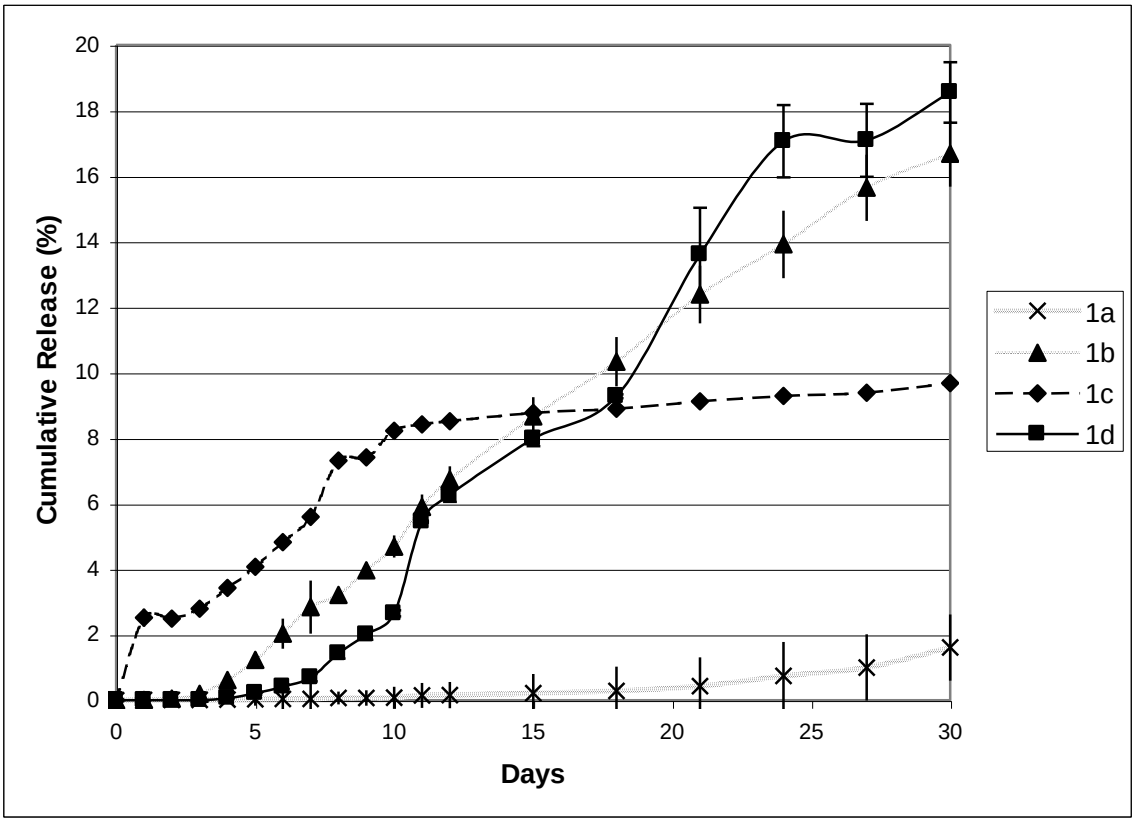


**Figure 6.11.** Release profiles of preservative-based poly(anhydride-esters) (**3**) into free bioactives (**1**).

Based on the relative hydrophobicity, polymers **3** exhibited relatively slow release rates with only a small amount of bioactive (**1**) released after 30 days (~2-20 %). Within the first 48 h, 0-2 % of the free bioactive **1** is release, which may explain the lack of bioactivity for inhibiting formation of the *Salmonella* biofilms. In future studies, the release rate of the bioactives (**1**) can be manipulated by altering the chemical composition of the polymers by either changing the "linker" molecule or introducing a more hydrophilic conomoner.

#### 6.3.2.f. Photocrosslinking

To explore the chemistry of the available double bond in the free bioactives (**1**), the polymers **3** were photocrosslinked using the photoinitiator Irgacure-2959. After 60 minutes of exposure to long-wave UV light (λ=365 nm), the polymers based on *p*- and *m*-coumaric acid (**3a** and **3b**, respectively) appeared completely gelled (**Figure 6.12**).

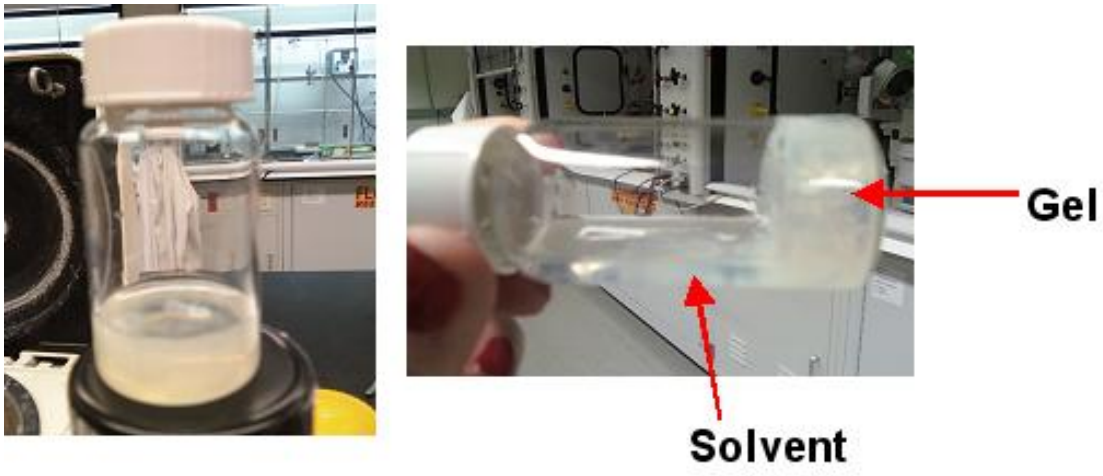


**Figure 6.12.** Photograph of glass vial containing crosslinked polymer **3a** after 60 minutes.

Preliminary photocrosslinking using the conditions mentioned above resulted in complete gelation of polymers **3a-b**. The polymers displayed relatively low water uptake, indicating hydrophobic crosslinked polymer networks. The gel content and water uptake of polymers **3** are summarized in **Table 6.3**.

| Polymer | Gel Content (%) | Water Uptake (%) |
|---|---|---|
| **3a** | 100 | 47.4 |
| **3b** | 100 | 31.2 |
| **3c** | - | - |
| **3d** | - | - |

**Table 6.3.** Values for gel content and water uptake for photocrosslinked polymers **3.**

Polymers **3c-d** did not exhibit any gelation with 5 wt % photoinitiator after 60 minutes exposure to UV light. Future experiments are necessary to find the optimal conditions for photocrosslinking these polymers and to analyze the properties of the resulting crosslinked polymer networks.

## 6.4. Summary

A synthetic approach for chemically incorporating preservative-based bioactives (**1**) into polymers (**3**) was explored. The preservative-based polyanhydrides (**3**) were found to be hydrolytically degradable to release the chemically incorporated bioactives (**1**) over a prolonged period of time (> 1 month). The bioactives (**1**) and intermediate degradation

products (**2**) were found to have antioxidant activity, and experiments indicated that the free bioactives (**1**) are responsible for antimicrobial activity. Furthermore, the polymers (**3**) possess the ability to be photocrosslinked. Controlled release of such naturally derived bioactives would be advantageous for many cosmetic, skin care, and personal care formulations. These preservative-based polymers may also be useful for food preservation, since these compounds are both antioxidant and antimicrobial.

## 6.5. Experimental

### 6.5.1. Materials

All reagents, solvents and other fine chemicals were purchased from Aldrich (Milwaukee, WI) and used as received.

### 6.5.2. Methods

#### 6.5.2.a. Proton Nuclear Magnetic Resonance ($^1$H NMR) Spectroscopy

Chemical composition of all polymers and intermediates was studied using a Varian 400 MHz or 500 MHz nuclear magnetic resonance (NMR) spectrophotometer.

#### 6.5.2.b. Infrared (IR) Spectroscopy

A Thermo Nicolet/Avatar 360 Fourier transform infrared spectrometer (FTIR) was utilized. Samples were solvent-casted by dropping samples dissolved in acetone or methylene chloride onto sodium chloride plates.

### 6.5.2.c. Gel Permeation Chromatography (GPC)

Polymers were characterized using gel permeation chromatography (GPC) for weight-averaged molecular weight and polydispersity using a Perkin-Elmer liquid chromatography system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 autosampler. Automation of the samples and processing of the data was done using a Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software with a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Polymer samples were prepared for autoinjection by dissolving in methylene chloride (10 mg/mL) and filtering through 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe filters (Whatman, Clifton, NJ). Samples were resolved on a Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm, Alltech Associates, Deerfield, IL) at 25 °C, with methylene chloride as the mobile phase at a flow rate of 0.5 mL/min. Molecular weights were calibrated relative to broad polystyrene standards (Polymer Source Inc., Dorval, Canada).

### 6.5.2.d. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was used for determining glass transition ($T_g$) and melting ($T_m$) temperatures with a Thermal Advantage (TA) DSC Q200. TA Universal Analysis 2000 software was used for automation and data collection running on an IBM ThinkCentre computer. Samples (5-10 mg) were heated under dry nitrogen gas from –10 °C to 200 °C at a heating rate of 10 °C/min and cooled to –10 °C at a rate of 10 °C/min with a two-cycle minimum. Glass transition temperatures were calculated as half $C_p$ extrapolated. Melting temperatures were calculated at the peak of melting

#### 6.5.2.e. Thermal Gravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was utilized for determining decomposition temperatures ($T_d$) with a Perkin-Elmer Pyris 1 system with TAC 7/DX instrument controller. Perkin-Elmer Pyris software on a Dell Optiplex GX110 computer was used for automation and collection of data. Samples (5-10 mg) were heated under dry nitrogen gas from 25 °C to 400 °C at a heating rate of 10 °C/min. Decomposition temperatures were measured at the onset of thermal decomposition.

#### 6.5.2.f. Contact Angle Measurements

Static contact angles were measured by dropping deionized water onto pressed polymer disks using a Ramé-Hart Standard Goniometer Model Number 250-00 (Mountain Lakes, NJ) outfitted with a Dell Dimension 3000 computer with DROPimage Advanced software.

#### 6.5.2.g. Elemental Analysis

Elemental analyses were provided by Quantitative Technolgies Inc (Whitehouse, NJ).

### 6.5.3. Preservative-based Polymer Precursor: Diacid

In brief, the bioactive (22.8 mmol) was dissolved in 75 mL anhydrous THF. Pyridine (45.6 mmol) was added. Adipoyl chloride (11.4 mmol) was dissolved in 15 mL THF and added drop-wise. The reaction was allowed to stir at room temperature overnight under nitrogen. The reaction mixture was then poured onto ice/water slush (600 mL) and acidified to pH 2 using concentrated HCl. The solid obtained was filtered, washed with deionized water (3 x 200 mL) and dried under vacuum at room temperature.

***p*-Coumaric Acid-based Diacid (2a).** Yield: 90.4 % (white powder). $^{1}$H-NMR (DMSO-$d_6$): δ 7.73 (4H, Ar-H), 7.55 (2H, R-CH=CH-R), 7.17 (4H, Ar-H), 6.50 (2H, R-CH=CH-R), 2.64 (4H, $CH_2$), 1.72 (4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): δ 172.2, 168.1, 152.5, 143.6, 132.6, 130.1, 123.0, 120.0, 33.8, 24.3. IR (NaCl, $cm^{-1}$): 2930 (OH, COOH), 1760 (C=O, ester), 1680 (C=O, COOH), 1620 (C=C). Anal. Calcd.: C, 65.8 %; H, 5.0 %. Found: C, 65.3 %; H, 4.8 %. $T_m$: 251 ºC

***m*-Coumaric Acid-based Diacid (2b).** 89.4 % (white powder). $^{1}$H-NMR (DMSO-$d_6$): δ 7.54 (4H, Ar-H), 7.42 (4H, Ar-H), 7.16 (2H, R-CH=CH-R), 6.55 (2H, R-CH=CH-R), 2.65 (4H, $CH_2$), 1.74 (4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): δ 172.3, 168.0, 151.6, 143.5, 136.5, 130.6, 126.5, 124.3, 121.8, 121.0, 33.8, 24.3. IR (NaCl, $cm^{-1}$): 2930 (OH, COOH), 1750 (C=O, ester), 1680 (C=O, COOH), 1630 (C=C). Anal. Calcd.: C, 65.8 %; H, 5.0 %. Found: C, 65.5 %; H, 4.9 %. $T_m$: 233 ºC

**Ferulic Acid-based Diacid (2c).** Yield: 87.9 % (off-white powder). $^{1}$H-NMR (DMSO-$d_6$): δ 7.58 (2H, Ar-H), 7.47 (2H, Ar-H), 7.26 (2H, Ar-H), 7.11 (2H, R-CH=CH-R), 6.58 (2H, R-CH=CH-R), 3.80 (6H, $CH_3$), 2.63 (4H, $CH_2$), 1.75 (4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): δ 171.6, 168.3, 151.8, 144.0, 141.5, 134.0, 123.8, 122.1, 120.2, 112.5, 56.6, 33.5, 24.6. IR (NaCl, $cm^{-1}$): 2960 (OH, COOH), 1760 (C=O, ester), 1680 (C=O, COOH), 1625 (C=C). Anal. Calcd.: C, 62.7 %; H, 5.3 %. Found: C, 61.8 %; H, 5.4 %. $T_m$: 193 ºC.

**Sinapic Acid-based Diacid (2d).** Yield: 82.0 % (beige powder). $^{1}$H-NMR (DMSO-$d_6$): δ 7.55 (2H, R-CH=CH-R), 7.09 (4H, Ar-H), 6.61 (2H, R-CH=CH-R), 3.80 (12H, $CH_3$), 2.60 (4H, $CH_2$), 1.74 (4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): δ 171.2, 168.3, 152.6, 144.5, 133.3, 130.1, 120.4, 105.9, 56.9, 33.4, 24.5. IR (NaCl, $cm^{-1}$): 2940 (OH,

COOH), 1760 (C=O, ester), 1690 (C=O, COOH), 1630 (C=C). Anal. Calcd.: C, 60.2 %; H, 5.4 %. Found: C, 59.4 %; H, 5.5 %. $T_m$: 274 ºC

### 6.5.4. Preservative-based Poly(anhydride-ester)

Polymers were prepared to a modified version of a procedure previously described.[24] Diacid (6.0 mmol) was dissolved in 30 mL anhydrous methylene chloride under nitrogen. Triethylamine (26 mmol) was added, and the reaction mixture was cooled to 0 °C. Triphosgene (6.6 mmol) was dissolved in 20 mL anhydrous methylene chloride and added drop-wise (14 mL/h). The reaction was allowed to stir for 3 h. Then, the reaction mixture was poured over ether (400 mL). The ether was decanted to yield a solid (polymer). The polymer was dissolved in methylene chloride, washed in a separatory funnel with acidic water (3 x 150 mL). The organic layer was dried over magnesium sulfate, concentrated, and precipitated onto an excess of diethyl ether. The ether was decanted or filtered off, and the polymer was allowed to dry under vacuum at room temperature.

***p*-Coumaric Acid-based Polymer (3a).** Yield: 41 % (beige solid). $^1$H-NMR (DMSO-$d_6$): δ 7.92 (6H, Ar-H, R-CH=CH-R), 7.21 (4H, Ar-H), 6.81 (2H, R-CH=CH-R), 2.65 (4H, $CH_2$), 1.71 (4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): δ 172.1, 163.4, 153.4, 148.3, 132.0, 131.1, 123.2, 117.6, 33.8, 24.3. IR (NaCl, $cm^{-1}$): 1760, 1730 (C=O, anhydride), 1760 (C=O, ester), 1630 (C=C).

***m*-Coumaric Acid-based Polymer (3b).** Yield: 20 % (beige solid). $^1$H-NMR (DMSO-$d_6$): δ 7.90 (2H, Ar-H), 7.62 (4H, Ar-H), 7.48 (2H, R-CH=CH-R), 7.23 (2H, R-CH=CH-R), 6.84 (2H, R-CH=CH-R), 2.65 (4H, $CH_2$), 1.75 (4H, $CH_2$). $^{13}$C-NMR

(DMSO-$d_6$): δ 172.2, 163.1, 151.6, 148.1, 135.8, 130.8, 127.3, 125.5, 122.6, 118.6, 33.8, 24.4. IR (NaCl, $cm^{-1}$): 1760, 1720 (C=O, anhydride), 1760 (C=O, ester), 1630 (C=C).

**Ferulic Acid-based Polymer (3c).** Yield: 60 % (white solid). $^{1}$H-NMR (DMSO-$d_6$): δ 7.84 (2H, Ar-H), 7.60 (2H, Ar-H), 7.38 (2H, Ar-H), 7.18 (2H, R-CH=CH-R), 6.83 (2H, R-CH=CH-R), 3.81 (6H, $CH_3$), 2.64 (4H, $CH_2$), 1.73 (4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): δ 171.5, 163.4, 151.9, 148.7, 142.5, 133.3, 124.0, 123.2, 117.8, 113.2, 56.8, 33.5, 24.4. IR (NaCl, $cm^{-1}$): 1760, 1720 (C=O, anhydride), 1760 (C=O, ester), 1630 (C=C).

**Sinapic Acid-based Polymer (3d).** Yield: 49 % (off-white solid). $^{1}$H-NMR (DMSO-$d_6$): δ 7.85 (2H, Ar-H), 7.29 (4H, Ar-H), 6.96 (2H, Ar-H), 3.80 (12H, $CH_3$), 2.62 (4H, $CH_2$), 1.75 (4H, $CH_2$). $^{13}$C-NMR (DMSO-$d_6$): δ 171.1, 163.5, 152.8, 149.2, 132.6, 131.1, 117.9, 106.7, 56.9, 33.4, 24.5. IR (NaCl, $cm^{-1}$): 1760, 1730 (C=O, anhydride), 1760 (C=O, ester), 1630 (C=C).

### 6.5.5. Radical Quenching Assay

A solution of 2,2-diphenyl-1-picryl hydrazyl (DPPH) in methanol (0.025 mg/mL) was placed in a UV/vis spectrophotometer (λ = 517 nm) to determine the absorbance of the free radical alone (contol).[30] Then, sample solutions containing polymer **3** (0.10 mL) were incubated with DPPH solution (3.9 mL) at room temperature and the absorbance (at 517 nm) was monitored after 20 and 40 minutes to determine if the polymer (**3**) had the ability to lower the absorbance of the DPPH (i.e., quench the radicals). DPPH radical reduction (%) was calculated by: [(Abst0 – Abst)/Abst0] x 100, where Abst0 is the initial absorbance and Abst is the absorbance after a period of time, namely 20 or 40 min. The

half maximal effective concentration ($EC_{50}$) was calculated by plotting % DPPH reduction as a function of sample concentration. The experiments were done in triplicate.

### 6.5.6. Cytotoxicity Assay

[*Cell studies were performed by Jeremy Griffin, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ*].

Experimental procedures for polymer solution preparation, fibroblast culture, cell morphology evaluation and cell proliferation quantification will be published in the future.

### 6.5.7. Biofilm Inhibition

*[Microbiological assays were performed by Allison Guinta, Department of Food Science, Rutgers University, New Brunswick, NJ]*.

Experimental procedures will be published at a later date.

### 6.5.8. *In Vitro* Hydrolytic Degradation

The release of bioactive (**1**) from polymer (**3**) was evaluated by *in vitro* degradation in phosphate buffer solution (PBS).

#### 6.5.8.a. Sample Preparation

Polymer pellets were prepared by pressing ground polymers (~100 ± 5 mg) into 8 mm diameter x 1 mm thick disks in an IR pellet die (International Crystal Laboratories, Garfield, NJ) with a bench-top hydraulic press (Carver model M, Wabash, IN). A pressure of 10,000 psi was applied for 5 min at room temperature.

### 6.5.8.b. Degradation Media Preparation

Degradation media used was phosphate buffer saline (PBS) containing 0.1 M potassium hydrogen phosphate and 0.1 M potassium dihydrogen phosphate. The pH was adjusted to 7.4 using either 1 N hydrochloric acid or 1 M sodium hydroxide solutions. All pH measurements were performed using an Accumet® AR15 pH meter (Fisher Scientific, Fair Lawn, NJ).

### 6.5.8.c. Polymer Degradation

Hydrolytic degradation of the polymers (**3**) was performed by placing the disks in 20 mL Wheaton glass scintillation vials (Fisher, Fair Lawn, NJ) with 10 mL of PBS, and incubating them at 37 ºC with agitation using a controlled environment incubator-shaker (New Brunswick Scientific Co., Edison, NJ) at 60 rpm for 30 days. Every 24 h, the buffer solution was replaced by fresh solution (10 mL) and the spent media was analyzed by UV ($\lambda$=305 nm for **3a-3b** and 340 nm for **3c-3d**) with a Perkin-Elmer Lambda XLS spectrophotometer (Waltham, MA) to specifically monitor the release of free bioactives (**1**). UV data (average of three samples per time point) were calibrated against standard solutions of known concentrations.

### 6.5.9. Photocrosslinking Reaction

A solution of polymer **3** in methylene chloride (12 mg/mL) was prepared and 5 wt % of photoinitiator (Irgacure-2959) was added. The polymer/initiator solution was exposed to long-wave UV light ($\lambda$=365 nm) for 60 minutes. Then, the solvent was removed. The gel content was determined by redissolving the crosslinking polymer in methylene chloride (5 mL) to remove any unreacted polymer and excess photoinitiator.

The gel content was calculated by $[(W_2/W_1]$ x 100, where $W_2$ is final weight after incubation in solvent and $W_1$ is initial weight after UV exposure. Water uptake was calculated by dissolving the crosslinked films in PBS for 24 h using the following equation: $[(W_2 - W_1)/W_2]$ x 100, where $W_2$ is weight after incubation in PBS and $W_1$ is initial weight.

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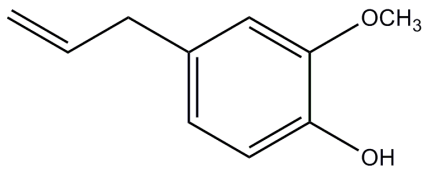
# A1. APPENDIX 1: ALTERNATE POLYANHYDRIDES

In this appendix, a series of short research projects that were explored in conjunction with main research goals is described. Preliminary data pertaining to these projects are summarized and form the basis for future experiments, if desired. All materials and methods for the synthesis and characterization of alternate polyanhydrides are listed in **Section A1.5**.

## A1.1. Aromatic Polyanhydride with Eugenol as Pendant Group

Previously in our laboratory, the synthesis of antiseptic-based polyanhydrides was described.[1,2] An additional antiseptic, eugenol (**1, Figure A1.1**) was also chemically incorporated into a polyanhydride as a pendant group *via* an ester linkage.




**Eugenol, 1**

**Figure A1.1.** Chemical structure of eugenol (**1**).

As described in **Chapter 5**, eugenol is has a variety of uses and is commonly used as a dental analgesic or in perfumery as a substitute for oil of cloves.[3] Additionally, it is an antimicrobial agent proven against a range of microorganisms.[4]

A polyanhydride comprised of eugenol was synthesized as outlined in **Figure A1.2**. As described in **Chapter 5**, this bioactive molecule has only one reactive functional group and is therefore attached as a pendant to the polyanhydride backbone *via* an ester linkage *prior* to polymerization.

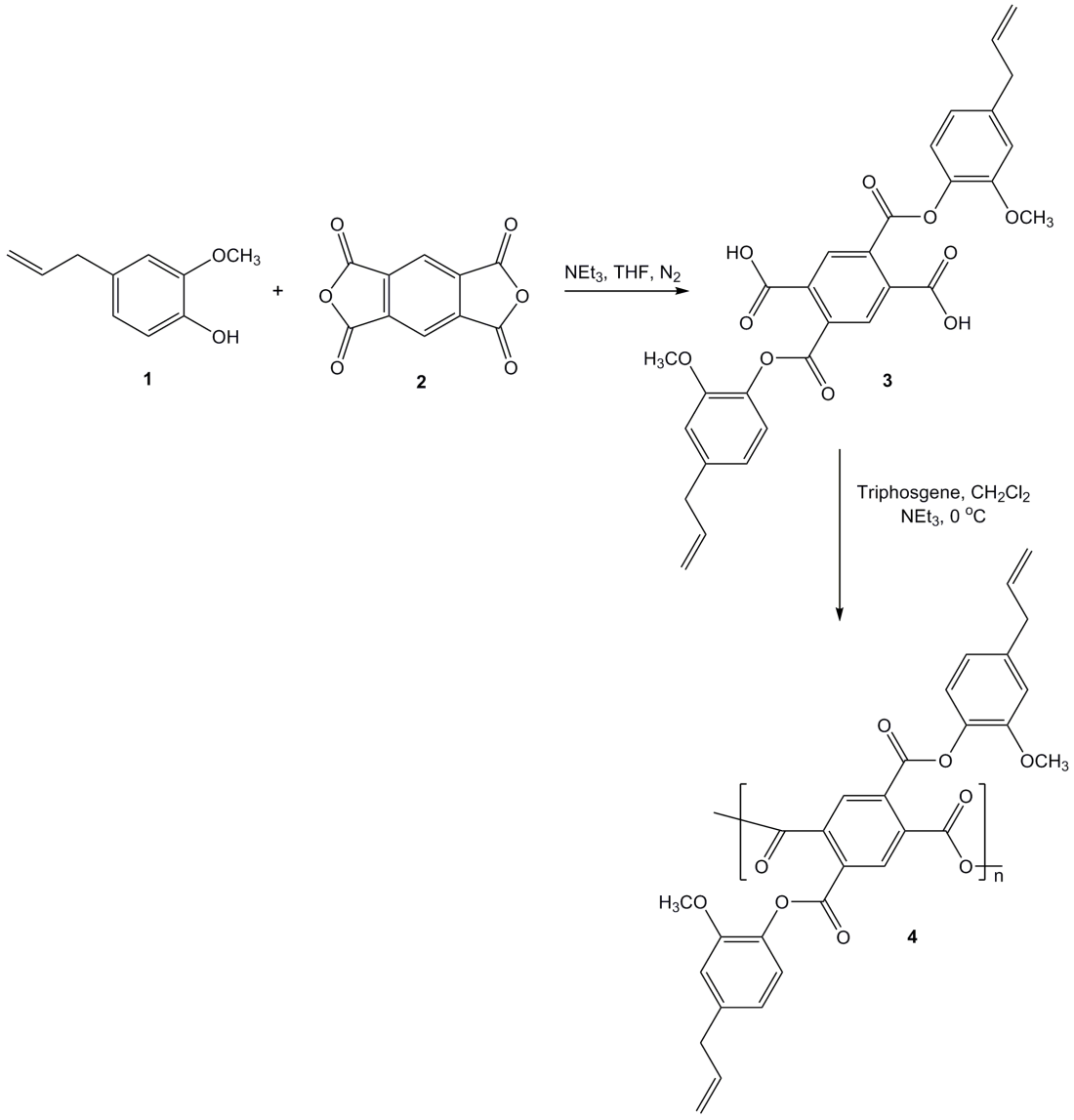



**Figure A1.2.** Synthesis of eugenol-based polyanhydride (**4**).

As eugenol (**1**) is monofunctional, traditional synthetic approaches for other bioactive-based polyanhydrides (**Chapters 2-4**) could not be utilized. Therefore, the bioactive was chemically incorporated into the polyanhydride (**4**) as a pendant group using procedures previously described.[1] Eugenol (**1**) was used to ring-open pyromellitic anhydride (**2**) in the presence of base (i.e., triethylamine) to yield the polymer precursor (diacid, **3**), which was subsequently polymerized using solution polymerization techniques[5,6] to yield the eugenol-based polyanhydride **4**.

As this polymer (**4**) may be useful for dermal therapies, as coatings for medical devices, surgical suites, food packaging or food processing equipment, the *in vitro* cytotoxicity was analyzed by Jeremy Griffin, a Ph.D. candidate in Biomedical Engineering Department (Rutgers University, Piscataway, NJ). The polymer (**4**) was deemed noncytotoxic after mouse L929 fibroblasts were cultured in cell media containing polymer **4** at concentrations between 0.01 mg/mL and 0.1 mg/mL over three days.

### A1.1.1. Experimental

#### A1.1.1.a. Polymer Synthesis

**Eugenol-based Diacid (3).** Eugenol (**1**) (3.4 g, 22 mmol) was dissolved in dry THF (~75 mL) and freshly distilled triethylamine (11 mL, 78 mmol). Purified pyromellitic anhydride (**2**, recrystallized from hot acetic anhydride) (2.4 g, 11 mmol) was slowly added to the stirring reaction mixture at room temperature to afford a suspension. The reaction was stirred for 16 h at room temperature under nitrogen, poured over ice/water slush (600 mL) and acidified to pH~2 using concentrated hydrochloric acid while stirring. The solid (diacid, **3**) that formed was isolated by vacuum filtration,

washed with water (3 x 200 mL) and dried overnight under vacuum at room temperature. Yield: 5.7 g (96 %, white powder). $^1$H NMR (DMSO-$d_6$): δ 8.19 (s, 2H, ArH), 7.18 (d, 2H, ArH), 7.02 (s, 2H, ArH), 6.86 (d, 2H, ArH), 5.93 (m, 2H, CH), 5.13 (d, 4H, $CH_2$), 3.19 (s, 6H, $CH_3$), 2.10 (d, 4H, $CH_3$). IR (NaCl, cm$^{-1}$): 3200-3650 (s, OH, COOH), 1755 (vs, C=O, ester), 1699 (vs C=O, COOH). Anal. Calcd.: C, 65.9 %; H, 4.9%. Found: C, 65.1%; H, 4.8%. $T_m$: 144-145 ºC.

**Eugenol-based Polymer (4).** Diacid (**3**) (2.0 g, 3.7 mmol) was dissolved 20 % (w/v) $CH_2Cl_2$ and freshly distilled triethylamine (2.2 mL, 16.1 mmol). Triphosgene (1.2 g, 4.0 mmol) dissolved in dichloromethane (5 mL) was added dropwise at a rate of 14 mL/h to the stirring reaction mixture at 0 °C to afford a suspension. The reaction was stirred for 2 h at 0 °C under nitrogen and poured over diethyl ether (~400 mL). The solid (polymer, **4**) that formed was isolated by vacuum filtration, washed with acidic water (3 x 100 mL) and dried overnight under vacuum at room temperature. Yield: 2.7 g (67 %, white powder,). $^1$H NMR (DMSO-$d_6$): δ 8.18 (s, 2H, ArH), 7.10 (d, 2H, ArH), 6.95 (s, 2H, ArH), 6.71 (d, 2H, ArH), 5.88 (m, 2H, CH), 5.06 (d, 4H, $CH_2$), 3.12 (s, 6H, $CH_3$), 2.11 (d, 4H, $CH_3$). IR (NaCl, cm$^{-1}$): 1820, 1700 (vs, C=O, anhydride), 1735 (vs, C=O, ester). $M_w$ = 19900, PDI = 1.0. $T_d$ = 171 ºC, $T_g$ = 58 ºC.

**A1.1.1.b. Cytotoxicity Study**

*[Cell Studies were performed by Jeremy Griffin, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ.]*

Experimental protocol for the preparation of polymer solution, fibroblast culture and cellular morphology and proliferation analyses are described in literature.[7]

## A1.2. Alternate Polyanhydrides Containing Salicylic Acid

Depending on the desired end-use of the biodegradable, bioactive-based polyanhydride, polymer properties and subsequent release of the bioactive can be controlled by changing the polymer chemical composition. A variety of different polyanhydrides containing salicylic acid were proposed, and some were synthesized as part of a variety of research projects as described below.

### A1.2.1. Synthesis of Iodinated Salicylate-based Copolymers

As described in **Chapter 2**, radiopaque polymers are desirable for a variety of biomaterials and imaging applications. In particular, a salicylate-based poly(anhydride-ester) was incorporated into cardiac stents,[8] however, this polymer is not X-ray opaque and the medical device comprised of this polymer cannot be seen under a clinical X-ray. To address this issue, iodinated salicylate-based poly(anhydride-esters) were synthesized as described in **Chapter 2**.[9] Although these polymers are biodegradable, they do not release any therapeutically active molecules. Therefore, copolymers of the iodinated salicylate-based poly(anhydride-esters)[9] were made with salicylic acid-based poly(anhydride-esters)[10,11] to render a biodegradable copolymer that may be both X-ray opaque and therapeutic.

Copolymers composed of iodinated salicylate-based diacid (**5**) and salicylic acid-based diacid (**6**) were synthesized using the synthetic approach shown in **Figure A1.3**.

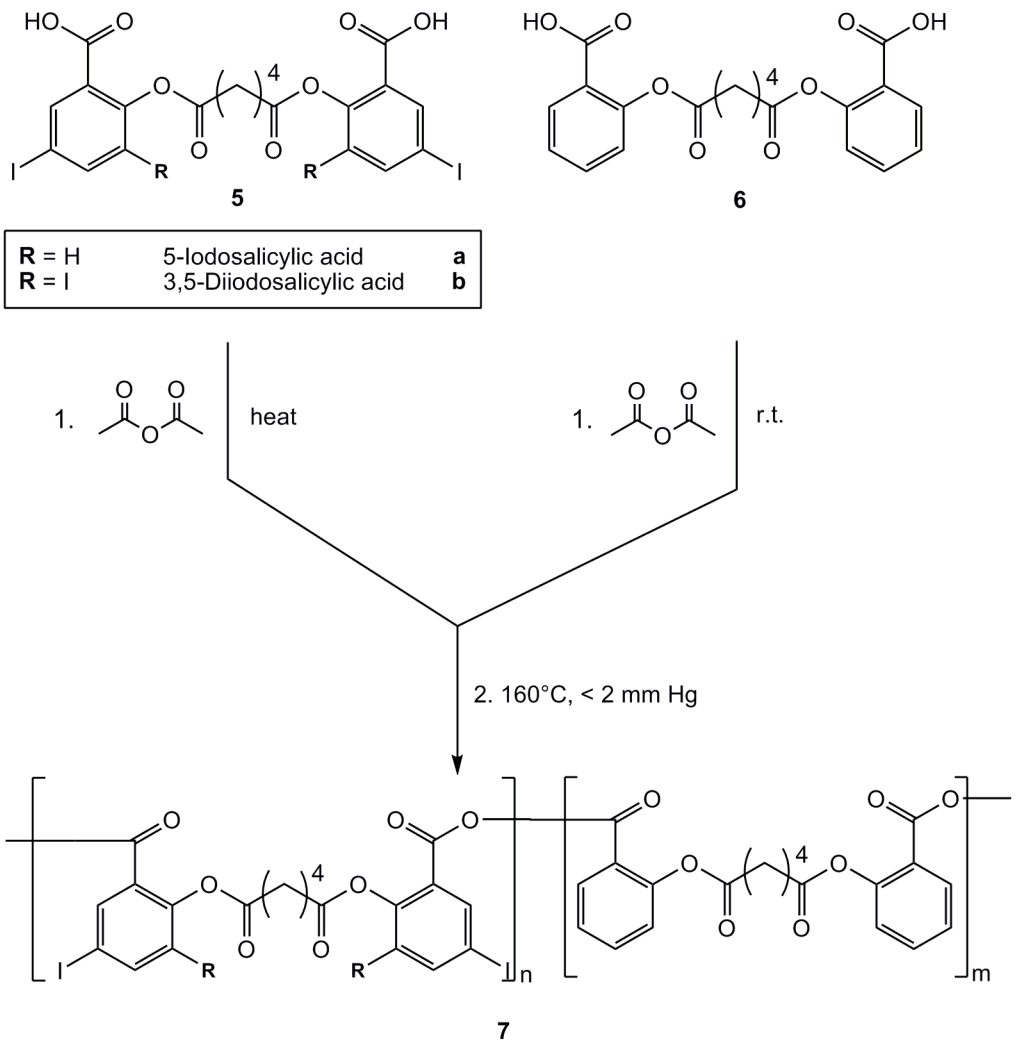



**Figure A1.3.** Synthesis of iodinated salicylate-based copolymers (**7**).

The diacids (**5** and **6**) were synthesized as previously described,[9,11] and copolymers (**7**) were synthesized by melt-condensation polymerization.[12] *Prior* to polymerization, the diacids (**5** and **6**) were separately activated with acetic anhydride, and then the prepolymers combined and melt-polymerized at 160 °C to yield the copolymers (**7**). The copolymers of each 5-iodosalicylic acid (**7a**) and 3,5-diiodosalicylic acid (**7b**) with salicylic acid-based diacid were made using a 50:50 molar composition, which was

verified by $^{1}$H-NMR. The copolymers were found to have similar glass transition temperatures of 46 °C for **7a** and 47 °C for **7b**. Futher experiments are needed to determine if these copolymers have sufficient X-ray opacity and to study the release of salicylic acid.

**A1.2.1.a. Experimental: Polymer Synthesis**

Diacids **5** and **6** were synthesized as described in **Chapter 2** using previously published methods.[9,11] The diacids were activated using acetic anhydride to form the monomers,[10-12] which were then melt-polymerized to yield the copolymers **7**.

**5-Iodosalicylic acid-based Copolymer (7a).** The iodinated salicylate-based and salicylic acid-based monomers were combined and melt-polymerized at elevated temperatures (*i.e.,* 160 $^{o}$C) for ~5 h under vacuum to remove the melt-condensation byproduct, acetic anhydride. The resulting copolymer (**7a**) was precipitated from a minimal amount of methylene chloride onto an excess of diethyl ether (~400 mL). Yield: 1.6 g (50 %, beige powder). Theoretical Composition: 50:50 (Diacid **5a**:Diacid **6**), Actual Composition (by H-NMR): 54:46. $^{1}$H NMR (DMSO-$d_6$): δ 8.43 (b, 4H, ArH), 8.15 (b, 4H, ArH), 7.74 (b, 2H, ArH), 7.31 (b, 4H, ArH), 2.55 (b, 8H, $CH_2$), 1.60 (b, 8H, $CH_2$). IR (NaCl, cm$^{-1}$): 1810, 1700 (vs, C=O, anhydride), 1750 (vs, C=O, ester). $M_w$ = 11800, PDI = 1.6. $T_g$ = 46 ºC.

**3,5-Diiodosalicylic acid-based Copolymer (7b).** The iodinated salicylate-based and salicylic acid-based monomers were combined and polymerized at elevated 160 $^{o}$C for ~4 h under vacuum. The resulting copolymer (**7b**) was precipitated from a minimal amount of methylene chloride onto an excess of diethyl ether (~400 mL). Yield: 2.0 g (69 %, light brown powder). Theoretical Composition: 50:50 (Diacid **5b**:Diacid **6**),

Actual Composition (by H-NMR): 55:45. $^{1}$H NMR (DMSO-$d_6$): δ 8.45 (b, 2H, ArH), 8.09 (b, 4H, ArH), 7.76 (b, 2H, ArH), 7.30 (b, 4H, ArH), 2.58 (b, 8H, $CH_2$), 1.60 (b, 8H, $CH_2$). IR (NaCl, $cm^{-1}$): 1800, 1700 (vs, C=O, anhydride), 1750 (vs, C=O, ester). $M_w$ = 5000, PDI = 3.6. $T_g$ = 47 ºC.

### A1.2.2. Synthesis of Salicylate-based Polyanhydrides containing Ethylene Glycol

As described in **Chapter 4**, fast-degrading polyanhydrides would be desirable for a variety of applications from personal care to wound healing. As an attempt to make more hydrophilic polymers that may degrade in short time periods (hours to days), an ethylene glycol derivative, specifically triethylene glycol (TEG, **8**) was chemically incorporated into salicylic acid-based polyanhydrides.

First, TEG (**8**) was reacted with succinic anhydride (**9**) in the presence of base (*i.e.,* triethylamine) to yield the TEG-based diacid (**10**) as shown in **Figure A1.4**.

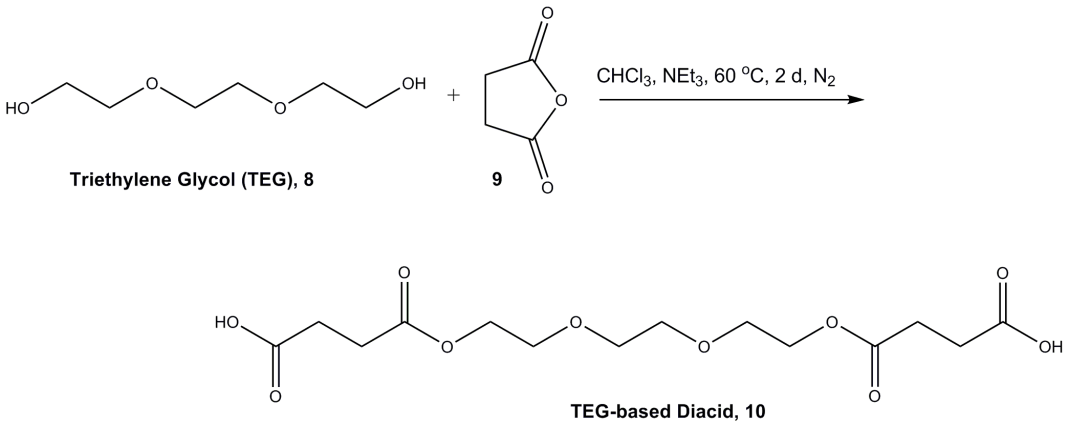



**Figure A1.4.** Synthesis of TEG-based diacid, **10**.

TEG-based diacid (**10**) was utilized in two ways: as a comonomer with salicylic acid-based diacid (**6**) to form a copolymer (**11**) or reacted with thionyl chloride to form an acyl chloride "linker" molecule (**12**) to form a salicylic acid-based poly(anhydride-ester) (**14**). The copolymer **11** was synthesized as shown in **Figure A1.5**.

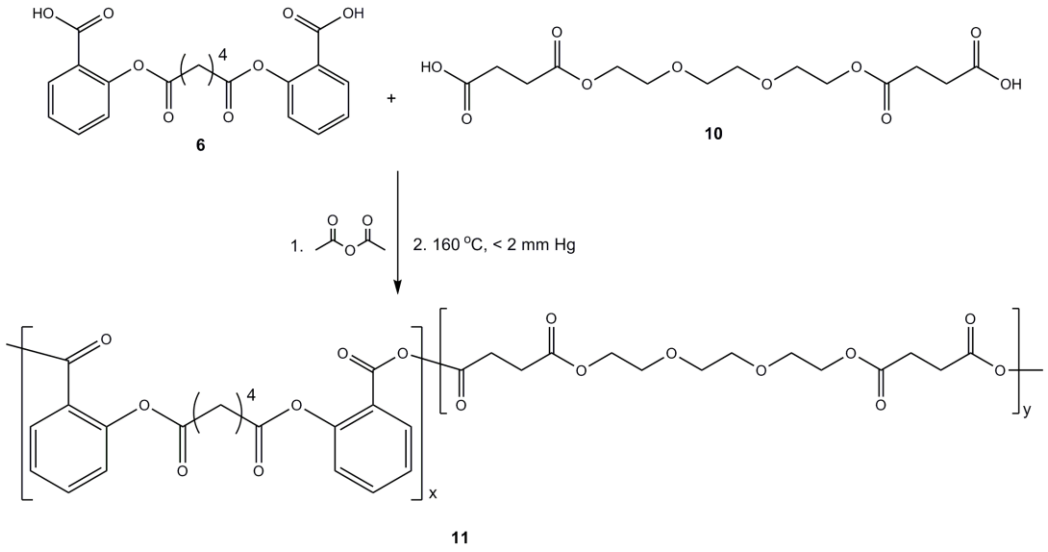



**Figure A1.5.** Synthesis of TEG-based Copolymer, **11**.

The salicylic acid-based diacid (**6**) and TEG-based diacid (**10**) were mixed together in excess acetic anhydride to form a prepolymer or monomer, which was successfully melt-polymerized to yield to copolymer **11**.

Instead of a comonomer, TEG-based diacid (**10**) may also be converted to an acyl chloride (**12**) and subsequently reacted with salicylic acid to form a salicylic acid-based diacid (**13**). This diacid (**13**) may then be polymerized to form the poly(anhydride-ester) **14** as shown in **Figure A1.6**.

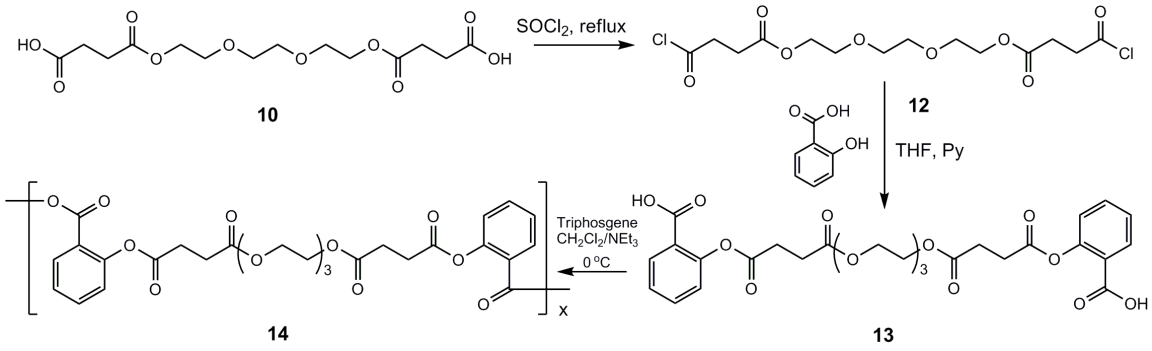



**Figure A1.6.** Synthesis of SA(TEG)-based poly(anhydride-ester), **14**.

The TEG-based acyl chloride (**12**) was successfully prepared, however the preparation of the diacid **13** and polymer **14** proved to be more complex. Because of solubility issues, the diacid **13** and polymer **14** were not successfully prepared. The diacid (**13**) was synthesized, but was impure. Pyridine was difficult to remove upon work-up. Solution polymerization was attempted with the impure diacid since base is necessary for this reaction as well. However, the polymer (**14**) was not isolated. Future experiments are needed to find a procedure to work up water soluble diacids and polymers such as **13** and **14**.

### A1.2.2.a. Experimental: Polymer Synthesis

**Triethylene glycol (TEG)-based Diacid (10).** Triethylene glycol (**8**) (0.8 ml, 5.7 mmol) was dissolved in 40 mL chloroform and pyridine (0.9 mL, 11 mmol). Succinic anhydride (**9**) (2.3 g, 23 mmol) was then added, and the reaction mixture heated to 60 °C for 2 d under nitrogen. After 48 h, the reaction was cooled to room temperature, and the chloroform was removed *via* rotovap. The colorless oil obtained was dissolved in acidic

water (50 mL) and extracted with chloroform (3 x 50 mL). The organic layers were combined, dried over magnesium sulfate and concentrated under reduced pressure to yield the diacid (**10**). Yield: 1.4 g (70 %, pale yellow oil). $^{\backslash 1}$H NMR ($D_2O$): δ 4.19 (t, 4H, $CH_2$), 4.05 (m, 4H, $CH_2$), 3.60 (s, 4H, $CH_2$), 2.59 (s, 8H, $CH_2$).

**Synthesis of TEG-based Copolymer (11).** Salicylate-based diacid (**6**) (2.0 g, 5.2 mmol) and TEG-based diacid (**10**) (0.60 g, 1.7 mmol) were placed in a RB flask with 100 mL acetic anhydride and allowed to stir at room temperature over night. After turning to a clear, homogeneous solution, the excess acetic anhydride was removed using the rotovap to yield the monomer in quantitative yield. The monomer was then melt-polymerized at 160 °C for 4 h. After cooling to room temperature, the copolymer (**11**) was precipitated from methylene chloride onto diethyl ether. The copolymer (**11**) was dried under vacuum at room temperature overnight. Yield: 1.7 g (66 %, white powder); Theoretical Composition: 75:25, Actual Composition (by H-NMR): 73:27. $^1$H NMR (DMSO-$d_6$): δ 8.05 (b, 2H, Ar-H), 7.69 (b, 2H, Ar-H), 7.25 (b, 4H, Ar-H) 4.10 (b, 4H, $CH_2$), 3.55 (b, 8H, $CH_2$), 3.35 (b, 4H, $CH_2$), 2.70 (b, 4H, $CH_2$), 1.58 (b, 4H, $CH_2$). $M_w$: 5,000, PDI: 1.4; $T_g$: 39 °C; Contact Angle: 76.6°.

**TEG-based Acyl Chloride (12).** TEG-based diacid (**10**) (1.1 g) and excess thionyl chloride (~20 mL) were heated to 90 °C under flowing nitrogen overnight. Then, the excess thionyl chloride was removed under vacuum to yield the TEG-based acyl chloride (**12**) in quantitative yield.

**Salicylic acid-based Diacid with Triethylene glycol linker [SA(TEG)-based Diacid], 13.** Salicylic acid (0.50 g, 3.6 mmol) was dissolved in 40 mL THF. Then, pyridine was added (0.60 mL, 7.2 mmol). TEG-based acyl chloride (**12**) (0.70 g, 1.8

mmol) was dissolved in 10 mL THF and added drop-wise to the reaction mixture. The reaction was allowed to stir at room temperature overnight. Then, the THF was removed using the rotovap, and the tacky solid washed with acidic water (pH 2 with conc. HCl, 3 x 100 mL), water (3 x 100 mL) and dried under vacuum at room temperature overnight. $^{1}$H-NMR showed pyridine present, even after subsequent washing with acidic water.

**Salicylic acid-based Polymer with Triethylene glycol linker [SA(TEG)-based Polymer], 14.** The diacid (**13**) was used directly for polymer synthesis, although some base (*i.e.*, pyridine) was still present. Diacid (**13**) (1.0 g, 1.7 mmol) was dissolved in 10 mL anhydrous methylene chloride. Then, triethylamine was added (1.0 mL, 7.5 mmol). Triphosgene (0.60 g, 1.9 mmol) is dissolved in 6 mL methylene chloride and added drop-wise to the reaction mixture. The reaction is allowed to stir at 0 °C under flowing nitrogen for 1.5h. Then, the reaction mixture is poured over diethyl ether (~400 mL), filtered, washed with acidic water (5 x 100 mL) and dried under vacuum at room temperature to yield the polymer, **14**. Yield: 0.36 g (36%, medium brown powder). $^{1}$H-NMR analysis showed a lot of water and base still present. Because of water solubility issues, an optimized method to isolate the diacid (**13**) and polymer (**14**) was not studied further.

### A.1.2.3. Synthesis of EDTA-based Polyanhydride with Benzyl-protected Salicylic Acid as a Pendant

In **Chapter 5**, polyanhydrides based on ethylenediaminetetraacetic acid (EDTA) were synthesized with natural antimicrobials as pendant groups *via* ester linkages. These polymers were proven to have mild to moderate antimicrobial activity against *Salmonella*

*enterica*. Polyanhydrides with salicylic acid chemically incorporated into the backbone, not attached as a side group, have also been proven to prevent biofilm formation.[13,14] Instead of having salicylic acid in the polymer backbone, an EDTA-based polyanhydride may be made containing salicylic acid as a pendant group using a similar approach as described in **Chapter 5**. The synthetic approach is outlined in **Figure A1.7**.

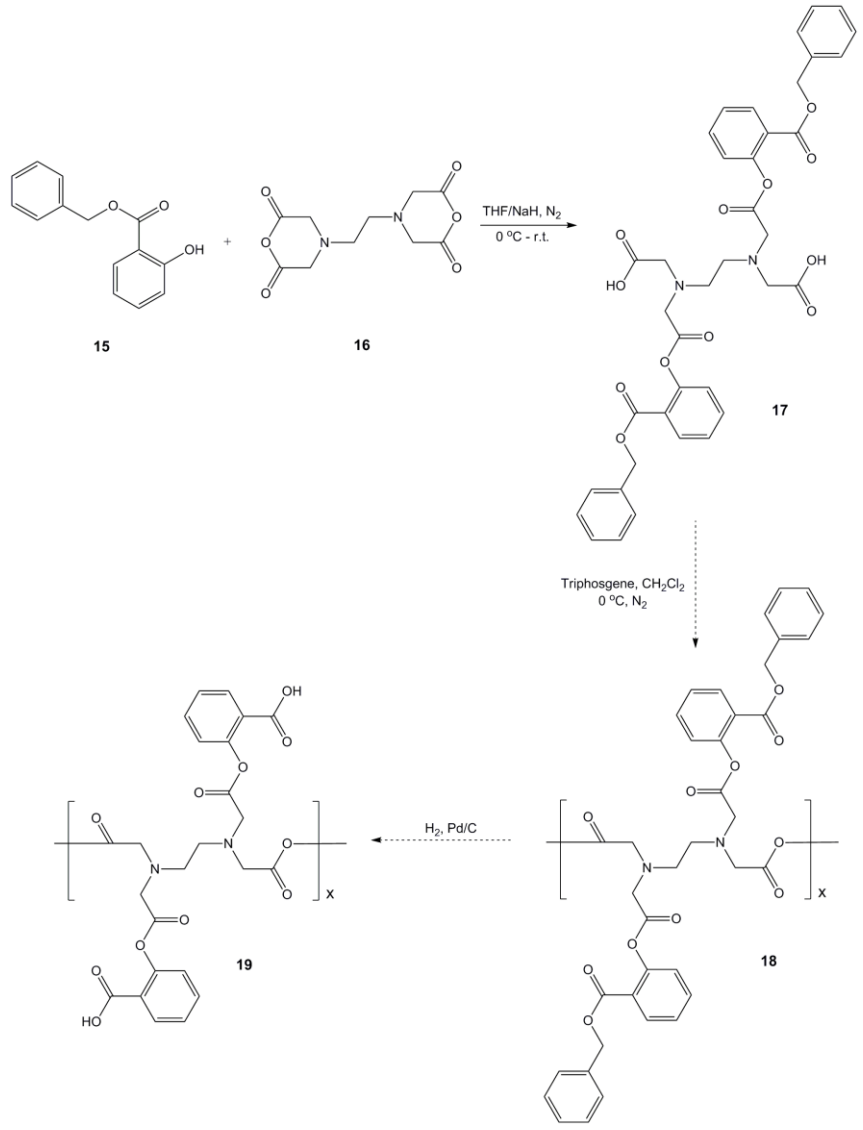



**Figure A1.7.** Synthesis of EDTA-based polyanhydride with salicylic acid as a pendant, **19**.

Benzyl-protected salicylic acid (**15**, OBn-SA) may be used to ring-open EDTA dianhydride (**16**) to yield the polymer precursor (diacid **17**), which may then be polymerized to yield the EDTA-based polyanhydride with OBn-SA as a pendant group (**18**). Then, the OBn-SA on the pendant groups may be deprotected to give the EDTA-based polyanhydride with salicylic acid as pendants (**19**). This last step may not be necessary as since the ester bonds on the benzyl protecting group will likely degrade nonetheless to release the SA.

Efforts to synthesize the diacid **17** proved difficult and the ring-opening reaction was not complete using the experimental conditions outlined below. Further experiments would be necessary to find a synthetic approach to yield the desired polymer **19**.

**A1.2.3.a. Experimental: Polymer Synthesis**

**EDTA-based Diacid (17).** Benzyl-protected salicylic acid (OBn-SA, **15**) (1.7 mL, 8.8 mmol) was dissolved in dry THF (20 mL). After cooling to 0 °C, NaH (60%, 0.4 g, 0.5 mmol) was slowly added to the stirring reaction mixture. After stirring for 1 h, EDTA dianhydride (**16**) was slowly added. The reaction was allowed to stir at room temperature under nitrogen for 2 h. Then, the reaction mixture was poured onto water (~400 mL) and acidified to pH 2 using concentrated HCl. The solid obtained was filtered, washed with water (3 x 150 mL) and dried under vacuum at room temperature. Crude yield: 1.7 g (56 %, white powder). NMR showed a lot of unreacted OBn-SA (**15**) with very small amount of product (< 10 %). The same result was found when using triethylamine as the base.

### A.1.2.4. Synthesis of Salicylic Acid-based Methacrylates

Biodegradable crosslinked networks based on polyanhydrides have been the focus of much research in the past decade and are useful for a wide range of applications from biomaterials and tissue engineering to drug delivery.[15-22] Depending on monomer and polymer composition, the properties can be tailored to give hydrophilic gels to hydrophobic networks.[22] Crosslinked polyanhydride networks have been found useful in bone applications,[19,21] for delivering DNA and proteins,[15,16,20] drug delivery,[17] and tissue engineering.[18]

Bioactive-based polyanhydrides[2,7,10,11,23] are good candidates for incorporation into crosslinked networks, as they may release bioactive molecules upon degradation of the network. To study the applicability of such polymers, methacrylates based on salicylic acid-based polyanhydride precursors, or diacids (**6** and **22**), were designed and synthesized (**Figure A1.8** and **Figure A1.9**).

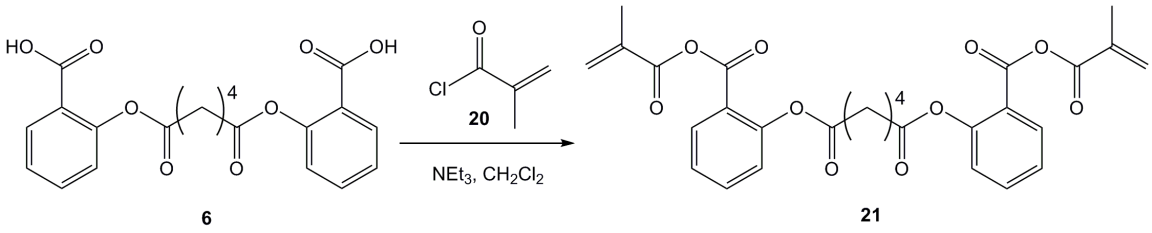



**Figure A1.8.** Synthesis of SA-based methacrylate with adipic linker, **21**.

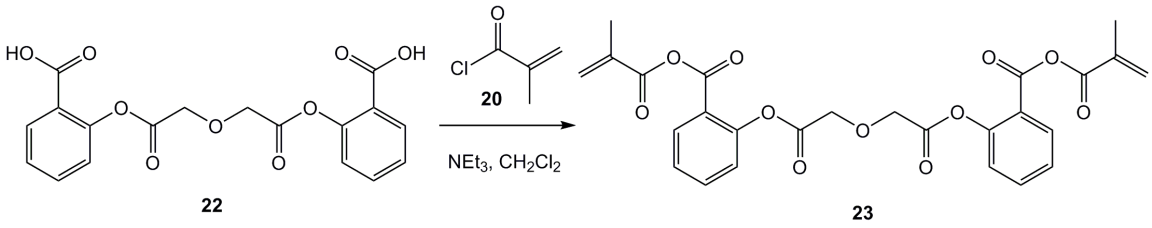



**Figure A1.9.** Synthesis of SA-based methacrylate with diglycolic linker, **23**.

The diacids (**6** and **22**) were reacted with methacryloyl chloride (**20**) in the presence of base (*i.e.*, triethylamine) to give the resulting salicylic acid-based methacrylates **21** and **23**, respectively. Future studies will need to be conducted to see how these methacrylates behave upon photocrosslinking and the properties of the crosslinked networks that may form.

**A1.2.4.a. Experimental: Polymer Synthesis**

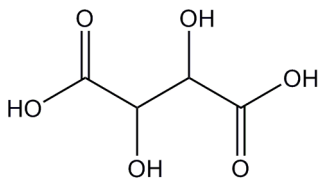
**Salicylic acid-based Methacrylate with Adipic Linker (21).** Diacid (**6**) (0.90 g, 2.6 mmol) was dissolved in dry methylene chloride (300 mL) and triethylamine (0.80 mL, 6.5 mmol) under argon. The reaction mixture was cooled to 0 °C, and methacryloyl chloride (**20**) (0.60 mL, 6.5 mmol) was added drop-wise. The reaction was stirred at 0 °C for 4 h. Then, the reaction mixture was washed with saturated sodium bicarbonate (2 x 300 mL), deionized water (2 x 300 mL) and dried over sodium sulfate. The methylene chloride was removed, and the product (**21**) was dried under vacuum at room temperature. Yield: 1.0 g (81 %, clear oil at room temperature, white solid when stored in the refrigerator). $^{1}$H NMR (DMSO-$d_6$): δ 8.09 (d, 2H, ArH), 7.82 (t, 2H, ArH), 7.50 (t, 2H, ArH), 7.38 (d, 2H, ArH), 6.30 (d, 2H, $CH_2$), 6.03 (d, 2H, $CH_2$), 2.66 (t, 4H, $CH_2$),

1.96 (s, 6H, $CH_3$), 1.71 (m, 4H, $CH_2$). $^{13}$C NMR (DMSO-$d_6$): δ 172.1, 163.4, 160.7, 151.6, 136.8, 135.5, 132.8, 131.7, 127.5, 125.2, 122.0, 33.7, 24.0, 18.1.

**Salicylic acid-based Methacrylate with Diglycolic Linker (23).** Diacid (**22**) (1.0 g, 2.7 mmol) was dissolved in dry methylene chloride (100 mL) and triethylamine (0.9 mL, 6.7 mmol) under argon. The reaction mixture was cooled to 0 °C, and methacryloyl chloride (**20**) (0.7 mL, 6.7 mmol) was added drop-wise. The reaction was stirred at 0 °C for 4 h. Then, the reaction mixture was washed with saturated sodium bicarbonate (2 x 300 mL), deionized water (2 x 300 mL) and dried over sodium sulfate. The methylene chloride was removed, and the product (**23**) was dried under vacuum at room temperature. Yield: 1.4 g (quantitative, clear oil). $^{1}$H NMR (DMSO-$d_6$): δ 8.18 (d, 2H, ArH), 7.83 (t, 2H, ArH), 7.56 (t, 2H, ArH), 7.42 (d, 2H, ArH), 6.37 (d, 2H, $CH_2$), 6.08 (d, 2H, $CH_2$), 4.62 (s, 4H, $CH_2$), 1.98 (s, 6H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$): δ 169.0, 163.4, 160.7, 151.0, 137.0, 135.5, 132.9, 131.9, 127.8, 125.2, 121.7, 68.2, 18.1.

### A.1.3. Synthesis of Tartaric Acid-based Polyanhydride

Tartaric acid (**24**) (**Figure A1.10**) is present in many fruits and widely used in soft drinks, confectionery and bakery products and gelatin desserts.[3] Tartaric acid is known to possess antioxidant properties and the ability to hydrate skin, therefore desirable for other applications such as skin care.[3,24]




**24**

**Figure A1.10.** Chemical structure of tartaric acid, **24**.

Chemically incorporating tartaric acid (**24**) into a biodegradable polyanhydride would be beneficial for controlled release applications for foods or personal care.

In our laboratory, tartaric acid has been modified by acylation with linear aliphatics to form hydrophobic portions of amphiphilic macromolecules for drug delivery and other applications.[25-27] Using similar approaches,[25-27] a modified tartaric acid could be utilized to make polyanhydrides containing tartaric acid in their backbone (**Figure A1.11**).

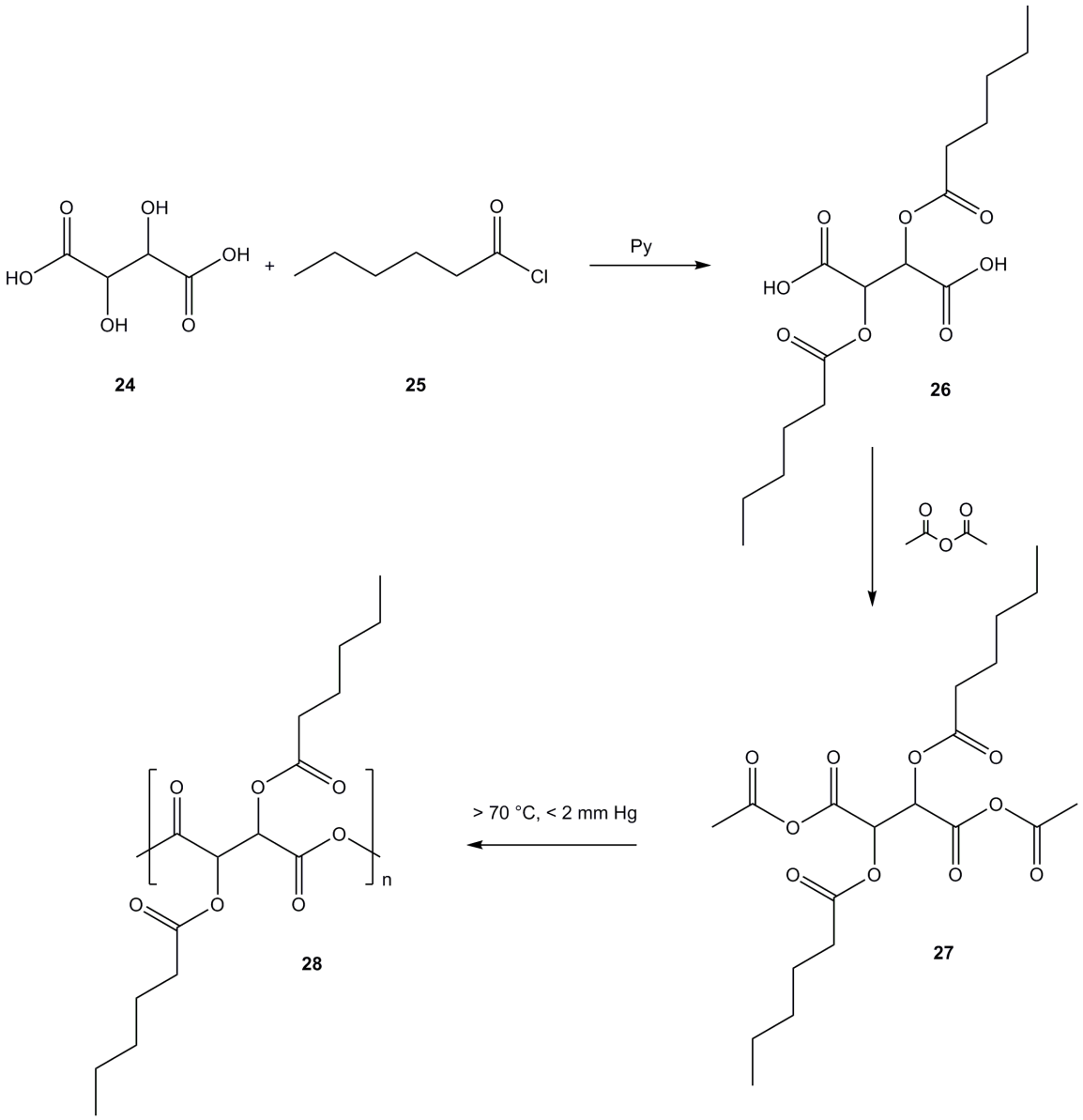



**Figure A1.11.** Synthesis of tartaric acid-based polyanhydride, **24**.

The diacid (**26**) and monomer (**27**) were successfully prepared, however an effective melt-polymerization was not possible since the monomer had a relatively low decomposition temperature (126 °C). In the future, solution polymerization[5-7] may be utilized to obtain the desired polymer (**28**).

Futhermore, instead of linear aliphatic pendant groups, a bioactive molecule can be chemically attached to tartaric acid *prior* to polymerization, which may result in a dual-releasing biodegradable polymer. Future work is needed to assess the feasibility of such an option.

### A1.3.1. Experimental

#### A1.3.1.a. Polymer Synthesis

**Tartaric acid-based Diacid (26).** The diacid (**26**) was synthesized using similar techniques that were previously described.[25-27] Tartaric acid (**24**) (4.2 g, 28 mmol) was dissolved in hexanoyl chloride (**25**) (31.3 mL, 224 mmol). Pyridine is then added drop-wise to the stirring reaction mixture. The reaction mixture was allowed to stir at room temperature for 2 days. Then, diethyl ether (~200 mL) was added to the reaction flask, and after stirring for 30 minutes, 20 mL of water was added. After 15 minutes, the entire reaction mixture was poured onto an ice/water slush (~150 mL) and allowed to stir for 30 minutes. After pouring into a separatory funnel, the organic phase was separated and washed with brine (2 x 200 mL) and was concentrated to ~30 mL. The concentrated organic phase was precipitated onto ~400 mL hexanes to yield the diacid (**26**). Yield: 4.9 g, (Quantitative, brown oil). $^{1}$H NMR (DMSO-$d_6$): δ 5.53 (s, 2H, CH), 2.30 (t, 4H, $CH_2$), 1.55 (m, 4H, $CH_2$), 1.28 (m, 8H, $CH_2$), 0.92 (t, 6H, $CH_3$).

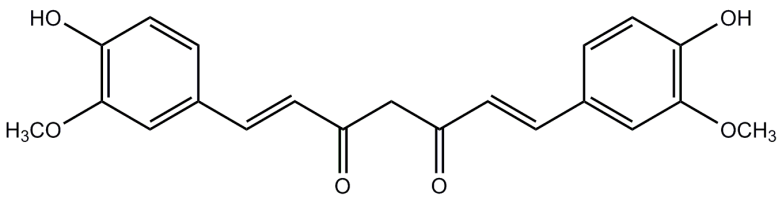
**Tartaric acid-based Monomer (27).** Diacid (**26**) (2 g) was stirred with excess acetic anhydride at room temperature until the reaction mixture turned to a clear, homogeneous solution. Excess acetic anhydride was removed under reduced pressure to yield the monomer (**27**). Yield: 4.0 g, (Quantitative, brown oil). $^{1}$H NMR (DMSO-$d_6$): δ

6.24 (s, 2H, CH), 2.40 (s, 6H, $CH_3$), 2.19 (t, 4H, $CH_2$), 1.60 (m, 4H, $CH_2$), 1.25 (m, 8H, $CH_2$), 0.93 (t, 6H, $CH_3$). $T_d$: 126 °C.

**Tartaric acid-based Polymer (28).** Monomer was placed in a double-necked round bottom flask equipped with an overhead stirrer. The monomer was melt polymerized at 70 °C for 4 h. No polymer formed at this temperature. Higher polymerization temperatures were not possible due to the relatively low decomposition temperature of the monomer (**27**) (*i.e.*, 126 °C).

## A1.4. Curcumin-based Poly(anhydride-ester)

Curcumin (**29**, **Figure A1.12**) is the primary active ingredient in the spice turmeric derived from *Curcuma longa*.[28] It has been found to have antioxidant, anticancer, anti-inflammatory, and anti-Alzheimer's activities.[28-33]




**29**

**Figure A1.12.** Chemical structure of curcumin, **29**.

Chemically incorporating curcumin (**29**) into the backbone of a polyanhydride may be beneficial for controlled drug delivery based on the above-mentioned biological activity of the bioactive (**29**).

Therefore, curcumin (**29**) was chemically incorporated into the backbone of a polyanhydride (**32**). Curcumin (**29**) was first reacted with glutaric anhydride (**30**) to yield the resulting diacid (**31**), which was subsequently polymerized to yield the polyanhydride (**32**) (**Figure A1.13**).

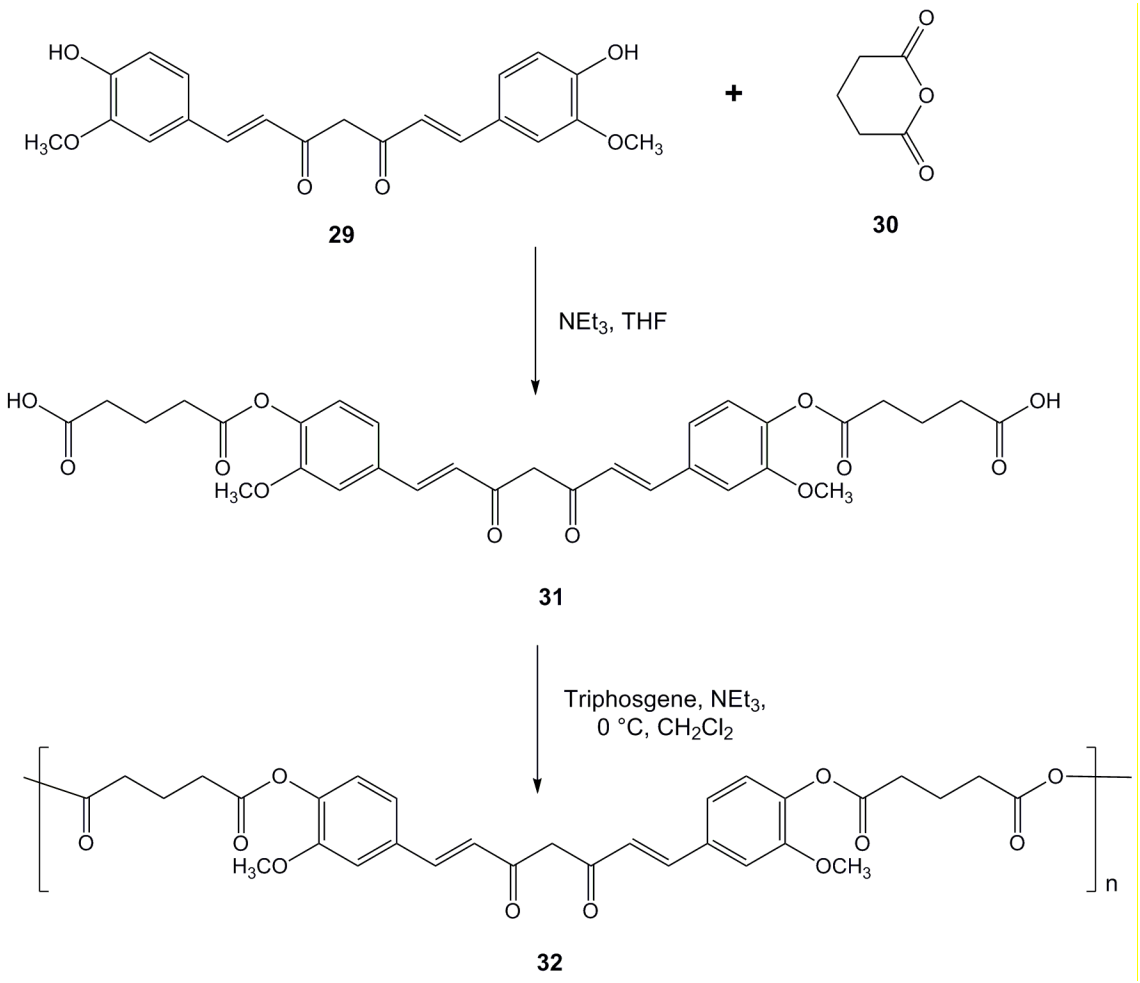



**Figure A1.13.** Synthesis of curcumin-based polyanhydride, **32**.

Further experiments are needed to examine the properties of the curcumin-based polyanhydride and its release behavior upon hydrolysis.

### A1.4.1. Experimental

#### A1.4.1.a. Polymer Synthesis

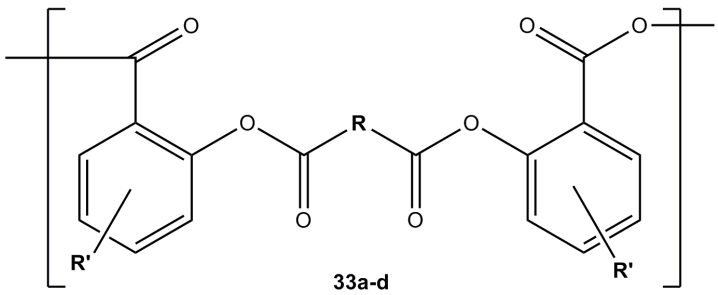
**Curcumin-based diacid (31).** Curcumin (**29**) (0.90 g, 2.5 mmol) was dissolved in dry THF (10 mL) and triethylamine (3.5 mL, 25 mmol), and the reaction mixture was cooled to 0 °C. Glutaric anhydride (0.60 g, 5.0 mmol) was dissolved in 5 mL dry THF and added drop-wise to the reaction mixture. The reaction was allowed to stir for 1 h at 0 °C and then warmed to room temperature to stir overnight. Then, the reaction mixture was poured onto an ice/water slush (~200 mL) and acidified to pH 2 using concentrated HCl. The tacky solid obtained was dissolved in acetone and precipitated onto an excess of hexanes to yield the diacid (**31**), which was dried under vacuum at room temperature. Yield: 2.4 g, (79 %, pale orange powder). $^1$H NMR (DMSO-$d_6$): δ 7.65 (d, 2H, CH), 7.57 (s, 2H, ArH), 7.35 (d, 2H, ArH), 7.20 (d, 2H, ArH), 6.97 (d, 2H, CH), 6.22 (s, 2H, $CH_2$), 3.87 (s, 6H, $CH_3$), 2.64 (t, 4H, $CH_2$), 2.40 (t, 4H, $CH_2$), 1.85 (m, 4H, $CH_2$).

**Curcumin-based Polyanhydride (32).** Curcumin-based diacid (**31**) (2.0 g, 3.4 mmol) was dissolved in 25 mL dry methylene chloride and triethylamine (2.1 mL, 15 mmol). The reaction was cooled to 0 °C. Triphosegene (1.1 g, 3.7 mmol) was dissolved in 10 mL methylene chloride and added drop-wise to the reaction mixture over 1 h. The reaction was stirred at room temperature for 2 h. Then, the reaction mixture was poured onto diethyl ether (~400 mL), and the solid obtained (polymer **32**) was washed with acidic water (pH 2 with concentrated HCl, 1L) and dried under vacuum at room temperature. Yield: 2.3 g, (74 %, orange-brown powder). $^1$H NMR (DMSO-$d_6$): δ 8.01 (b, 2H, CH), 7.71 (b, 2H, ArH), 7.54 (b, 2H, ArH), 7.30 (b, 2H, ArH), 7.16 (b, 2H, CH),

6.93 (b, 2H, $CH_2$), 3.90 (b, 6H, $CH_3$), 2.66 (b, 4H, $CH_2$), 2.38 (b, 4H, $CH_2$), 1.92 (b, 4H, $CH_2$).

### A1.5. Antimicrobial Assays of Salicylate-based Poly(anhydride-esters)

As discussed in **Chapter 4** and **Chapter 5**, salicylate-based poly(anhydride-esters) have been proven to prevent biofilm formation, which is important for a variety of applications for biomaterials and food safety applications.[13] To evaluate other NSAID-based poly(anhydride-esters)[11,23] (**Figure A1.14**) from our laboratory, the antimicrobial activity of polymer (**33**) coatings on metal substrates (*i.e.*, 316L stainless steel coupons) were studied.




**33a-d**

| | |
|---|---|
| **R** = 3, **R'** = H | **a** |
| **R** = 4, **R'** = H | **b** |
| **R** = 6, **R'** = H | **c** |
| **R** = 4, **R'** = SA | **d** |
| **R** = 4, **R'** = 2,4-difluorophenyl | **e** |
| **R** = 4, **R'** = 5-iodo | **f** |
| **R** = 4, **R'** = 3,5-diiodo | **g** |

**Figure A1.14.** Chemical structures of poly(anhydride-esters) (**33**) evaluated for their antimicrobial activity.

Polymer (**33**) samples were evaluated with the Biofilm Eradication Surface Testing (BEST) assay by Innovotech, Inc (Alberta, Canada), which quantitatively measures adherence, killing and biofilm formation of microorganisms attached to the surface of coated materials. The microorganisms tested were *Pseudomonas aeruginosa* (Gram-negative) and **Staphylococcus** *aureus* (Gram-positive).

Results indicated that polymers **33** were not effective in preventing or retarding bacterial adherence of *Pseudomonas aeruginosa* to the metal coupons. This effect may be explained by the fact that the antimicrobials (*i.e.*, salicylic acid or salicylsalicylic acid) are not available until the polymers begin to hydrolytically degrade. To evaluate this hypothesis, polymer-coated metal coupons were incubated up to 24 h in PBS solution (pH 7.4 at 37 °C). After incubation, the polymer-coated substrates still did not demonstrate biological activity.

Alternately, Gram-positive bacteria, namely *Staphylococcus aureus*, was chosen for further experiments. Using the BEST method, polymer **33b** and **33d** coatings (after 12 h incubation in PBS) completely prevented biofilm formation. Polymer **33a** and **33c** were able to reduce biofilm formation by 1.5 and 2.8 Log, respectively. Under the experimental conditions used, it could not be determined if the coatings were able to prevent adhesion of the microorganism through surface modification or if the biofilm inhibition was caused by planktonic cell death from the release of the bioactives from the polymers (**33**). This research provided the preliminary data for the study of salicylate-based polyanhydrides for biofilm prevention[14] as described in **Chapter 4** and **Chapter 5**.

### A.1.5.1. Experimental

#### A.1.5.1.a. Polymer Synthesis

Polymers (**33**) were synthesized from previously described methods.[1,9,11,23,34]

#### A.1.5.1.b. Preparation of Polymer-coated Substrates

316L stainless steel coupons were washed with methylene chloride and hexanes to remove any organics or oils. The coupons were masked with Fisher lab tape to limit the surface area to 6.25 $cm^2$ on the coupon. Polymer (**33**) solutions (~5 wt % in methylene chloride) were spray-coated onto the metal coupons using a Badger Model 350-3 airbrush system (30 s on each side to allow visual representation of a uniform coating). Polymer coatings were dried at room temperature in the hood for 12 h, followed by drying under vacuum at room temperature for 12 h. Coupons were weighed before and after coating to determine mass of polymer **33** applied. Mass of polymer (**33**) was ~15 mg and coating thickness was ~0.5 mm. Samples were incubated in PBS (10 mL, pH 7.4) at 37 °C for 0, 12 or 24 h. All samples were made in triplicate.

#### A.1.5.1.c. Antimicrobial Assessment

*[Antimicrobial assays were performed by Innovotech Inc, Alberta, Canada.]*

Experimental protocol for the preparation of cell cultures, media and reagents, and testing procedure are described in literature[35,36] and will be discussed in future publications.

## A1.6. Materials and Methods

### A1.6.1. Materials

All chemicals, reagents and solvents were purchased from Aldrich (Milwaukee, WI) and used as received unless otherwise noted above.

### A1.6.2. Methods

#### A1.6.2.a. Proton Nuclear Magnetic Resonance ($^1$H NMR) Spectroscopy

Proton nuclear magnetic resonance ($^1$H-NMR) spectra were recorded on a Varian 300, 400, or 500 MHz spectrophotometer. Samples (5-10 mg) were dissolved in deuterated solvent (DMSO-$d_6$ or $D_2O$), which was also the internal reference.

#### A1.6.2.b. Infrared (IR) Spectroscopy

Infrared (IR) spectra were measured on a Thermo Nicolet/Avatar 360 FT IR spectrometer, by solvent-casting samples from appropriate solvent onto NaCl plates.

#### A1.6.2.c. Gel Permeation Chromatography (GPC)

Weight-averaged molecular weights ($M_w$) of polymers were determined using a Perkin-Elmer LC system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 advanced sample processor. A Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software was utilized for data collection and automation of the system,. The connection from the LC system to the computer was made using a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Polymers (**XX**) were dissolved in methylene chloride (10 mg/mL) and filtered through 0.45 µm poly(tetrafluoroethylene) (PTFE) syringe filters (Whatman, Clifton, NJ) prior to elution through a Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm) (Alltech

Associates, Deerfield, IL). Molecular weights were calculated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

**A1.6.2.d. Differential Scanning Calorimetry (DSC)**

DSC analyses were performed using a Perkin-Elmer system consisting of a Pyris 1 differential scanning calorimeter (DSC) or Thermal Advantage system consisting of a differential scanning calorimeter (DSC) Q200. A Dell Optiplex GX110 computer equipped with Perkin-Elmer Pyris software or IBM ThinkCentre computer equipped with Thermal Advantage Universal Analysis software were used for data collection and processing. The glass transition temperature ($T_g$) was determined on samples (5-10 mg) under nitrogen gas heating from –10 °C to 200 °C at a heating rate of 10 °C/min and cooling to –10 °C at a rate of 10 °C/min with a minimum of two heating/cooling cycles. The $T_g$ was calculated as half $C_p$ extrapolated.

**A1.6.2.e. Thermal Gravimetric Analysis (TGA)**

Decomposition temperature ($T_d$) was measured using a Perkin-Elmer system consisting of a thermogravimetric analyzer (TGA) with TAC 7/DX instrument controller. A Dell Optiplex GX110 computer equipped with Perkin-Elmer Pyris software was used for data collection and processing. Samples (5-10 mg) were heated under nitrogen gas from 25 °C to 400 °C at a rate of 10 °C/min; the decomposition temperature ($T_d$) was calculated as the onset of thermal decomposition.

**A1.6.2.f. Contact Angle Measurement**

Static contact angles were measured by dropping deionized water onto pressed polymer disks using a Ramé-Hart Standard Goniometer Model Number 250-00

(Mountain Lakes, NJ) outfitted with a Dell Dimension 3000 computer with DROPimage Advanced software.

## A1.7. References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# A2. APPENDIX 2: COLLABORATIONS

This appendix describes research collaborations that are an extension of the main research projects described in previous chapters. Materials and methods are compiled in **Section A2.4**.

## A2.1. Bioactive-based Polyanhydrides and Nerve Regeneration

Non-steroidal anti-inflammatory drug (NSAID)-based polymers[1] may be useful as artificial nerve guides because of their ability to locally mitigate inflammation.[2,3] The potential cytotoxicity of NSAID-based poly(anhydride-esters) with neural cells was evaluated *in vitro*.[4] The polymers tested were based on three NSAIDs, namely salicylic acid (SA, **1a**), salicylsalicylic acid (**1b**) and diflunisal (**1c**). These polymers were chosen because of their previously demonstrated cytotoxicity with fibroblasts[1,5] and glass transition temperature values above 37 ˚ C, which is necessary for processing into conduits. For comparison, adipic acid was used as the linker (**2**) for all polymers (**Figure 6.1**). Salicylic acid and diflunisal-based poly(anhydride-esters) (**5a** and **5c**, respectively) were synthesized using previously described methods,[6,7] and the polymer based on salicylsalicylic acid (**5b**) was synthesized using a modified procedure used for similar polymers.[1]

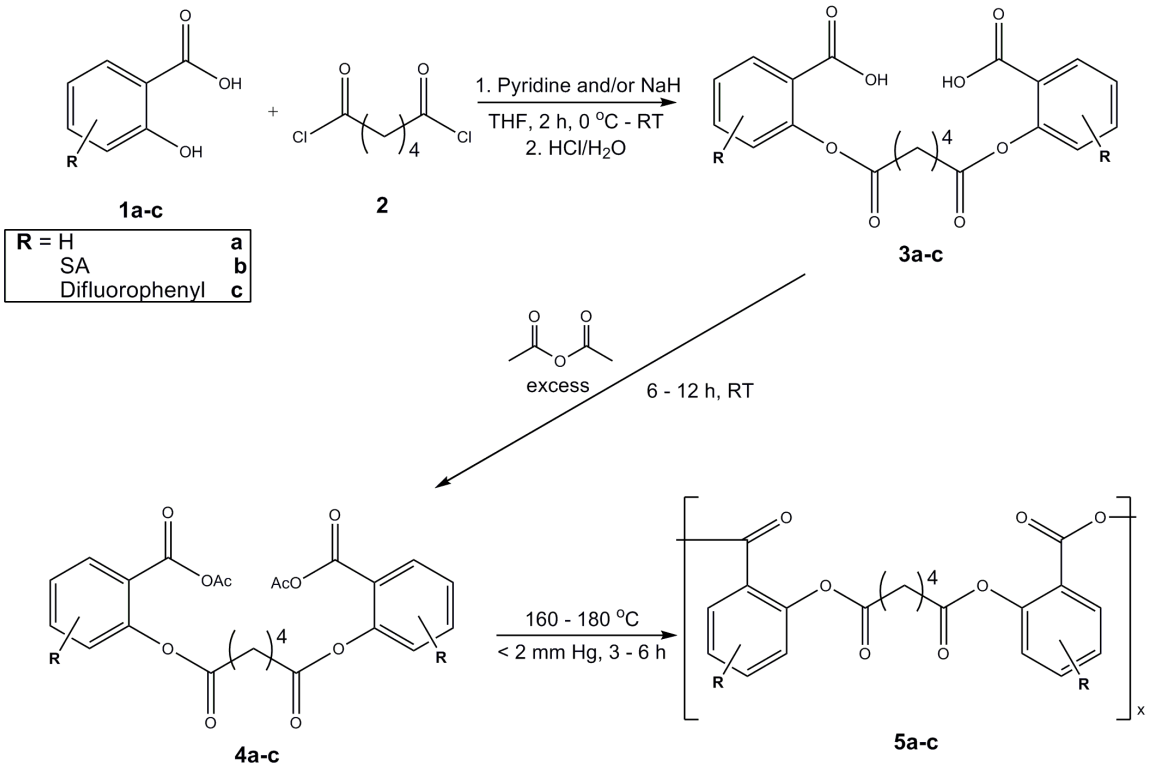



**Figure A2.1.** Synthetic scheme for NSAID-based poly(anhydride-esters) **5**.

To evaluate the NSAID-based polymers (**5**) as nerve regeneration materials, primary dorsal root ganglia (DRG) and Schwann cell responses on polymer-coated glass coverslips were analyzed and compared. DRG and Schwann cells are representative of neuron and glial cells in the peripheral nervous system, respectively.[8,9] PLGA was utilized as the control because it has been extensively studied in the tissue engineering field[8,10] and used in approved implant devices.[11,12]

In brief, the salicylic acid-based polymer (**5a**) was deemed most promising for nerve cells based on the attachment and proliferation profile, followed by the diflunisal-based polymer (**5c**). Polymer **5a** demonstrated similar compatibility with the PLGA

control for neuron growth, and both polymers **5a** and **5c** had Schwann cell attachment and proliferation rates comparable to the PLGA control. Notably, contact angles of the polymer (**5**) surfaces roughly correlate to neuron cell compatibility; the contact angles of polymer **5a** and PLGA were relatively more hydrophilic than polymers **5b** and **5c** (both at 44˚ relative to ~66˚), which may assist in adsorbing soluble proteins in the media, resulting in higher neuron adhesion and growth. In summary, these materials may be useful as or in conjunction with nerve guidance devices.

### A2.1.1. Experimental

#### A2.1.1.a. Polymer Synthesis and Characterization

Polymers containing salicylic acid and diflunisal were synthesized as previously described.[6,13] Contact angles of **5a** and **5c** were 44 ˚ and 68 ˚, respectively.

The salicylsalicylic acid (SA-SA)-based diacid (**3b**) with was prepared using a similar procedure to the previous published methods.[1] In brief, the salicylate derivative (**1**; 30 mmol) was dissolved in THF (100 mL), to which base was added drop-wise. The diacyl chloride (**2**; 15 mmol) was added drop-wise to the reaction mixture. After stirring for 2 h, the reaction was quenched by pouring over water (~700 mL) and acidified to pH 2 using concentrated HCl. The diacid (**3**) was obtained *via* vacuum filtration and washed with water (3 x 200 mL) and is dried under vacuum at room temperature. The diacid (**3**) were recrystallized from acetone and hexanes. Any changes to the procedure are noted below.

**Salicylsalicylic Acid-based Diacid (3b).** The reaction was performed at 0 $^{o}$C, and NaH (75 mmol) used as a base. Yield: 72 % (white solid). $^{1}$H-NMR (DMSO-$d_6$): 7.81 (dd,

4H, ArH), 7.63 (t, 4H, ArH), 7.39 (td, 4H, ArH), 7.14 (t, 4H, ArH), 2.19 (t, 4H, $CH_2$), 1.28 (m, 4H, $CH_2$). IR (NaCl, $cm^{-1}$): 3400-2900 (COOH), 1745 (C=O, ester), 1698 (C=O, acid). Anal. Calcd: C, 65.2 %; H, 4.2 %. Found: C, 65.7 %; H, 4.8 %. $T_m$: 151 °C.

The diacids (**3**) were activated into monomers (**4**) by acetylation as previously described.[1,6] Briefly, the diacid (2 g) and an excess of acetic anhydride (50 mL) were stirred at room temperature until a clear, homogeneous solution was observed. The monomer (**4**) was isolated by removing excess acetic anhydride *via* a rotary evaporator at room temperature.

**Salicylsalicylic Acid-based Monomer (4b).** Yield: quantitative (pale yellow oil). $^1$H-NMR (DMSO-$d_6$): 8.05 (dd, 4H, ArH), 7.93 (t, 4H, ArH), 7.74 (td, 4H, ArH), 7.36 (t, 4H, ArH), 2.46 (t, 4H, $CH_2$), 2.18 (s, 6H, $CH_3$), 1.61 (m, 4H, $CH_2$). IR (NaCl, $cm^{-1}$): 1812, 1707 (C=O, anhydride), 1747, 1605 (C=O, ester). $T_d$: 319 °C.

Melt-condensation polymerization[14] of the monomers (**4**) was performed by placing the monomer (2 g) in a double-necked round-bottom flask equipped with an overhead stirrer (T-line Laboratory Stirrer, Model 104, Talboys Engineering, Thorofare, NJ) and vacuum connection (< 2 mm Hg). While stirring at ~100 rpm and under vacuum, the melt was heated to 160-180 $^oC$ depending on the monomer decomposition temperature using a temperature controlled silicone oil bath (Cole-Parmer, Vernon Hills, IL). The polymerization was considered complete when the viscosity of the melt remained the same and/or it solidified, which was approximately 3-6 h. After cooling to room temperature, the polymer (**5**) was isolated by precipitation from methylene chloride into a 20-fold excess of diethyl ether, filtered and dried overnight at room temperature

under vacuum.

**Salicylsalicylic Acid-based Polymer (5b).** Yield: quantitative (off-white solid). $^{1}$H-NMR (DMSO-$d_6$): 8.22 (dd, 4H, ArH), 7.99 (t, 4H, ArH), 7.77 (td, 4H, ArH), 7.42 (t, 4H, ArH), 2.56 (t, 4H, $CH_2$), 1.66 (m, 4H, $CH_2$). IR (NaCl, $cm^{-1}$): 1791, 1722 (C=O, anhydride), 1747, 1606 (C=O, ester). $M_w$: 5,900; PDI: 1.1. $T_g$: 63 $^{o}$C, $T_d$: 309 °C. CA: 65 ˚.

### A2.1.1.b. Polymer Formulation

The NSAID-based polymers (**5**) were evaluated as potential nerve guidance materials and PLGA 50:50 copolymer (Boehringer Ingelheim Inc., Germany) was used as a control. Polymers (100 mg) were dissolved in ~ 1 mL of methylene chloride, then spin-coated onto glass coverslips at 2000 rpm for 30 s using a spin-coater (Headway Research, Inc., Garland, TX). Before spin-coating, glass coverslips (18 mm diameter; 0.15 mm thickness) were cleaned using an Alconox (Alconox Inc., White Plains, NY) and $H_2SO_4$:$H_2O_2$ (10:1, v/v) solutions and stored in 70 % ethanol until used. Uncoated glass coverslips were also used as a control.

### A2.1.1.c. *In Vitro* Assays

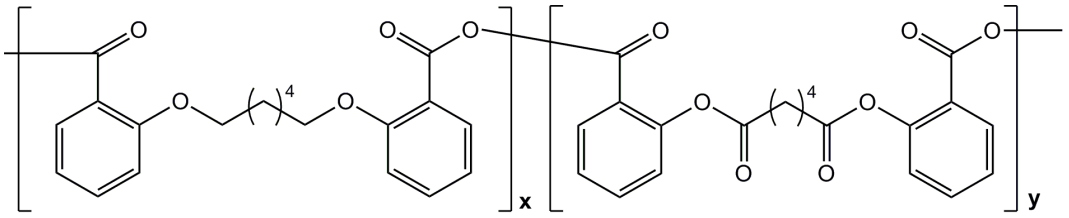
*[In vitro cytotoxicity evaluation of NSAID-based polymers on nerve cells, namely Schwann and dorsal root ganglia, was performed by Minjung Song (Department of Biomedical Engineering, Rutgers University, NJ)].*

Experimental procedures for dorsal root ganglia isolation, immunostaining, Schwann cell preparation, cell morphology and proliferation will be discussed in future publications.[4]

## A2.2. Salicylate-based Poly(anhydride-esters) in Bone Regeneration

Bone grafts are widely used for alveolar bone reconstruction and periodontal regeneration, however, recent literature reviews conclude that clinical evidence supports the use of bone grafts, although a critical need exists for more robust reconstructive and regenerative strategies.[15-18] The efficient delivery and containment of particulate grafts often presents a significant clinical challenge. Using biodegradable polymers as a means for controlled delivery may be advantageous. Polylactic acid is often used, yet often results in inflammatory and foreign body giant cell reactions.[19-22]

Salicylic acid-based poly(anhydride-esters) (*e.g.*, **6**) have a relatively high drug loading of non-steroidal anti-inflammatory drug (up to 70 % by weight) that can be locally delivered.[3,6] Furthermore, controlled release of salicylic acid may be valuable in reconstructive procedures due to the potential to disrupt bacterial biofilm formation.[23,24]




***o*-CPH:SAA Copolymer, 6**

**Figure A2.2.** Chemical structure of the copolymer (**6**) comprised of 1,6-bis(*o*-carboxyphenoxy)hexane (*o*-CPH) and SA-based diacid **3a**.

This study evaluated the effect of salicylate-based copolymer **6** on wound healing and bone formation when used as a bone binder. The copolymer (**6**) exhibited a

favorable $T_g$ above body temperature and prolonged degradation profile with ~6 % of SA released after 7 days as compared with the salicylic acid-based homopolymer (**5a**), which completely degraded in ~1 week. The results indicated significantly greater fibrous tissue formation and less residual graft particles were present in the defects grafted with bone and SA-based copolymer **6**. Evidence of active resorption and phagocytosis of graft particles was found, which is consistent with the presence of significantly less residual graft material.

### A2.2.1. Experimental

#### A2.2.1.a. Polymer Synthesis and Characterization

A copolymer of 1,6-bis(*o*-carboxyphenoxy)hexane (*o*-CPH) and SA-based diacid **3a** was synthesized in a 82:18 ratio (**6**, Figure **A2.2**) as described in **Chapter 3**. The polymer precursors were synthesized using previously described methods.[1,3,6,25,26] The diacids were activated separately in excess acetic anhydride to yield the monomers, which were then melt-polymerized together to yield the resulting copolymer.[1,6,14] The properties of copolymer **6** used in this study were: $M_w$: 6 900, PDI: 1.3; $T_g$: 53 °C, $T_d$: 290 °C.

#### A2.2.1.b. *In Vivo* Studies

*[In vivo study and histological analysis were performed by Mark A. Reynolds, Mary E. Aichelmann-Reidy and Gary Warburton (Departments of Periodontics and Oral and Maxillofacial Surgery, University of Maryland Dental School, MD)].*

Experimental *in vivo* procedures, specimen preparation and histological analysis will be described in future publications.[7]

### A2.3. Effect of Salicylate-based Polyanhydrides on Biofilm Formation

As discussed in **Chapter 5** and **Chapter 6**, biofilms are one of the major concerns in food, personal care and medical industries. Salicylate-based poly(anhydride-esters) have previously been shown to inhibit biofilm formation, possibly by affecting attachment to surfaces.[23] To further understand the mechanism for biofilm prevention, a model system was designed utilizing biofilm-forming *Salmonella enterica* serovar Typhimurium. Two different strains of the microorganism were used that produce two different biofilm structures, one at the air-liquid interface (*i.e.*, top-forming) and one at the liquid-surface interface (bottom-forming).

Prior to these experiments, it was in question whether the lack of biofilm formation of microorganisms exposed to salicylate-based polymers (**5a**) was the result of interference with biofilm forming ability of the cells or reduction of viable cells to numbers low enough that biofilm formation could not be initiated. No significant difference was observed between the control and treatment plate (presence of salicylate-based polymer) with respect to planktonic cell density. The data clearly suggests that low overall cell density is not the reason for lack of biofilm formation. Furthermore, salicylic acid-based polymers interfere with biofilm forming abilities of bacteria, but not through the attachment mechanism, as the polymers only prevented biofilm formation for the top-forming strain of the microorganism.

### A2.3.1. Experimental

#### A2.3.1.a. Polymer Synthesis

Poly[1,6-bis(o-carboxyphenoxy)-hexanoate] (**5a**) was prepared using previously described methods.[6]

#### A2.3.1.b. Polymer Formulation

The polymer (**5a**) was dissolved in methylene chloride (10 mg/mL) and solvent-casted onto microscope glass coverslips (Fisher Scientific, Fair Lawn, NJ; 12 mm diameter, 0.15 mm thickness). The coated coverslips were allowed to dry at room temperature for 12 h, and under vacuum at room temperature for 12 h, to ensure full solvent removal. Before coating, the coverslips were cleaned using Alconox (Alconox. Inc. NY) and $H_2SO_4$:$H_2O_2$ (10:1, v/v) solutions and stored in ethanol until use.

#### A2.3.1.c. *In Vitro* Microbiological Assays

*[In vitro microbiological assays were performed by Linda Rosenberg-Minkow (Department of Food Science, Rutgers University, NJ)].*

Experimental procedures for *in vitro* cell culture methods and biofilm-associated and free cell enumeration are described in literature.[24]

## A2.4. Materials and Methods

### A2.4.1. Materials

Tetrahydrofuran (THF), acetic anhydride, methylene chloride, and diethyl ether were purchased from Fisher (Fair Lawn, NJ). All other fine chemicals and solvents were obtained from Aldrich (Milwaukee, WI) and used as received.

### A2.4.2. Methods

#### A2.4.2.a. Proton Nuclear Magnetic Resonance ($^1$H NMR) Spectroscopy

Proton nuclear magnetic resonance ($^1$H-NMR) spectra were recorded on a Varian 400 or 500 MHz spectrophotometer. The samples (~10 mg) were dissolved in a deuterated solvent (DMSO-$d_6$), which was also used as the internal reference

#### A2.4.2.b. Infrared (IR) Spectroscopy

Infrared (IR) spectra were measured on a Thermo Nicolet/Avatar 360 FT IR spectrometer, by solvent-casting samples from acetone onto NaCl plates.

#### A2.4.2.c. Gel Permeation Chromatography (GPC)

A Perkin-Elmer LC system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 advanced sample processor was used to determine weight-averaged molecular weights ($M_w$) and polydispersity indices (PDI). For data collection and automation of the system, a Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software was utilized. The connection from the LC system to the computer was achieved via a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Polymers (**1a-c**, **6**) were dissolved in methylene chloride (10 mg/mL) and filtered through 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe filters (Whatman, Clifton, NJ) before elution onto the Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm) (Alltech Associates, Deerfield, IL). Molecular weights were calculated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

#### A2.4.2.d. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was used to determine glass transition ($T_g$) temperatures using a TA DSC Q200 outfitted with a Dell Dimension 3000 computer

running TA Universal Analysis 2000 software was used for data collection and processing. Samples (~5 mg) were heated under dry nitrogen gas at heating and cooling rates of 10 ºC/min with a two-cycle minimum. Glass transition temperatures were calculated as half $C_p$ extrapolated.

**A2.4.2.e. Thermal Gravimetric Analysis (TGA)**

Thermogravimetric analysis for decomposition temperatures ($T_d$) was performed on a Perkin-Elmer TGA7 analyzer with TAC7/DX controller equipped with a Dell OptiPlex GX110 computer running Perkin-Elmer Pyris software. Samples (~5 mg) were heated under dry nitrogen gas at a heating rate of 10 ºC/min from 25 ºC to 400 ºC. Decomposition temperatures were defined as the onset of decomposition.

**A1.4.2.f. Elemental Analysis**

Elemental analyses were performed by QTI (Whitehouse, NJ).

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# A3: APPENDIX 3: GLOSSARY[1,2]

## A3.1. Terms

**Analgesic** - an agent that alleviates pain without causing loss of consciousness.

**Antihelmintic** - an agent that is destructive to worms and used for removing internal parasitic worms in animals and humans.

**Anti-inflammatory** - ability to counteract or suppress the inflammatory process.

**Antimicrobial** - a drug for killing microorganisms or suppressing their multiplication or growth.

**Antioxidant** - one of many widely used synthetic or natural substances added to a product to prevent or delay its deterioration by action of oxygen in the air. Rubber, paints, vegetable oils and prepared foods commonly contain antioxidants.

**Antipyretic** - an agent that relieves or reduces fever.

**Antiseptic** - a drug that can be safely used externally on tissues to kill microorganisms or suppress their multiplication or growth.

**Bactericidal** - capable of killing bacteria.

**Biofilm** - A layered culture of microorganisms growing on a surface that they have created themselves by secreting polysaccharides and glycoproteins.

**Cytotoxicity -** toxicity to cells, preventing their reproduction or growth.

**Disinfectant** - a chemical agent used on inanimate objects to destroy microorganisms.

**Dorsal root ganglia** - a nodule on a dorsal root (i.e., sensory root of a spinal nerve) that contains cell bodies of neurons in afferent spinal nerves

**EDTA** - ethylenediaminetetraacetic acid; a chelating agent mainly used to sequester metal ions in aqueous solution.

*Escherichia coli* - rod shaped gram-negative bacteria abundant in the large intestine of mammals.

**Fibroblast** - resident cell of connective tissue, mesodermally derived, that secretes fibrillar procollagen, fibronectin and collagenase.

**Free radical scavenger** - substance that influences the course of a chemical reaction by ready combination with free radicals.

**Gram-positive** - bacteria that retain the stain or that are resistant to decolourisation by alcohol during Gram's method of staining. This effect is a primary characteristic of bacteria whose cell wall is composed of a thick layer of peptidologlycan containing teichoic and lipoteichoic acid complexed to the peptidoglycan.

**Gram-negative** - bacteria are considered to be gram-negative because of their characteristic staining properties under the microscope, where they either do not stain or are decolourised by alcohol during Gram's method of staining. This effect is a primary characteristic of bacteria that have a cell wall composed of a thin layer of peptidoglycan covered by an outer membrane of lipoprotein and lipopolysaccharide containing endotoxin.

**Hydrogel** - a network of polymer chains that are water-insoluble, sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are highly absorbent (they can contain over 99% water) natural or synthetic polymers. Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content.

**Keratolytic** - pertaining to therapies to remove warts and other lesions in which the epidermis produces excess skin. In this treatment, acidic medicines, such as salicylic

acid, is put on the lesion. Keratolytic therapy thins the skin on and around the lesion, causing the outer layer of the skin to loosen and shed.

**Non-steroidal Anti-inflammatory Drug (NSAID)** - anti-inflammatory agents that are not steroids with analgesic, antipyretic, and platelet-inhibitory actions. They are used primarily in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation.

**Preservative** - a natural or synthetic chemical that is added to products such as foods, pharmaceuticals, paints, biological samples, wood, etc. to prevent decomposition by microbial growth or by undesirable chemical changes.

*Pseudomonas aeruginosa* - species of gram-negative, aerobic, rod-shaped bacteria commonly isolated from clinical specimens (wound, burn, and urinary tract infections). It is also found widely distributed in soil and water. It is a major agent of nosocomial infection.

**Phytochemical** - chemical produced by a plant.

**Radiopacity** - the relative inability of electromagnetism to pass through a particular material, particularly X-rays. Dense materials that prevent the passage of electromagnetic radiation are called 'radiopaque'.

**Schwann cell** - specialized glial cell that wraps around vertebrate axons providing electrical insulation.

***Salmonella enterica*** **-** subgenus of *salmonella* containing several medically important serotypes. The habitat for the majority of strains is warm-blooded animals.

*Salmonella enterica* serovar typhimurium - a serotype of *salmonella enterica* that is a frequent agent of *salmonella* gastroenteritis in humans. It also causes paratyphoid fever.

***Staphylococcus aureus*** - potentially pathogenic bacteria found in nasal membranes, skin, hair follicles, and perineum of warm-blooded animals. They may cause a wide range of infections and intoxications.

***Staphylococcus epidermidis*** - A species of *staphylococcus* that is a spherical, non-motile, gram-positive, chemoorganotrophic, facultative anaerobe. Mainly found on the skin and mucous membrane of warm-blooded animals, it can be primary pathogen or secondary invader.

**Tissue engineering** - the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions.

**Young's modulus** - a measure of the stiffness of an isotropic elastic material. It is defined as the ratio of the uniaxial stress over the uniaxial strain in the range of stress in which Hooke's Law holds.

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