DESIGN, SYNTHESIS, AND FORMULATION OF BIOACTIVE-BASED

POLYMERS: CONTROLLED DELIVERY VIA BIODEGRADATION

by

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A Dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Graduate Program in Chemistry & Chemical Biology

written under the direction of

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New Brunswick, New Jersey

October, 2015

ABSTRACT OF THE DISSERTATION

Design, synthesis, and formulation of bioactive-based polymers: controlled

delivery via biodegradation

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Biodegradable, bioactive-based polymers have been successfully employed as sustained bioactive delivery systems. This dissertation describes bioactives that have been chemically incorporated into novel, biodegradable polymers via covalent bonds for controlled, sustained, and tunable release properties. Bioactives are released from polymers via hydrolytic degradation. The polymers described herein utilize alternative synthetic methods and a wide array of bioactives, including antibiotics, antioxidants, antimicrobials, and anti-inflammatory drugs. One goal is to focus on naturally-occurring bioactives that are generally regarded as safe (GRAS) by the FDA.

First, poly(anhydride-amides) comprised of ampicillin were synthesized and formulated as coatings. Polymer adhesion onto medical-grade stainless steel surfaces was assessed and *in vitro* release characterized. Cytocompatibility and antibacterial activity elucidated polymer safety and efficacy for potential *in*

vivo use. These localized delivery systems could mediate the issues caused by implant surgery.

Second, poly(anhydride-esters) comprised solely of naturally-occurring phenols and EDTA were synthesized and physicochemical properties determined. Bioactive release was ascertained, in addition to antioxidant activity and activity against Gram-positive and Gram-negative bacteria. These polymers can act as potential preservatives, increasing cosmetic and food product shelf life through antioxidant and antimicrobial pathways.

Third, through environmentally sustainable (i.e, green) methods, polyesters with pendant anti-inflammatory groups and a sugar-based backbone were prepared with minimal solvent use, enzymatic catalysis, and biorenewable reactants and reagents. Three comonomers of varying hydrophobicity were tested to elucidate changes in polymer thermal properties and bioactive release rate.

Fourth, poly(anhydride-esters) with a mannitol backbone and multiple bioactive groups per repeat unit were developed as the first linear, biodegradable polymers with high bioactive loading (~70%) using a polyol. *In vitro* ibuprofen release was quantified and an anti-inflammatory assay determined that bioactive retained activity upon polymer degradation. Alteration of polyol, bioactive class, and other facets leads to highly tunable polymer properties.

Last, to combat bacterial spoilage and oxidation, poly(anhydride-esters) containing natural antimicrobials were designed for food applications. Bioactive released from polymer exhibited radical scavenging ability and antibacterial

activity. Furthermore, polymers were blended with current food packaging materials (e.g., polyethylene) and molded into films for active food packaging that contains a higher percentage of biodegradable content.

PREFACE

'Oh, you can't help that,' said the cat. 'We're all mad here.'

- Lewis Carroll,
Alice's Adventures in Wonderland

DEDICATION

To Taylor

ACKNOWLEDGEMENTS

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ABBREVIATIONS

[M + 1]	Mass plus one
[M + Na]	Mass plus sodium
%	percent
°	degrees
°C	degrees Celcius
±	plus or minus
~	approximately
δ	chemical shift
λ	wavelength
μg	microgram
μL	microliter
^{1}H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon nuclear magnetic resonance
Abs_t	absorbance after a time period
Abs_{t0}	absorbance at time zero
ACN	acetonitrile
API-ESI	atmospheric pressure ionization-electrospray ion source
Ar	argon
Ar-H	aromatic proton
ASTM	American Society for Testing and Materials
br	broad
C=O	carbonyl
CCl_3CN	trichloroacetonitrile
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
cm^{-1}	wavenumber
COMU	(1-cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate
COX	cyclooxygenase
CPME	cyclopentylmethyl ether
d	doublet; day
Da	Dalton

DBU	1,8-diazabicyclo [5.4.0]undec-7-ene
DCM	dichloromethane
dd	doublet of doublets
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DI	deionized
DIPEA	diisopropylethylamine
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
DPPH	2,2-diphenyl-1,1-picrylhydrazyl
DSC	differential scanning calorimetry
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EDTA	ethylenediamine-tetraacetic acid
eq	equivalents
Et	ethyl
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
FDA	US Food and Drug Administration
FT-IR	Fourier transform infrared
g	gram
GPC	gel permeation chromatography
GRAS	generally recognized as safe
h	hour
H_2	hydrogen
HCl	hydrochloric acid
H_2O	water
HPLC	high performance liquid chromatography
J	coupling constant

KBr	potassium bromide	PBS	phosphate buffered saline
kg	kilogram		
KH_2PO_4	monobasic potassium phosphate	PDI	polydispersity index
		PLGA	poly(lactic-*co*-glycolic acid)
$KHSO_4$	potassium bisulfate		
L	liter	PPh_3	triphenylphosphine
LDPE	low density polyethylene	psi	pounds per square inch
		PTFE	polytetrafluoroethylene
m	multiplet; minute	PVDF	poly(vinylidine fluoride)
M	molar	RI	refractive index
Me	methyl	rt	room temperature
MeLi	methyl lithium	s	singlet; second
MeOH	methanol	SA	salicylic acid
MIC	minimum inhibitory concentration	$SOCl_2$	thionyl chloride
		t	triplet
mg	milligram	*t-*	tertiary
$MgSO_4$	magnesium sulfate	TDBMS	*tert*-butyl dimethylsilyl
MHz	megahertz	T_d	decomposition temperature
mL	milliliter		
mm	millimeter	TFA	trifluoroacetic acid
mM	millimolar	T_g	glass transition temperature
MS	mass spectrometry		
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-)3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium)	TGA	thermogravimetric analysis
		THF	tetrahydrofuran
		TLC	thin layer chromatography
		T_m	melting temperature
Mw	weight-averaged molecular weight	TMS	tetramethylsilane
		TMSEA	(trimethylsilyl) ethoxyacetylene
MW	molar mass		
N	normal	UV	ultraviolet
N_2	nitrogen	wt %	weight percent
N435	Novozym 435		
NaCl	sodium chloride		
$NaHCO_3$	sodium bicarbonate		
NaOH	sodium hydroxide		
NIH	National Institutes of Health		
nm	nanometer		
NSAID	nonsteriodal anti-inflammatory drug		
p-	para		
PAE	poly(anhydride-ester)		
Pd/C	palladium on carbon		

1. INTRODUCTION

1.1. Controlled Bioactive Delivery

Bioactive delivery systems have long been researched to provide a well-controlled and sustained release of bioactives by providing therapeutic bioactive doses over time, thereby decreasing the dosing required, reducing potential for toxicity, and improving patient compliance.[1] Additionally, advanced delivery systems can target specific delivery areas *in vivo* and respond to environmental stimuli to increase safety and improve overall efficacy.[2-5] Traditionally, bioactives are delivered via short-acting (< 24 hour) methods, including, but not limited to, oral tablets, injections, implants, and dermal applications. However, limited bioactive bioavailability, poor solubility, short half-lives, and rapid clearance from the body hamper the effectiveness of many small molecule bioactives.

1.2. Polymers for Bioactive Delivery

The use of polymers for bioactive delivery has offered a solution to many of the issues associated with traditional small molecule bioactives. The majority of systems utilizing polymers involve the physical incorporation of bioactives into polymeric matrices or the encapsulation of actives into carriers such as polymeric micelles.[3,6-11] Many polymeric systems are highly versatile because of the wide

range of potential formulations (e.g., microspheres, nanoparticles, coatings, films, fibers, hydrogels, etc.) that cannot be achieved with small molecules alone. Synthetic polymers such as poly(lactic-co-glycolic acid) (PLGA)[9,12] and poly(caprolactone) (PCL)[13] and natural polymers such as chitosan and alginate[14] have been widely used as delivery matrices. Depending on the composition, size, and formulation, bioactive release can be rate can be more well-controlled than with the free bioactives alone. As an example, microspheres, which have a much greater surface area than discs, exhibit a faster release rate. Additionally, the aforementioned polymers are all biodegradable polyesters; they degrade hydrolytically into biocompatible small molecules easily removed by the body, thereby minimizing adverse immune responses. In these examples, bioactive release is controlled by diffusion through the polymer matrix and polymer degradation, both of which are influenced by the polymer's erosion mechanism. These polyesters predominately undergo bulk erosion; water permeates the entirety of the polymer and causes degradation both on the outer surface and the inner core (**Figure 1.1a**). Here, diffusion of the bioactive occurs faster than polymer degradation.[15] Consequently, bioactive release is oftentimes poorly controlled as pores are formed throughout the system and structural integrity of the system is diminished. Conversely, polyanhydrides undergo predominately surface erosion, in which degradation occurs predominantly at the polymer surface, leaving the core untouched (**Figure 1.1b**). Here, polymer degradation occurs faster than diffusion of the bioactive.[16] As a result, a near-zero order drug release is often observed, leading to a more predicable release rate. One FDA-

approved example of a surface-eroding polymer system is the Gliadel wafer, containing chemotherapeutics physically incorporated within a polyanhydride matrix, used to treat malignant brain tumors.[17]
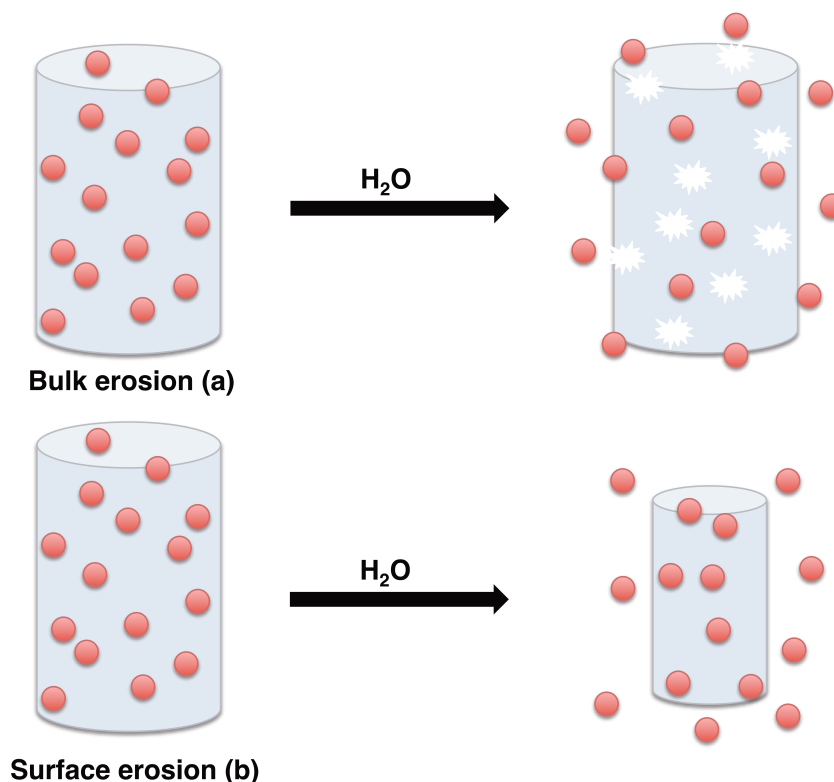
Figure 1.1: Release of physically entrapped bioactives (red circles) from biodegradable polymer matrices (gray cylinders) via bulk erosion (**a**) and surface erosion (**b**) mechanisms

Although physical incorporation of bioactives into polymers addresses some delivery limitations, these systems still suffer from their own drawbacks; namely, the amount of bioactive that can be loaded without destroying the structural integrity of the delivery system is often very low (~10 wt%). Also, these systems often exhibit a burst release in which the majority (>75%) of

encapsulated bioactive is released in the first few hours, owing to their diffusion-controlled release mechanisms and the presence of bioactive on polymer surface.[15]

1.3. Chemical Incorporation of Bioactives into Polymers

To overcome the disadvantages of physical incorporation of bioactives into polymeric matrices, researchers have covalently bonded bioactives to polymers. [18-24] Most commonly, polymer-bioactive conjugates of poly(ethylene glycol) (PEG)[25,26] and poly(N-2-hydroxypropyl)methacrylamide) (HPMA)[27,28] are researched for this purpose. Conjugation of PEG (i.e., PEGylation) to bioactives has been well-studied, as PEGylation increases water solubility of hydrophobic molecules and increases *in vivo* circulation time, thereby preventing rapid clearance. A wide range of PEGylated cancer drugs, peptides, and proteins have undergone clinical trials;[26] however, these conjugates suffer from low bioactive content (**Figure 1.2a**).[19] HPMA offers additional benefits, including high flexibility in modifying polymer properties such as active release rate and thermal properties; additionally, pendant side chains can contain cleavable bioactive groups, imaging agents, or targeting moieties.[18,19] As with PEG, a number of HPMA bioactive conjugates have undergone clinical trials.[27,29] Even with the aforementioned improvements, the non-biodegradable nature of HPMA (Figure 1.2**b**) limits their usage, as conjugates can cause issues with toxicity and immune response.
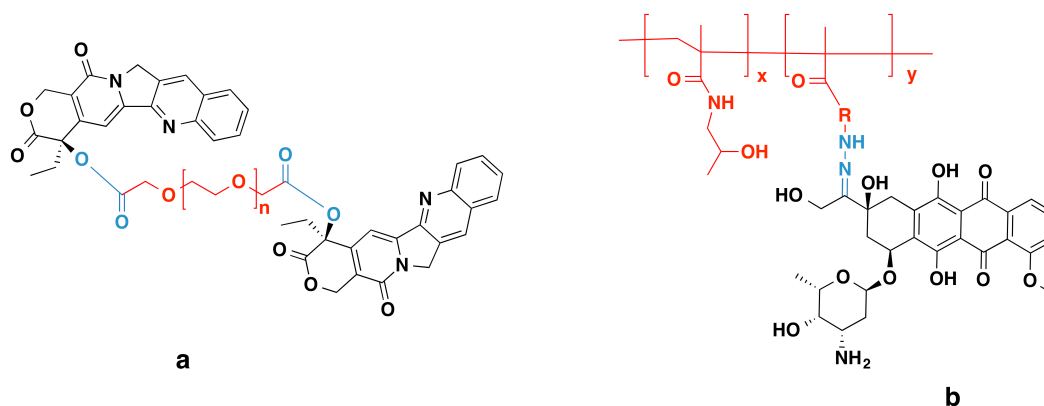
Figure 1.2: Structures of representative PEG-bioactive conjugate (**a**) and HPMA-bioactive conjugate (**b**). Biodegradable linkage to bioactive is shown in blue, whereas the non-biodegradable polymer backbone is indicated in red

1.4. Bioactive-containing Biodegradable Polymers

The low bioactive loading, non-biodegradability, and limited tunability associated with PEG conjugates and the non-biodegradability of HPMA and other polymers can be addressed by developing novel, completely biodegradable polymers with bioactives chemically incorporated into the system via covalent bonds. This method can lead to a more well-controlled and sustained bioactive release, as well as a much higher bioactive loading (>50%) and more tunable structures. As one of the first examples, Uhrich, *et al.* developed salicylic acid (SA)-based poly(anhydride-esters)[30] (PAEs) with highly tunable release profiles and thermal properties. The bioactive in this system is chemically incorporated into the polymer backbone through utilization of a linker molecule (**Figure 1.3**). The hydrophobicity of the linker influences thermal and release properties:[31] oxygen-containing linkers increase hydrophilicity and, consequently, all SA is

released via anhydride and ester bond hydrolysis in two days.[32] Conversely, a branched aliphatic linker increases polymer hydrophobicity and releases SA over months.[31] In the case of these PAEs, regardless of linker, bioactive release follows a near-zero order release, due to their predominantly surface-eroding properties. Beyond SA, a myriad of other bioactives with various functional groups have been chemically incorporated into polymers by Uhrich, *et al.*
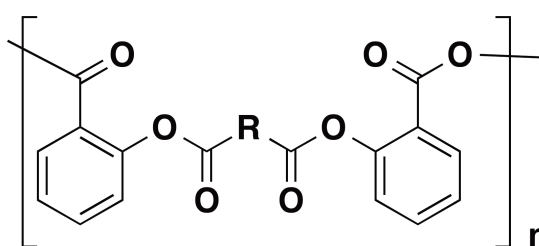
SA-based PAE

Figure 1.3: Structure of SA-based PAE with hydrolytically degradable anhydride and ester bonds. The linker molecule, which may be heteroatom-containing, linear, aliphatic, branched, or aromatic, is represented by **R**

Bioactives have been incorporated directly into polymer backbones, as with SA-based PAEs, and have also been incorporated as pendant groups[33,34] (**Figure 1.4**). These unique systems offer higher bioactive loading to increase efficacy and improved biodegradability; the polymers undergo hydrolysis of covalent bonds to release bioactive and non-toxic small molecules that may be eliminated by the body. Moreover, these polymers can also be formulated into multiple different geometries including microspheres for injectable delivery,

hydrogels for topical applications, films for packaging, surface coatings for implants, and fibers for engineering. This dissertation will focus on the development of novel polymer systems with bioactives chemically incorporated both as pendant groups and in the polymer backbone, and will additionally discuss their formulation for various applications.
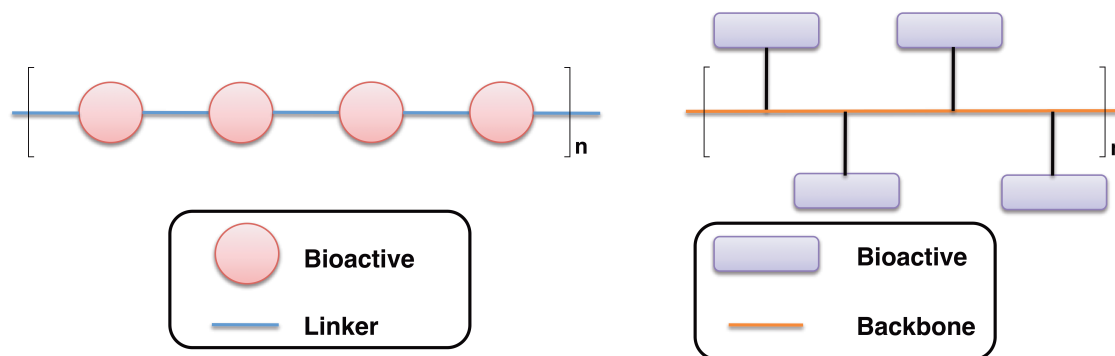
Figure 1.4: Two approaches to incorporate bioactives into a polymer: within a polymer backbone (left) or attached as a pendant group off a polymer backbone (right)

Polyanhydrides and polyesters comprise the vast majority of completely biodegradable bioactive-containing polymers and are the two classes that will be emphasized throughout this dissertation. A number of synthetic methods are traditionally used to synthesize these polymers. Firstly, polyanhydrides are often made through melt-condensation or solution methods. In melt condensation, acetylated diacid monomers are reacted under high temperature (i.e., above melting point) and vacuum (2 Torr) to polymerize.[35] This type of reaction can be easily performed on a wide range of scales, but is not appropriate for thermally sensitive monomers or polymers. Alternatively, solution polymerizations that

utilize phosgene-based reagents at ambient temperatures may be used.[36] Solution polymerization necessitates strict control over reaction stoichiometry and often leads to lower molecular weight polymers. Additionally, phosgene-based reagents may exhibit marked toxicity, and the reagents are known toxins. A less common, yet still effective coupling reagent (trimethylsilyl)ethoxyacetylene,[37] can also be used to make polyanhydrides and will be discussed at greater length in Chapters 5 and 6. Polyesters, which are often synthesized from hydroxyacids or combinations of diacids and diols, require different synthetic reagents including alkyl-inorganic catalysts such as those containing tin, titanium, or antimony.[38] Recently, lipase-catalyzed polyesterification has garnered increasing amounts of attention due to its biorenewable and environmentally friendly nature.[39-42] Using a lipase to develop polyesters for bioactive delivery will be further discussed in Chapter 4. Synthesis of both polyesters and polyanhydrides containing bioactives will be presented in this work.

1.5. Research Projects

Using previous research as inspiration, this dissertation focuses on new methods to chemically incorporate bioactives into biodegradable polymers. The applications of these polymers span from personal care and cosmetics, to biomedical and pharmaceutical, to food packaging and preservation. Antibiotic-containing poly(anhydride-amides) were synthesized, formulated into coatings for

biomedical implants, and tested for their antibacterial activity in Chapter 2. In Chapter 3, plant-derived phenols that exhibit antimicrobial and antioxidant activities and ethylenediaminetetraacetic acid (EDTA) were incorporated into PAEs, leading to a highly atom efficient system. Chapter 4 continues the theme of sustainability by utilizing a synthetic methodology motivated by the Principles of Green Chemistry[43] to make polyesters to released non-steroidal anti-inflammatory drugs (NSAIDs); solvent-free, catalytic, and less toxic reagents are used, including a biorenewable lipase. In Chapter 5, a new synthetic approach that yielded NSAID-containing PAEs from polyols was developed, lead to the first linear, completely biodegradable polymer containing sugar alcohols that has high drug loading (~70%). Finally in Chapter 6, the range of bioactives that could be used in polymers with a tartaric acid backbone was expanded. Resulting polymers were also blended with polyethylene to elucidate their potential as antimicrobial food wraps. A brief outline of each project is provided below.

1.5.1. Polymeric Prodrugs of Ampicillin as Antibacterial Coatings

Ampicillin, a widely-used beta-lactam antibiotic, was converted into a novel prodrug and subsequently converted into a poly(anhydride-amide) via solution polymerization. This polymer, which chemically incorporates the ampicillin prodrug into the polymeric backbone, was designed as a stainless steel coating to prevent infections associated with implantation of medical devices through controlled, localized release of antibiotics. The *in vitro* hydrolytic

degradation of the polymer into the ampicillin prodrug was measured and the antibacterial activity of polymer-derived coatings was examined using a Gram-positive bacterium, *Staphylococcus aureus* (**Figure 1.5**). The ampicillin prodrug demonstrated antibacterial activity and the polymer demonstrated no cytotoxic effects on fibroblasts. Based upon these results, the biodegradation of the antibiotic-based poly(anhydride-amide) into the prodrug displays substantial promise as an implant coating to reduce device failure resulting from bacterial infections.

Formulate into coating

PolyAmpicillin-coated stainless steel

Antibacterial activity
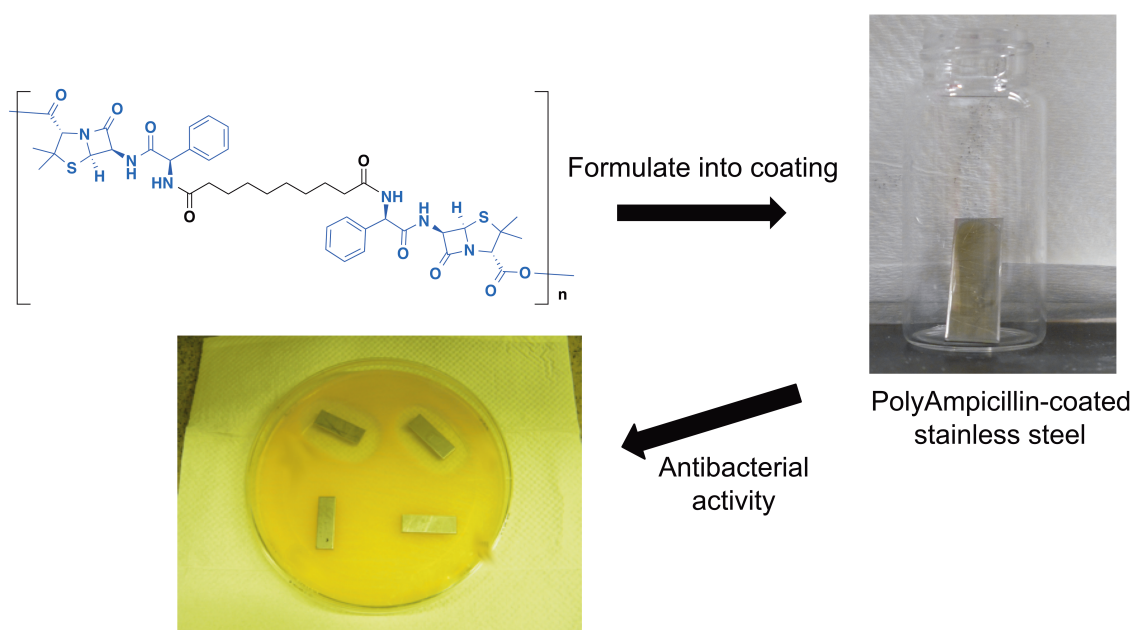
Figure 1.5: Formulation of ampicillin-based polymers onto a stainless steel coating and subsequent determination of antibacterial activity

1.5.2. Poly(anhydride-esters) Comprised Exclusively of Naturally Occurring Antimicrobials and EDTA: Antioxidant and Antimicrobial Activities

Carvacrol, thymol, and eugenol are naturally-occurring phenolic compounds known to possess antioxidant activity as well as antimicrobial activity against a range of bacteria. Biodegradable poly(anhydride-esters) comprised of an ethylenediaminetetraacetic acid (EDTA) backbone and antimicrobial pendant groups (i.e., carvacrol, thymol, or eugenol) were synthesized via solution polymerization. The resulting polymers were characterized to confirm their chemical composition and determine their thermal properties and molecular weight. *In vitro* release studies demonstrated that polymer hydrolytic degradation resulted in release of free antimicrobials and EDTA (**Figure 1.6**).[44] Antioxidant and antibacterial assays determined that polymer release media exhibited bioactivity similar to that of free compound, demonstrating that polymer incorporation and subsequent release had no effect on bioactivity. These polymers completely degrade into components that are biologically relevant and have the capability to promote preservation of consumer products in the food, personal care, and cosmetic industries via antimicrobial and antioxidant pathways.

R_1 = iPr, R_2 = H, R_3 = Me		Thymol
R_1 = Me, R_2 = H, R_3 = iPr		Carvacrol
R_1 = OMe, R_2 = CH$_2$CH=CH$_2$, R_3 = H		Eugenol
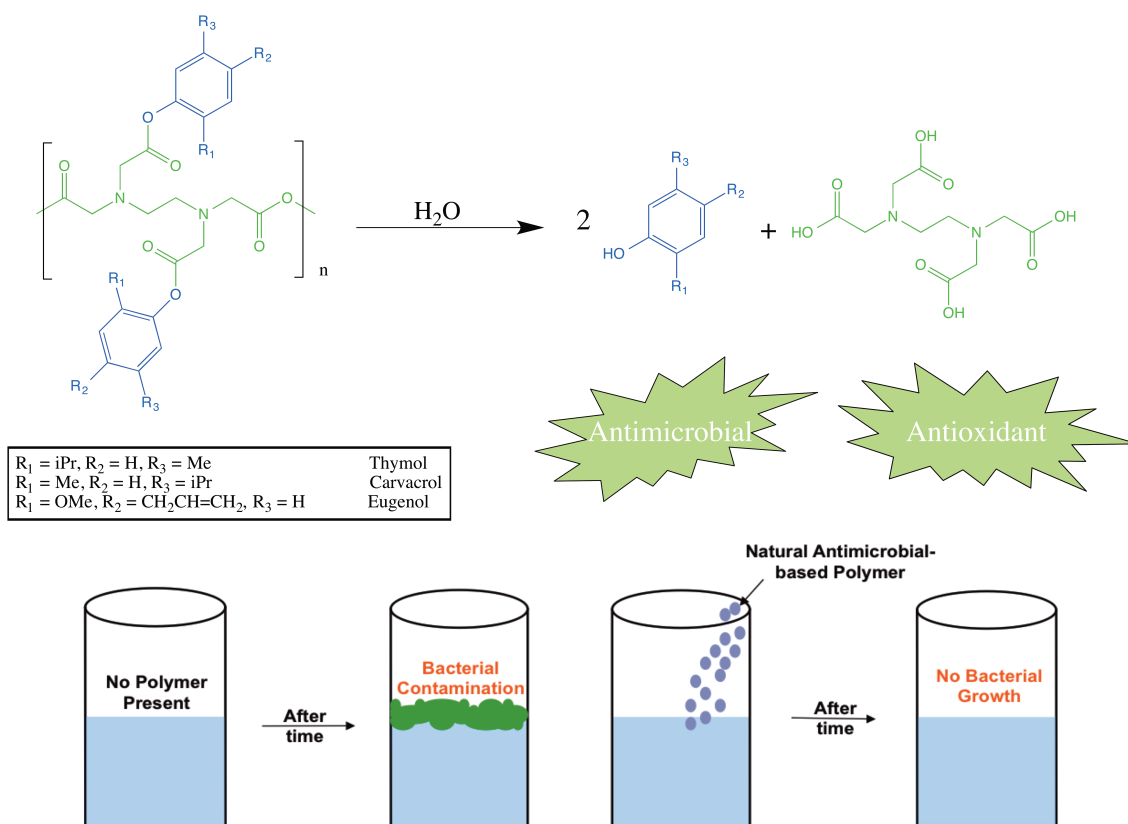
Figure 1.6: Hydrolytic degradation of PAEs containing naturally-occurring antimicrobials and representation of antimicrobial properties

1.5.3. Enzymatic Polymerization of an Ibuprofen-containing Monomer and Subsequent Drug Release

Novel ibuprofen-containing monomers comprised of naturally occurring and biocompatible compounds were synthesized and subsequently polymerized via enzymatic methods to form poly(alkylibuprofen malates). Through the use of a malic acid sugar backbone, ibuprofen was attached as a pendant group, and then subsequently polymerized with a linear aliphatic diol (1,3-propanediol, 1,5-

pentanediol or 1,8-octanediol) as a comonomer using lipase B from *Candida antarctica,* a safer, bio-derived alternative to traditional metal catalysts. Polymer composition was confirmed by nuclear magnetic resonance and infrared spectroscopies. All polymers exhibited sustained ibuprofen release, with the longer chain containing more hydrophobic diols exhibiting the slowest release over the 30-day study. Degradation products were either bioactive or biorenewable (**Figure 1.7**). Polymers were deemed cytocompatible using mouse fibroblast cells, when evaluated at relevant therapeutic concentrations. Additionally, ibuprofen retained its chemical integrity throughout the polymerization and *in vitro* hydrolytic degradation processes. This methodology of enzymatic polymerization of a drug presents a more environmentally friendly synthesis and a novel approach to bioactive polymer conjugates.
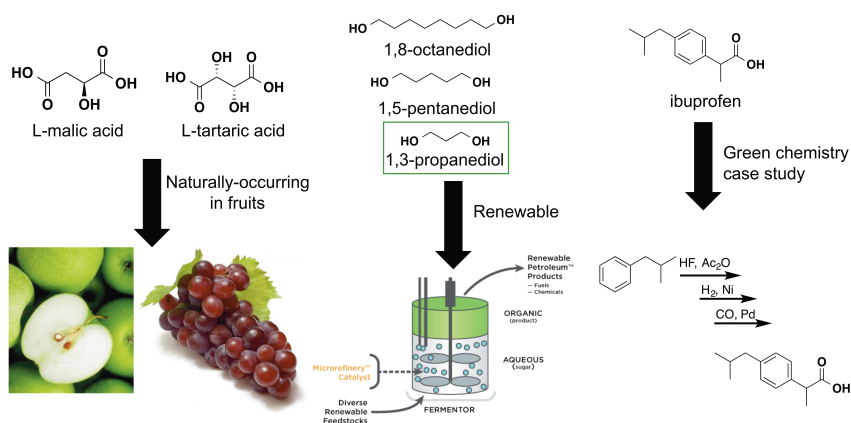
Figure 1.7: Representation showing the renewable and green nature of poly(alkylibuprofen malate) polyester degradation products

1.5.4. Linear, Mannitol-based Poly(anhydride-esters) with High Tunability: Biodegradability with Sustained Anti-inflammatory Activity

Sugar alcohols, such as mannitol and xylitol, are polyols on the FDA GRAS list that have been used to make highly cross-linked polyester elastomers and dendrimers for tissue engineering and drug delivery, respectively (**Figure 1.8a**). However, little research has been performed on utilizing the secondary hydroxyl groups as sites for pendant bioactive attachment and subsequent polymerization. This work is the first report of a linear, completely biodegradable polymer with a sugar alcohol backbone and chemically incorporated pendant bioactives that exhibits sustained bioactive release and high loading (~70%). With four pendant esters per repeat unit, this poly(anhydride-ester) has high drug loading and biodegrades into three products: bioactive, sugar alcohol, and biocompatible diacid. Ibuprofen served as the model bioactive whereas mannitol was used as a representative polyol (**Figure 1.8b**). Polymerization was achieved through reaction with (trimethylsilyl)ethoxyacetylene. Drug release via polymer degradation was quantified by high performance liquid chromatography. Additionally, a cytocompatability study with fibroblast cells was performed to elucidate the polymer's suitability for *in vivo* use and a cyclooxygenase-2 (COX-2) assay was performed on the degradation media to ensure that released ibuprofen retained its anti-inflammatory activity. The synthetic methods used are easily adaptable to desired polymer properties: altering sugar alcohols can

change drug loading, release, and thermal properties, chain extenders of varying hydrophobicity can tune release rate, and different bioactive classes as pendant groups can be investigated in the future.
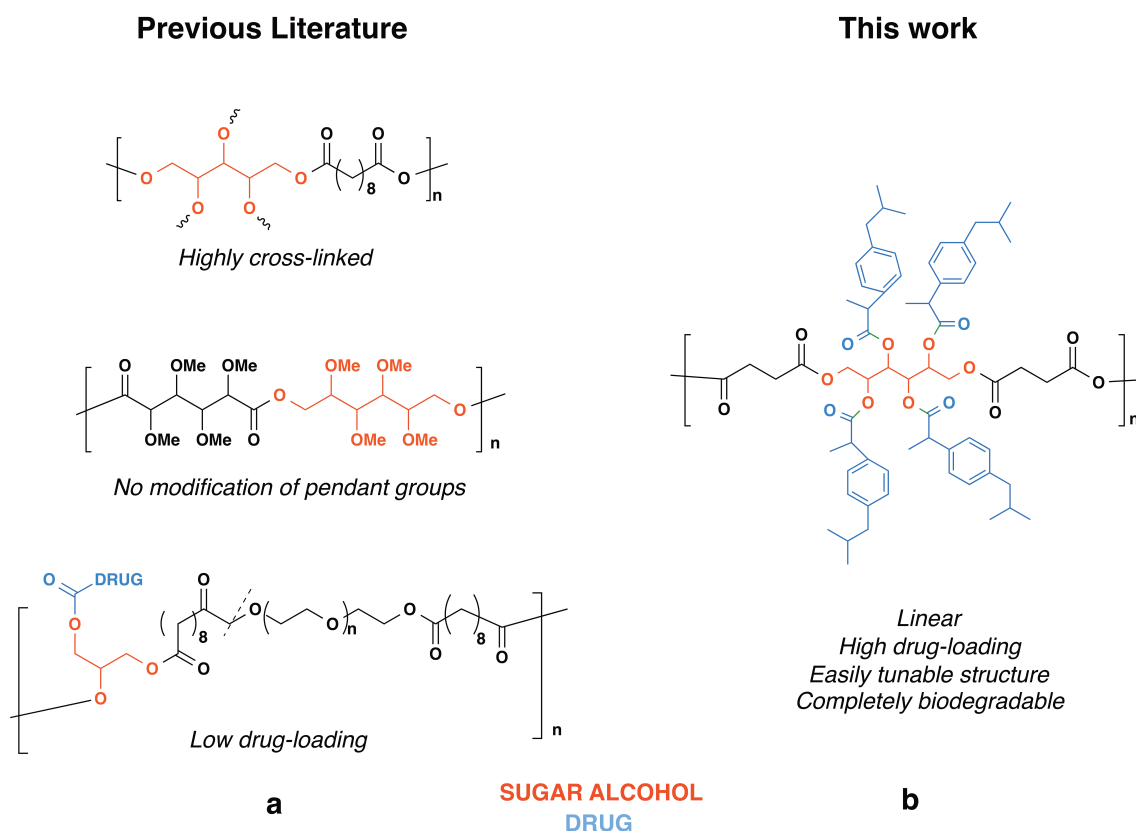
Figure 1.8: Previously developed sugar alcohol-containing polymers **(a)** and novel mannitol-based PAEs **(b)**

1.5.5. Sugar-based PAEs Containing Natural Antioxidants and Antimicrobials: Synthesis and Formulation into Polymer Blends

Poly(anhydride-esters) with a tartaric acid sugar backbone and pendant thymol groups have been developed as a novel system to release antimicrobial and antioxidant compounds. These tunable PAEs are investigated as potential food packaging wraps, as all degradation products are naturally occur in many food products. Polymers, acquired through reaction with (trimethylsilyl)ethoxyacetylene, were fully characterized to confirm structure and determine thermal properties. Release studies on raw polymer were performed to determine thymol release rate. In addition, miscible polymer blends were formed, melt blended with polyethylene, the most widely used food-packaging polymer, and compression-molded into films (**Figure 1.9**). Thymol released from both polymer alone and blended polymer films exhibited the same activity (i.e., antioxidant and antimicrobial) as free thymol; neither polymerization nor processing negatively impacted bioactivity. Thymol release from polymer blend under real-world conditions and mechanical testing will elucidate possibility for active packaging applications.
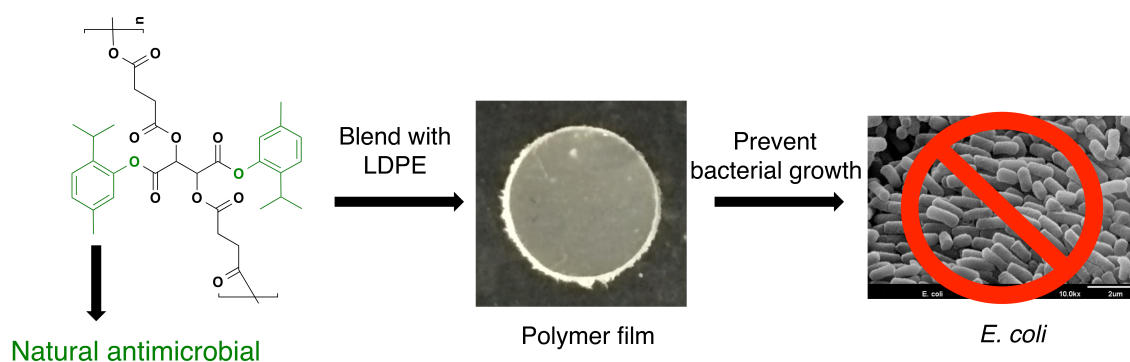
Figure 1.9: Structure and formulation of thymol tartrate-based PAEs of into blended films to prevent bacterial growth on food products

1.6. Summary

A wide range of fully biodegradable polymers containing bioactives were developed, all exhibiting a sustained bioactive release and high (>50%) drug loading. Such polymers can be used in industries ranging from cosmetic to food preservation to biomedical. New synthetic methods expand the types and properties of polymers used. Furthermore, the use of lipase and (trimethylsilyl)ethoxyacetylene in Chapters 3-5 broaden the range of polymerization methods and open the door for the successful polymerization of more sensitive (i.e., thermally unstable, acid labile) monomers. This dissertation describes the design, synthesis, characterization, formulation, and degradation of novel polymers containing bioactives such as antioxidants, antimicrobials, and anti-inflammatories for various applications.

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2. POLYMERIC PRODRUGS OF AMPICILLIN AS ANTIBACTERIAL COATINGS

2.1. Introduction

As numerous metal prostheses are implanted each year in humans, the bacterial infections associated with these medical implants remain a significant issue.[1-5] The presence of a foreign body such as a prosthetic implant provides a site for microbial adhesion and subsequent colonization, often leading to device-related infections.[5-9] For example, nearly half of the two million nosocomial infections occurring in the United States are associated with medical implants.[10] Unfortunately, common therapeutic approaches to prevent biomaterial-related infections are often ineffective, and device removal is required to ensure eradication of the infection and avoid relapse.[4,6-11] As a result, new delivery methods are needed to improve the current state of medical device-mediated bacterial infection treatment.

Ampicillin, a ß-lactam antibiotic widely used to treat infections, has shown efficacy against both Gram-positive and Gram-negative bacteria.[14] Despite this efficacy, numerous bacterial strains have developed resistance to ampicillin,

necessitating the development of new analogs or antibiotics.[15,16] Researchers have utilized several different approaches to synthesize ampicillin analogs; however, little is known about the proccessability of these analogs[17,18] Alternatively, novel biocompatible polymer systems containing antibiotics have been investigated to address device-related infections. In these systems antibacterial agents are impregnated into the polymeric matrix to achieve localized release of the antibiotic, rather than systemic delivery.[5,12,13-25] This physical admixture would reduce the total amount of drug administered, while maximizing the drug's toxicological effects against bacteria at the implant site. While such physical admixtures are easily formulated, they suffer from low drug-loading amounts and efficiencies.[26,27] In other cases where antimicrobial agents were covalently attached to polymeric surfaces, the bioactives were chemically incorporated as pendant groups along biocompatible, but not biodegradable, polymer backbones.[28-32]

To overcome the limitations associated with physical admixture and pendant attachment methods, and to better control the release characteristics of antimicrobial-eluting polymer systems, methods to chemically incorporate bioactives *within* the polymer backbone are being investigated.[14,15] In this study a novel, degradable biomaterial was designed to release antimicrobials upon polymer degradation and thus locally prevent infections during the first few hours following surgery, when the implant is most susceptible to bacterial colonization.[37] The synthesis and physicochemical characterization of an ampicillin analog or prodrug, as well as chemical incorporation into a

polyanhydride backbone, is described. The poly(anhydride-amide) is comprised of 81% ampicillin by weight. The utility of the polymer as a robust coating was demonstrated by solvent-casting the ampicillin-based polymer onto stainless steel substrates.   Stainless steel was chosen, as it has a long history as a metallic material for medical implants in bone, cardiovascular, urologic, and dental applications.[38] In addition, the antibacterial properties of polymer-coated stainless steel substrates were measured against *Staphylococcus aureus* (*S. aureus*), a Gram-positive bacterial strain, that is responsible for infections associated with dental and orthopedic implants and fixation devices.[3,39,40]

2.2. Results and Discussion

2.2.1. Synthesis and Physicochemical Characterization

Ampicillin analog (**3**) was synthesized by direct coupling of the ampicillin (**1**) with sebacoyl chloride (**2**) in DMF containing pyridine (**Scheme 2.1**). In this conversion, pyridine acts as acid acceptor by neutralizing the HCl by-product. At low reaction temperatures, the primary amino group of the antibiotic reacts with the acyl chlorides of sebacoyl chloride to form **3** in approximately 80% yield. The ^{1}H and ^{13}C NMR spectra indicate that pure product is obtained.

With the thermal instability of **1** and **3**, the polymer could not be generated *via* melt-condensation conditions typically used to synthesize polyanhydrides.[45-47] Therefore, the ampicillin prodrug was polymerized under mild conditions in DCM

at 0 °C using triphosgene (1.1 eq) as a coupling agent and triethylamine as acid acceptor to form **4** in nearly quantitative yields. This polymer is unique in that it is able to achieve 100% drug loading.

The chemical incorporation of **3** into a polymeric backbone yielded a polymer (**4**) with a M_w of 30 kDa. This M_w is typical for solution polymerization methods.[16,17] Polymer (**4**) displayed a T_g of 120 °C and a T_d of 235 °C. The prodrug-containing polymer (**4**) was more soluble in organic solvents such as methanol, dimethyl sulfoxide, and DMF (solubility >80 mg/mL) than free ampicillin (solubility 400 μg/mL); this characteristic may be particularly significant for processing and formulations.
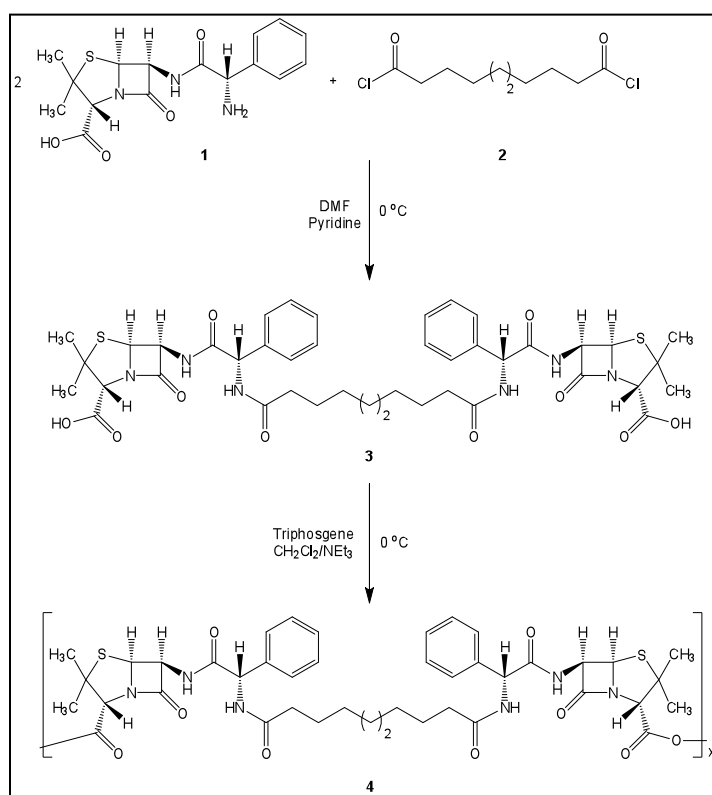
Scheme 2.1: Synthesis of ampicillin analog (**3**) from ampicillin (**1**) and sebacoyl chloride (**2**), and subsequent polymerization into poly(anhydride-amide) (**4**)

2.2.2. Coating Characterization and Adhesion Testing

Smooth, uniform polymer coatings containing an average of 12 mg of polymer (**4**) were solvent-cast onto 316L stainless steel. To produce coatings with maximum mechanical stability, the effects of solvent evaporation temperature and pressure on coating homogeneity and adhesion strength were investigated. Coatings dried under ambient conditions (25 °C and atmospheric pressure) were susceptible to significant cracking, whereas heating (to 70 °C) under reduced pressure reproducibly produced the most visually uniform coatings.

The adhesion strength of the polymer coatings on stainless steel was assessed by an ASTM 3359 X-cut peel test.[41] Coatings formed at 25 °C for 24 h were non-homogeneous and of insufficient stability to perform peel tests. Using the peel test results, coatings formed under elevated temperature (70 °C) and atmospheric pressure were ranked as 3A, whereas coatings formed under elevated temperature and reduced pressure were ranked as 4A. Although the peel test showed minimal removal of material along the x-cut incisions, the remaining areas of the coatings showed strong adhesion to the stainless steel substrates. Interestingly, the coatings strongly adhered to the substrates without the need for substrate-surface modification prior to the coating procedure. These findings highlight the significant potential of the ampicillin-based polymer as an antibacterial coating for implantable, metallic medical devices.

2.2.3. *In Vitro* Bioactive Release Studies

Hydrolytic degradation of **4** was measured on polymer-coated stainless steel coupons immersed in PBS at 37 °C for 6 h. In this experiment, **3** was detected in the degradation media (R_T = 2.61 min); the free antibiotic (**1**) (R_T = 12.19 min) was not detected. After 3 h of *in vitro* incubation, the anhydride linkages of **4** are completely hydrolyzed to yield the prodrug (**3**) (**Figure 2.1**). Because anhydride bonds are very labile to hydrolysis, the analog was released from the polymer in a matter of hours. No amide cleavage to release **1** was observed *in vitro*; this result was expected because hydrolysis of amides typically requires amidases or extreme pH conditions.[42]
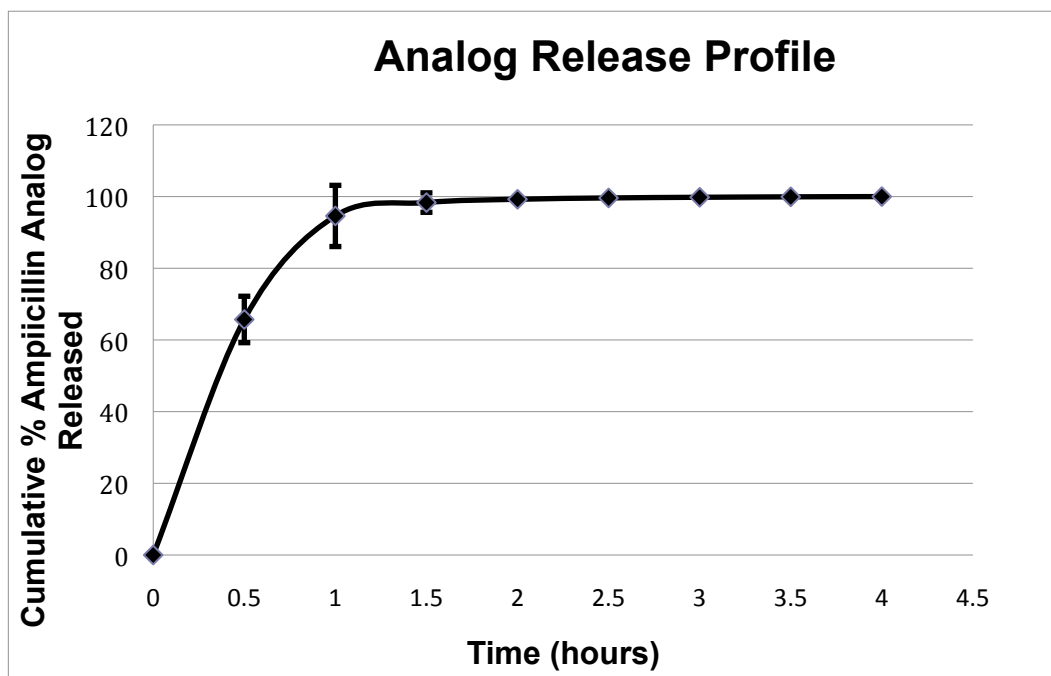
Figure 2.1: *In vitro* release profile of ampicillin analog (**3**) from polymer (**4**) in PBS at pH 7.4 and 37 °C.

2.2.4. Antibacterial Activity

To determine the antibacterial efficacy of ampicillin analog **3** and polymer **4**, the Kirby-Bauer method was used to predict their efficacies *in vivo*.[48] Coatings of **3** and **4** of stainless steel coupons diffused into the agar plate over 24 h, both creating nearly identical zones of inhibition (**Figure 2.2**).
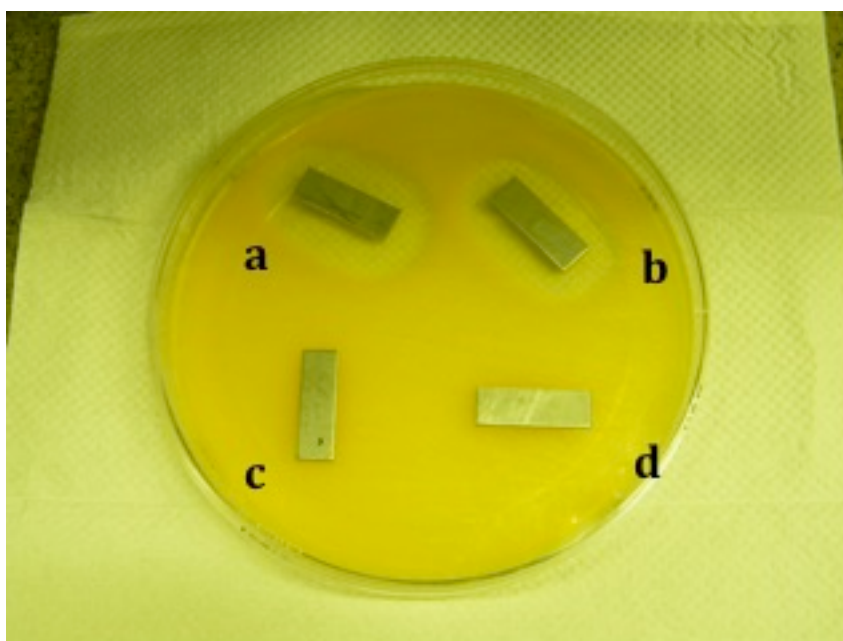
Figure 2.2: Agar disc inoculated with *S. aureus* displaying results of Kirby-Bauer test for analog **3** (**a**), polymer **4** (**b**), DMF-treated coupon (**c**), and bare coupon (**d**). Zones of inhibition surrounding **a** and **b** elucidate clear antibacterial activity

The analog is a new entity that displays antibacterial activity against *S. aureus*, displaying a zone of inhibition of 19 mm. Additionally, the polymer displays a nearly identical zone of inhibition (18 mm). The results of the *in vitro*

degradation study suggest that hydrolysis of the polymer into analog is rapid enough that analog **3** will be completely released throughout the 24 h incubation period. Control coupons (untreated and treated with DMF) displayed no zones of inhibition.

2.2.5. Cytocompatibility Assay

[Study performed by MinJung Song, Department of Biomedical Engineering, Rutgers University]

*The cytotocopatibility of polymer (**4**) was evaluated in vitro with L929 fibroblasts, the most predominant tissue cells in the body and the standard cells recommended by the American Society for Testing and Materials (ASTM)[41] in testing materials. For this study, polymer solutions of 0.01 mg/mL and 0.10 mg/mL were prepared in DMSO/media to evaluate fibroblast morphology and proliferation. Solutions containing media and DMSO at the same concentrations used to prepare the polymer solutions were used as controls. Fibroblast proliferation in antimicrobial-based polymer solutions at the two different concentrations (0.01 and 0.10 mg/mL) was analyzed over a 3-day period with results shown in **Figure 2.3**. Based on the in vitro degradation experiments described previously, the 3-day cell cultivation time was sufficient to guarantee cell contact with the polymers and to allow complete cell cycles. Cells exhibited positive growth cycles in all the polymer solutions at both concentrations and no*

*statistical significance was observed for all polymers with respect to the controls (Student's t-test, 95%). In general, the polymer (**4**) showed good results at both concentrations with respect to cell proliferation/cytotoxicity. Because the polymer was fully degraded by the end of the 3-day study, these tests show that the degradation product, the analog, is also non-cytotoxic.*
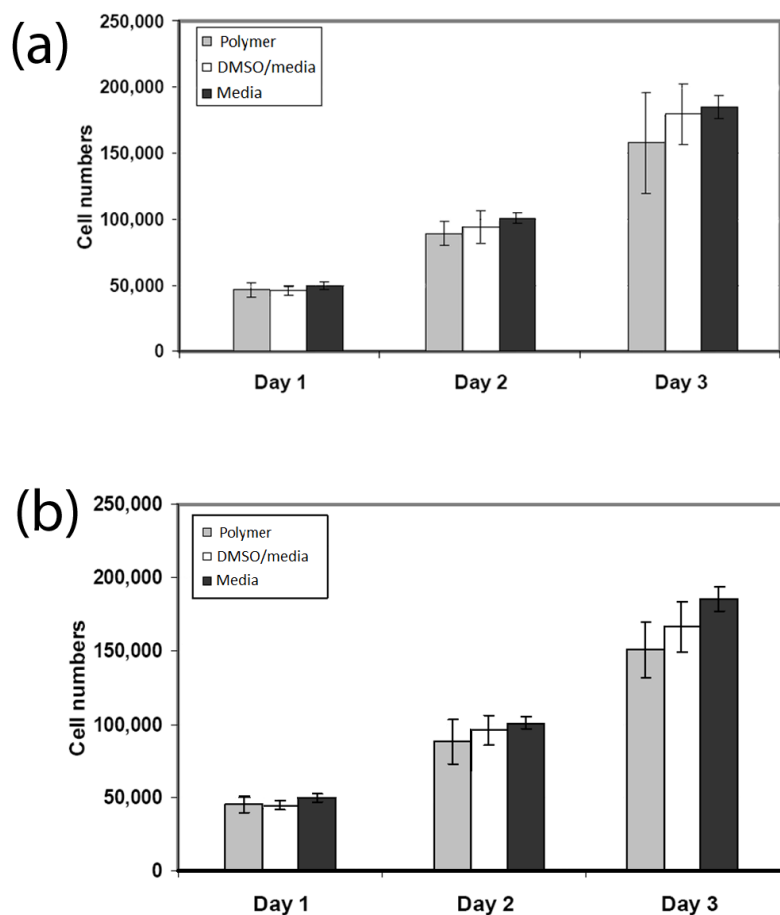
***Figure 2.3**: Proliferation of L929 fibroblasts in (a) 0.01 mg/mL and (b) 0.1 mg/mL antimicrobial-based poly(anhydride-amide) (**4**) solutions at days 1, 2, and 3*

2.3. Conclusion

This study describes the synthesis of an ampicillin prodrug, a new drug entity with anti-biofilm properties. A biodegradable poly(anhydride-amide) with 100% drug loading was synthesized from the ampicillin analog. The polymer's high glass transition temperature (120°C) as well as its improved solubility in organic solvents relative to ampicillin are significant, as they demonstrate excellent processibility of the polymer (**4**) into solvent-cast coatings onto medical devices, as well as potential for formulation into microspheres and fibers. Robust polymer coatings formed on stainless steel coupons were able to withstand standard peel tests, indicating strong adhesion to the underlying substrates and highlighting the polymer's significant potential as an antimicrobial coating material for medical implants. *In vitro* hydrolytic degradation and antimicrobial susceptibility assays of these materials showed that antimicrobial compounds were rapidly released from the polymer and these compounds inhibited the biofilm growth of *S. aureus*, a bacterial strain responsible for a wide range of infection-induced medical device failures.   The ampicillin prodrug and biodegradable polymer show promise as biomaterials that may reduce implant-associated infections due to their inherent antimicrobial properties.

2.4. Experimental

2.4.1. Materials

1 N hydrochloric acid and 1 N sodium hydroxide were obtained from Fisher Scientific (Fair Lawn, NJ). All other chemicals were purchased from Aldrich (Milwaukee, WI) and used as received.

2.4.2. Polymer and Precursor Synthesis

[Synthesis initially performed by Almudena Prudencio, Department of Chemistry and Chemical Biology, Rutgers University]

2.4.2.1. Diacid Synthesis

Ampicillin (**1**) (1 eq) was added to DMF (10 mL/mmol) and pyridine (5.0 eq). Sebacoyl chloride (**2**) (1.1 eq) dissolved in DMF (10 mL) was added dropwise over 5 min to the stirring reaction mixture at 0 °C to give a clear solution. The reaction was stirred for 2 h at 0 °C, poured over distilled water (400 mL) and acidified to pH~2 using 1 N hydrochloric acid while stirring. The white solid that formed was isolated by vacuum filtration, washed with distilled water (3×) and dried overnight under vacuum at room temperature to yield ampicillin analog (**3**).

Yield: 82% (white powder). ^{1}H NMR (DMSO-d_6): δ 9.10 (d, 2H, NH), 8.50 (d, 2H, NH), 7.40 (m, 10H, ArH), 5.75 (d, 2H, CH), 5.55 (m, 2H, CH), 5.40 (d, 2H, CH), 4.20 (s, 2H, CH), 2.25 (t, 4H, CH_2), 1.65-1.35 (m, 12H, CH_2), 1.20 (s, 12H, CH_3). ^{13}C-NMR (DMSO-d_6): d 173.7, 172.3, 170.5, 169.1, 138.6, 128.4, 127.8, 127.4,

70.6, 67.5, 64.0, 58.5, 55.7, 35.2, 30.7, 29.0, 26.9, 25.6. IR (NaCl, cm^{-1}): 3500-3100 (OH, acid), 1785 (C=O, acid), 1730 (C=O, amide I), 1645 (C=O, amide I), 1645 (C=O, amide I), 1535 (NH, amide II), 1510 (NH, amide II). Anal. Calcd.: C, 58.3%; H, 6.1%; N, 9.7%; S, 7.4%. Found: C, 57.7%; H, 6.3%; N, 9.5%; S, 7.4%. T_d = 200 ºC.

2.4.2.2.   Polymer Synthesis

Ampicillin analog **3** (1 eq) was dissolved 20% (w/v) dichloromethane (DCM) and triethylamine (4.4 eq). Triphosgene (0.33 eq) dissolved in DCM (15 mL) was added dropwise over 15 min to the stirring reaction mixture at 0 °C to form a suspension. The reaction was stirred for 2 h at 0 °C under nitrogen and poured over diethyl ether (400 mL). The solid that formed was isolated by vacuum filtration, washed with acidic water at pH~2 (3×) and dried overnight under vacuum at room temperature to yield polymer (**4**).

 Yield: quantitative (yellow powder). ^{1}H NMR (DMSO-d_6): δ 9.10 (d, 2H, NH), 8.45 (d, 2H, NH), 7.40 (m, 10H, ArH), 5.75 (d, 2H, CH), 5.55 (m, 2H, CH), 5.35 (d, 2H, CH), 4.20 (s, 2H, CH), 2.20 (t, 4H, CH$_2$), 1.60-1.30 (m, 12H, CH$_2$), 1.15 (s, 12H, CH$_3$). IR (NaCl, cm^{-1}): 1795, 1760 (C=O, anhydride), 1730 (C=O, amide I), 1650 (C=O, amide I), 1605 (C=O, amide I), 1540 (NH, amide II), 1515 (NH, amide II). M_w = 30 kDa, PDI = 1.2. T_g = 120 °C, T_d = 235 ºC. Contact angle: 45º.

2.4.3. Polymer Coating Preparation and Adhesion Testing

Prior to use, 316L stainless steel coupons (McMaster-Carr, cut to 10 × 30 mm by Rutgers Physics Department Machine Shop) were sonicated in 1) HPLC grade H_2O, 2) tetrahydrofuran, and 1:1:1 methanol:tetrahydrofuran:dichloromethane, all for 30 min each. Polymer **4** (12 mg) was dissolved in DMF (5% w/v). The polymer solution (150 µL) was then pipetted onto each coupon. The solvent was evaporated using one of three methods: i) 25 °C for 24 h; ii) heating to 70 °C under atmospheric pressure for 2 h; and iii) heating to 70 °C under vacuum for 2 h. Upon cooling, the coating thickness was 0.03 mm, as determined by a digital caliper.

Peel tests were conducted using the X-cut method in American Standard Test Method (ASTM) D 3359-02 (n=5).[41] Two 10-mm X-cuts were scratched into coated coupons using a fresh razor blade. Permacel P-99 tape was adhered to the surface and peeled away at a 180° angle after 90 seconds. Each sample was examined for coating removal and ranked according to the following scale: 5A-no peeling or removal; 4A-trace peeling or removal; 3A-jagged removal along incisions; 2A-jagged removal along most of the incisions; 1A-removal from most of the area of the X under the tape; and 0A-removal beyond the area of the X.

2.4.4. Gel Permeation Chromatography

Weight-averaged molecular weights (M_w) and polydispersity indices (PDI) were determined by gel permeation chromatography (GPC) on a Waters liquid chromatography system consisting of a Waters 410 refractive index detector, a Waters 510 LC pump, and an ISS 200 advanced sample processor. An IBM Thinkcenter computer running Waters Empower software was used for data collection and processing. Polymers were dissolved (10mg/mL) in dimethylformamide (DMF) containing 0.1% trifluoroacetic acid (TFA) and filtered through 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe filters (Whatman, Clifton, NJ) before elution. Samples were resolved on two mixed-bed GPC columns (Polymer Laboratories, Amherst, MA) (Alltech Associates, Deerfield, IL) placed in series at 25 °C, with DMF containing 0.1% TFA as eluent at a flow rate of 0.8 mL/min. Molecular weights were calibrated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

2.4.5. *In Vitro* Bioactive Release Studies

High performance liquid chromatography (HPLC) was performed on a Waters system consisting of a 2695 Separations Module with a 2487 dual λ absorbance detector. Empower software was used for data collection and processing. Samples were filtered (PTFE 0.2μm pore size) and resolved at 25ºC

on an Xterra reversed phase RP18 5 μm column (4.6 x 150 mm) with an eluent of 20 mM KH_2PO_4 (pH 4) and methanol with a gradient flow at a flow rate of 1.0 ml/min. For the first five minutes of the run, 100% buffer was used as the mobile phase, then over the next 15 min, the mobile phase was changed linearly to 50% buffer, 50% methanol.

The polymer-coated coupons prepared by heating under vacuum to 70 °C for 2 h were used for degradation studies. Degradation media consisted of phosphate-buffered saline (PBS) (Aldrich, Milwaukee, WI). The pH was adjusted to 7.4 with 1 N sodium hydroxide and/or 1 N hydrochloric acid solutions, and pH-measurements were performed on an Accumet® AR15 pH-meter (Fisher, Fair Lawn, NJ). Hydrolytic degradation of polymer **4** was performed by placing the coated stainless steel coupons in 20 mL Wheaton glass scintillation vials (Fisher, Fair Lawn, NJ) with 10 mL of PBS. These were incubated at 37 ºC with agitation using a controlled environment incubator-shaker (New Brunswick Scientific Co., Edison, NJ) at 60 rpm for 6 h. The degradation media (10 mL) was removed and replaced by fresh solution (10 mL) every 0.5 h. The spent media was analyzed by HPLC with UV detection at λ = 209 nm. Data were calibrated against standard solutions of known concentrations of **1** and **3**. Data (average of 3 samples per time point) were presented as a cumulative percent over time.

2.4.6. Antibacterial Activity

[Study performed with assistance of Dr. Susan Skelly, Division of Life Sciences, Rutgers University]

Staphylococcus aureus, a Gram-positive bacteria, is responsible for infections associated with dental and orthopedic implants and fixation devices[3, 39, 40] and is, therefore, a significantly relevant bacterial species against which the efficacy of the polymer system can be determined. Muller-Hinton agar medium (Becton Dickinson, Sparks, MD) was suspended in deionized water and boiled while stirring to dissolve. Solution was autoclaved (Amsco Scientific Series 3021-S, Apex, NC) at 121°C for 20 min then poured into sterile petri dishes (Fisher, Fair Lawn, NJ) to an even thickness of 4mm. Plates were then refrigerated at 4°C prior to use. Bacteria inocula were suspended in nutrient broth (EMD Chemicals, Gibbstown, NJ) and turbidity was adjusted to that of a 0.5 McFarland standard to give a bacterial count of approximately 10^8 colony forming units per mL. The agar plate was inoculated with *S. aureus* broth culture using a sterile cotton swab (Fisher, Fair Lawn, NJ). Both the polymer-coated and analog-coated coupons were prepared pipetting 150 µL of a 5% w/v of polymer or analog dissolved in DMF onto a stainless steel coupon then heating under vacuum to 70 °C for 2 h. A coupon treated with DMF and a bare coupon were used as controls. Coupons were then placed onto agar plate and gently pressed down. Plates were incubated at 37°C for 24 h and zones of inhibition were measured in millimeters with a ruler.

2.4.7. Cytocompatibility Assay

[Study performed by MinJung Song, Department of Biomedical Engineering, Rutgers University]

The polymer was first sterilized by UV irradiation for 300 s and dissolved in DMSO at 40 mg/mL. The stock solution was then diluted with cell culture medium to two different final concentrations (0.10 and 0.01 mg/mL) and added to a 24-well plate. Cell culture media consisted of Dulbecco's Modified Eagle's Medium (Sigma, St. Louis, MO) with 10% v/v fetal bovine serum, 1% v/v glutamine, and 50 U/mL of penicillin and streptomycin. Media containing DMSO at the concentration used to prepare the polymer solution was used as control. L929 mouse areolar/adipose fibroblasts (Department of Biomedical Engineering, Rutgers University, Piscataway, NJ) were incubated in cell culture medium at 37 °C in a humidified atmosphere (5% CO_2). Confluent fibroblasts were detached by trypsin (0.02 mg/mL, Sigma, St. Louis, MO) and incubated at 37 °C for 5 min. Trypsin activity was stopped by adding cold media, and cell pellets were obtained by centrifugation (Thermo Electron 5682 3L GP, Franklin, MA) at 2000 rpm for 2 min. Cells were seeded onto the wells of the aforementioned 24-well plate at a density of 5×10^4 cells per well and incubated for 3 days. Fibroblast morphology in the polymer solution was observed using a fluorescent microscope (IX81, Olympus, Japan) equipped with 10x phase-contrast objective at days 1, 2 and 3. All experiments were performed in triplicate

Cell growth in the solution was evaluated in triplicate every 24 h, after imaging. Cell numbers were measured using Calcein AM staining as a live-dead

assay. Calcein AM solution is a cell membrane-permeable fluorescent marker for live cell cytoplasms that is used to determine cytotoxicity .[42-44] *At each time point, medium was removed and fibroblasts were gently washed twice with PBS (MP Biomedical, Aurora, OH). Fibroblasts were then incubated with 200 µL of Calcein AM staining solution (8 µM, Molecular Probe, Carlsbad, CA) for 40 min at 4 °C. Fluorescence intensity was measured using a fluorescence plate reader (Cytofluor® Series 4000, Applied Biosystems, Woodinville, CA) at 485 nm excitation and 530 nm emission. Live cell numbers were calculated against a standard curve. Fibroblast growth in the polymer solution was statistically compared to controls at each time point in triplicate. Student's t-test were performed using Microsoft EXCEL® software at an accepted significance of p < 0.05.*

*Other methods described in Chapter 9.

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3. POLY(ANHYDRIDE-ESTERS) COMPRISED EXCLUSIVELY OF NATURALLY OCCURRING ANTIMICROBIALS AND EDTA

3.1. Introduction

The previous chapter discussed biodegradable polymers containing antibiotics for use as infection-preventing medical implant coatings. However, preventing bacterial growth and infection has uses far beyond the medical field. Prevention of microbial contamination in the personal care and food industries is of paramount importance for consumer protection. Unfortunately, most commonly used synthetic preservatives, such as parabens, suffer from problems such as sensitization and toxicity.[2] Another commonly used antimicrobial, triclosan, is thought to breed bacterial resistance. Triclosan can potentially disrupt the endocrine system and its level in the blood correlating to a consumer's use has been discovered.[3] Moreover, parabens, triclosan, and other preservatives have environmental bioaccumulation issues, with concentrations of these chemicals detected in aquatic life,[4] leading to a call for safer alternatives. Alternatively, many naturally-occurring phenolic compounds possess known activity against a range of Gram-positive and Gram-negative bacteria. Carvacrol, thymol, and eugenol are three such phenolic compounds that are major constituents of oregano, thyme, and clove essential oils, respectively.[5,6] In addition to their

antibacterial activity,[7] these compounds are potent antioxidants;[8] thus, they can potentially be used to prevent the bacterial spoilage[9,10] and oxidation[11,12] of perishable food items (e.g., meat, dairy, fruit) as well as personal care products.[13]

Currently, antimicrobials are physically mixed into formulations to prevent bacterial growth. The sustained release of these bioactive compounds can naturally preserve consumer products thereby increasing shelf life. To extend bioactive release, researchers have physically incorporated the aforementioned phenols into polymer matrices. Chitosan nanoparticles loaded with eugenol or carvacrol were successfully formulated but suffered from low antimicrobial content (ca. 3% w/w)[14] or a lack of release studies.[15] Likewise, carvarcol-containing polyethylene-*co*-vinylacetate films[16] and thymol-containing polycaprolactone films[17] suffered from low loading at 7 wt% and 10 wt%, respectively. Eugenol has been physically incorporated into methacrylate polymers; however, release studies were conducted under non-physiological conditions (i.e., ethanol).[18] Additionally, poly(lactic-*co*-glycolic acid) (PLGA) films containing carvacrol within the polymer matrix exhibited antibiofilm and antibacterial activities,[19] although the release is not quantified. Eugenol and carvacrol-loaded PLGA nanoparticles displaying antibacterial activity have been formulated by multiple groups but the nanoparticles exhibited a burst release; nearly 50% antimicrobial released in the first 8 h for eugenol[20] and 60% released in 3 h for carvacrol.[21]

Chemical incorporation of bioactives into a biodegradable polymer backbone has multiple advantages (e.g., higher drug loading, sustained release,

tunable release rates and geometry depending on desired application) over the aforementioned physical incorporation methods.[22,23] Chemical incorporation of eugenol into a polymer backbone has also been achieved, along with antibacterial activity, but the polymethacrylate was not degradable, limiting its use in other applications.[24] In this work, we present the synthesis of biodegradable poly(anhydride-esters) (PAEs) containing one of the three phenolic compounds (carvacrol, thymol, or eugenol) chemically linked and subsequently polymerized through EDTA, a widely used chelator known to prevent oxidation caused by metals[25], to achieve high drug loading (> 50%). Previously, our group has synthesized polymers chemically incorporating various bifunctional[26,27] and monofunctional[28,29] bioactives; however, in this research the polymers hydrolyze into the phenols and EDTA, both of which are known preservatives and safe to use in consumer products.[30,31] Furthermore, all ultimate degradation products are found on the FDA's generally regarded as safe (GRAS) list. Chemical structures, thermal properties, and molecular weights of polymer and precursors were determined and *in vitro* release studies confirmed that the polymer degraded into the free antimicrobials and EDTA. The release media antioxidant efficacy and antibacterial activity were determined using a free radical quenching assay and a disk diffusion assay with a Gram-positive bacterium (*Staphylococcus aureus*) and a Gram-negative bacterium (*Escherichia coli*), respectively, and compared to activities of the free phenolic compounds.

3.2. Results and Discussion

3.2.1. Synthesis and Physicochemical Characterization
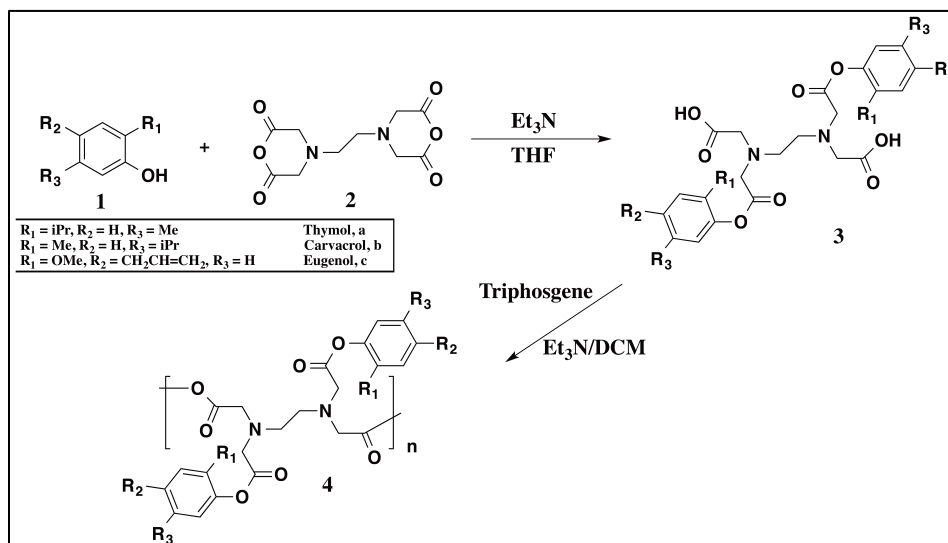
Scheme 3.1: Synthesis of antimicrobial-containing PAEs (**4**) and precursors (**3**)

The phenolic antimicrobials (**1**) are reacted with EDTA dianhydride (**2**) in the presence of triethylamine to yield diacid (**3**) via a ring-opening transesterification (**Scheme 3.1**). The diacids, **3**, were successfully prepared in high yields (76-85 %) with only minor purification necessary. Diacid structures were confirmed by ^{1}H-NMR and FT-IR spectra while DSC, MS, and elemental analysis were used for melting point, molecular weight, and chemical composition determination, respectively. In the ^{1}H-NMR spectrum of **3a**, which is provided as an example (**Figure 3.1**), the disappearance of the phenol signal of **1a** at 9.02 ppm and the appearance of EDTA linker peaks at 4.15, 3.77, and 3.13 ppm demonstrate successful ring-opening esterification to generate **3a**. The

diacids **3** were polymerized via solution polymerization techniques,[32,33] using triphosgene as the coupling reagent in the presence of triethylamine at 0˚C. Solution polymerization was chosen instead of melt-condensation to prevent potential ring-closure and regeneration of the EDTA dianhydride, **5**.[33] Polymers were characterized by ^{1}H-NMR to confirm structure. Additionally, FT-IR (**Figure 3.2**) confirmed the synthesis of a poly(anhydride-ester) through the disappearance of the carboxylic acid stretch at 1712 cm^{-1} in diacid **4** and the appearance of the C=O anhydride stretches at 1815 cm^{-1} and 1745 cm^{-1} in polymer **5**.
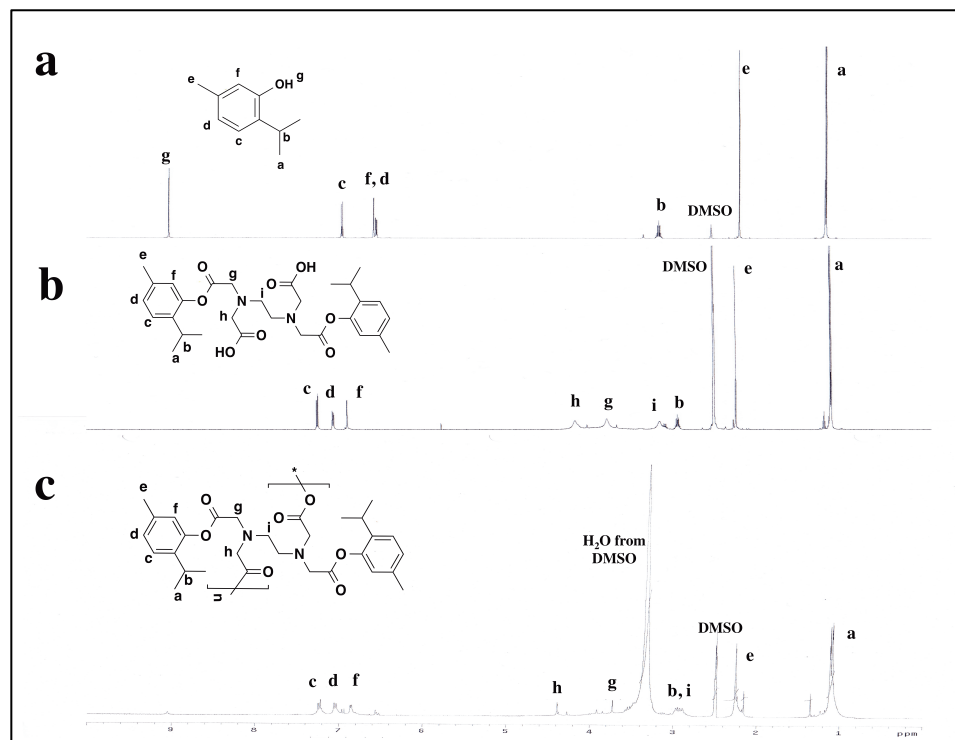
Figure 3.1: ^{1}H NMR spectra of thymol (**a**), corresponding diacid (**b**), and polymer (**c**) showing successful coupling and polymerization
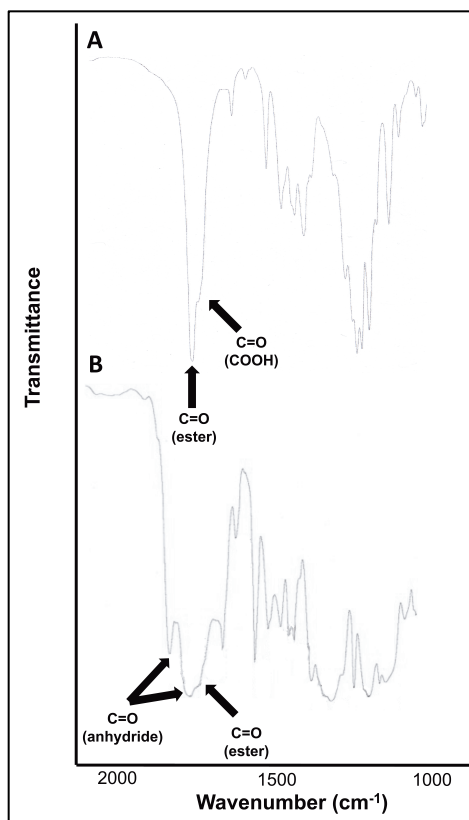
Figure 3.2: FTIR spectra of thymol-containing diacid **3a** (A, top) and polymer **4a** (B, bottom) showing conversion of diacid into PAE

The polymers (**4**) displayed moderate molecular weights typical for solution polymerization techniques, ranging from 11,000 to 23,000 Da.[32,33] PDI values were narrow (1.3-1.5) following isolation from the reaction mixture, indicating high homogeneity. The polyanhydride with the bulkiest antimicrobial, eugenol (**4c**), was the most difficult to polymerize and displayed the lowest molecular weight. All polymers (**4**) are amorphous with no indication of melting temperatures (up to 200˚C), exhibiting only glass transition temperatures in the range of 65 to 86˚C. With thermal decomposition occurring at temperatures ~200˚C, it is anticipated that processing at elevated temperatures is viable.
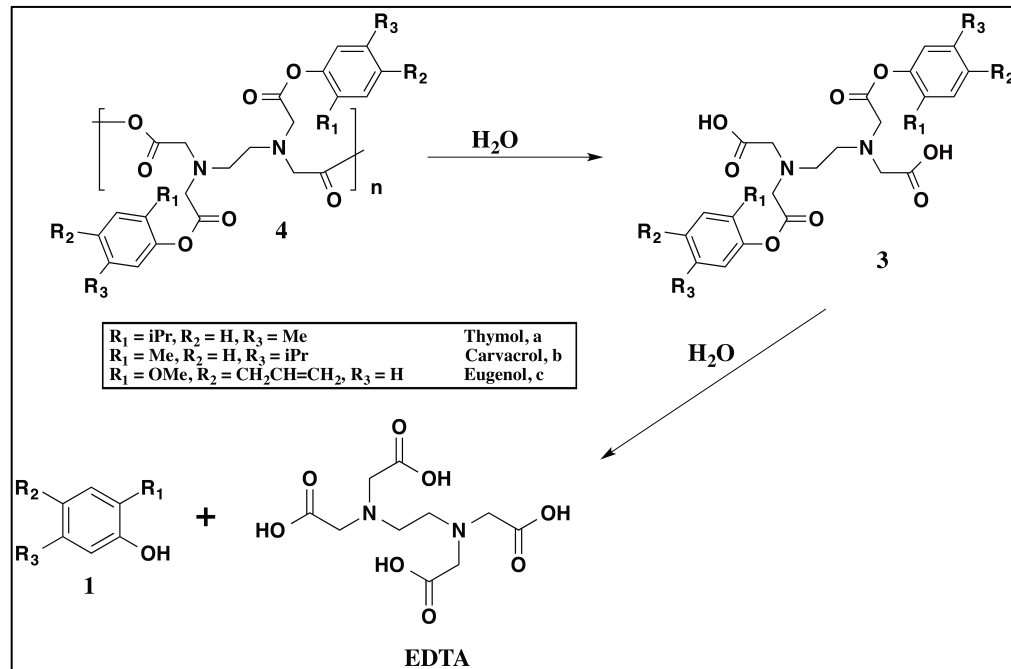
Scheme 3.2: Proposed hydrolytic degradation scheme of antimicrobial-containing PAEs (**4**) into diacid (**3**) and free antimicrobial (**1**) and EDTA

3.2.2. *In Vitro* Bioactive Release Studies

Bioactive release was carried out on powdered samples in PBS at physiological conditions (37°C, pH 7.4). The degradation rate of polymer into bioactive via anhydride and ester bond hydrolysis is an important factor in obtaining controlled antimicrobial release. Based upon previous knowledge of PAEs, the anhydride bonds are expected to hydrolyze first, followed by the ester bonds (**Scheme 3.2**).[34] To obtain polymer degradation lag time (i.e., the time during which little degradation takes place) under physiological conditions, degradation of polymer into diacid was first determined by UV-vis

spectrophotometry. All polymers (**4**) exhibited a brief lag time (<6 hrs) before any degradation was detected, as polyanhydrides commonly exhibit a degradation lag time due to their surface eroding properties.[34,35] To study complete degradation, bioactive release was monitored under identical conditions with HPLC. The studies were concluded upon complete degradation into respective bioactives and EDTA. Small amounts of diacid were also observed in degradation media, with the amounts of thymol increasing over time (e.g., thymol retention time of 14.45 min, thymol diacid retention time of 4.54 min), thus proving that the anhydride bonds hydrolyze first, followed by ester bonds. All chemically incorporated bioactive was completely released after 16 days. At the end of the study, a mass balance was performed (>97% mass accounted for) and the release data was normalized (**Figure 3.3**).
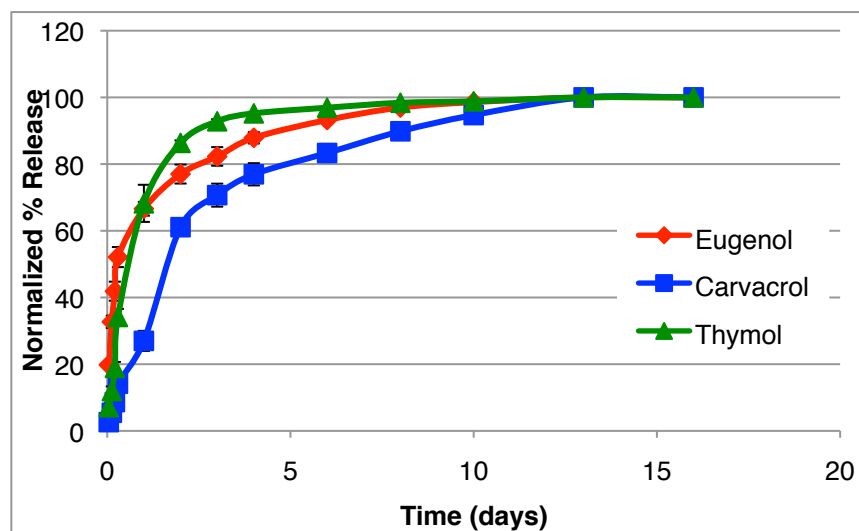
Figure 3.3: Normalized release of antimicrobials (**1**) from polymers (**4**) a result of *in vitro* hydrolytic degradation

3.2.3. Antioxidant Activity Via Radical Scavenging

To ensure bioactive released from polymers exhibited the same efficacy as free bioactive, a DPPH radical quenching assay was used.[36] DPPH is often used to assess antioxidant activity by determining the change in absorbance at 517 nm via UV-vis spectrophotometry; the solution color turns from deep purple to light yellow upon free radical quenching, thus reducing absorbance at 517 nm. Given that the assay is dependent on antioxidant concentration,[37] degradation media at 24 h were analyzed (**Figure 3.4**) and compared to freshly prepared solutions of free bioactives of the same concentrations (52 μg/mL for carvacrol, 74 μg/mL for eugenol, and 100 μg/mL for thymol). Student's t-tests were performed to ascertain significant differences (p < 0.05) between the released and freshly-prepared samples. The observed activities displayed no significant differences between released samples and free bioactive for all three phenols; thus the intermediate degradation products (i.e., diacid) and EDTA present in the degradation media had a negligible effect on free radical quenching ability. The DPPH quenching efficacy of the concentrations tested are consistent when compared to literature values of 500 μg/mL solutions of eugenol, carvacrol and thymol (93%, 79% and 82%, respectively).[8]
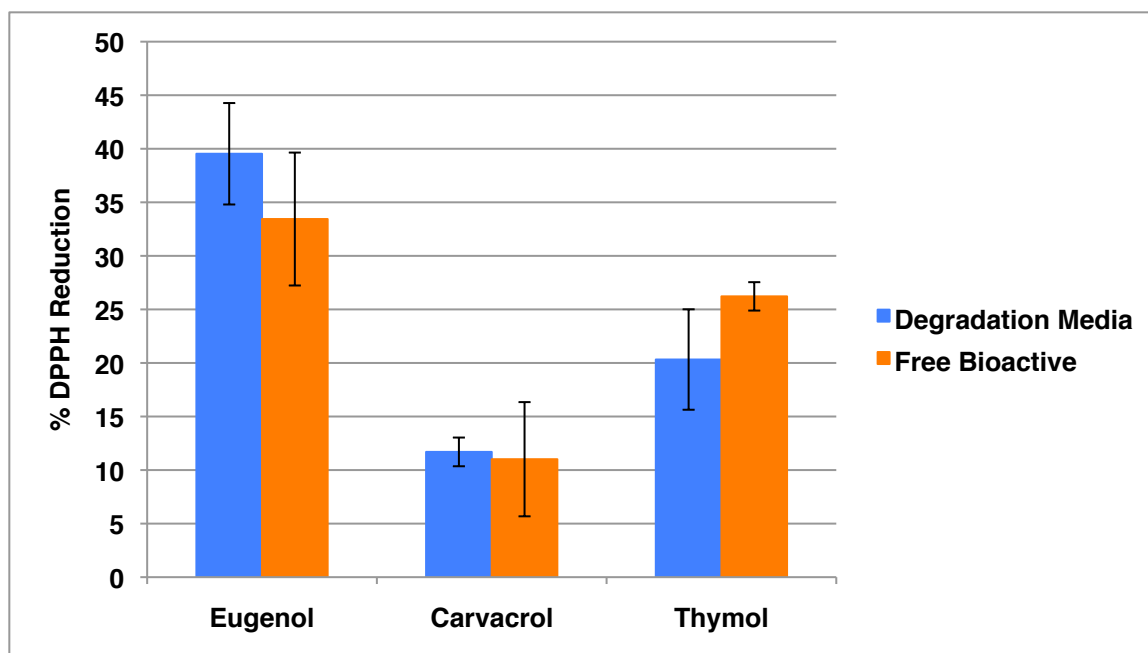
Figure 3.4: DPPH reduction results comparing bioactive released from polymer at 24 h timepoint to free bioactive

3.2.4. Antibacterial Activity

To ensure that polymer degradation products diffusing from the discs would cause clear zones of growth inhibition, the polymer was completely hydrolyzed and a specific bioactive concentration was prepared from extracted products and compared to that of equivalent concentrations of free bioactives; the concentration for all bioactives was kept at 10 mg/mL (greater than the minimum inhibitory concentrations (MICs) to ensure a clear zone is observed) in 1:1 PBS:DMSO. *S. aureus*, a Gram-positive bacterium, and *E. coli*, a Gram-negative bacterium, were both evaluated, since both strains are commonly encountered and often responsible for contamination of products leading to

spoilage.[38] As shown in **Figure 3.5**, the free phenols diffused from the discs and prevented bacterial growth on the agar. Both the free bioactives (**e-g**) and the extracted bioactives (**b-d**) exhibited nearly the same zones of inhibition for both strains (**Table 3.1**). Neither EDTA (**h**) nor PBS:DMSO (**a**) controls exhibited any activity against either strain. Carvacrol and thymol displayed greater activity compared to eugenol, which is expected owing to eugenol's higher MICs against both strains.[7] Overall, this assay shows that the methods used to process the bioactives used did not alter their antibacterial activity upon release from polymer.
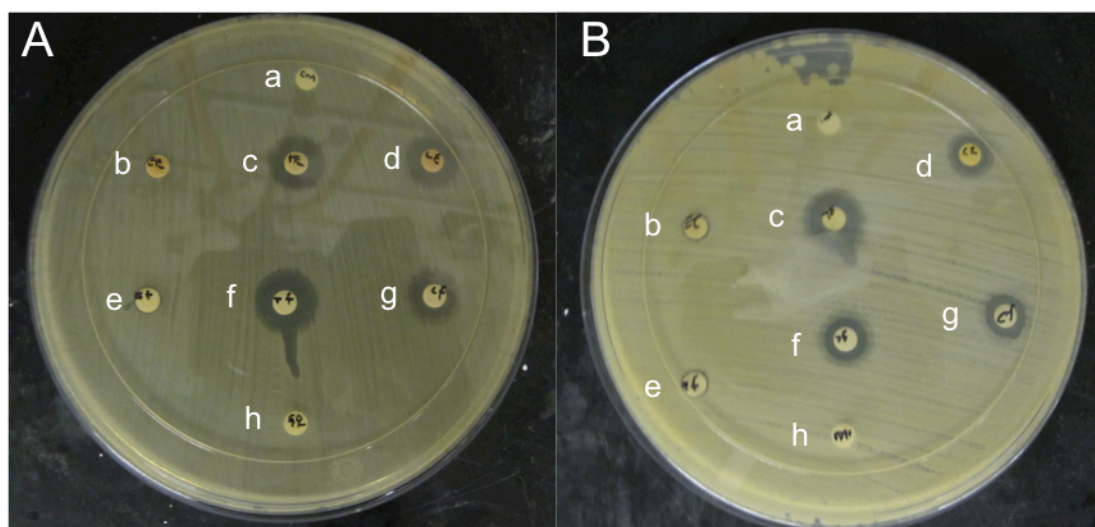
Figure 3.5: Disk diffusion assay results for *E. coli* (A) and *S. aureus* (B) showing zones of growth inhibition for 1:1 PBS:DMSO (**a**), extracted eugenol (**b**), extracted thymol (**c**), extracted carvacrol (**d**), free eugenol (**e**), free thymol (**f**), free carvacrol (**g**), and EDTA (**h**). Results display retained bioactivity

Table 3.1: Sizes of zones of growth inhibition for extracted and free phenols

Compound	*E. coli* Zone of inhibition (mm)	*S. aureus* Zone of inhibition (mm)
PBS:DMSO	-	-
Extracted eugenol	4	4
Free eugenol	4	4
Extracted thymol	8	7
Free thymol	8	6
Extracted carvacrol	7	6
Free carvacrol	7	6
EDTA	-	-

3.3. Conclusion

PAEs containing naturally-occurring antimicrobials found in plant extracts were synthesized using solution polymerization methods, resulting in products with high drug loading (> 50%). Polymers (**4**) hydrolytically degraded after 16 days, releasing free phenolic antimicrobials and EDTA in a controlled manner. Final polymer degradation products, phenolic antimicrobials and EDTA, are both commonly used as preservatives and are found on the FDA GRAS list. These polymers are unique in that the polymer completely breaks down into useful products – EDTA and an antimicrobial. A sustained release of compounds with antimicrobial, antioxidant, and chelation abilities can be beneficial for consumer product protection; the spoilage of products caused by bacterial contamination

and oxidation can be prevented by polymer degradation products. Furthermore, bioactives released from polymer display similar antioxidant and antibacterial activities. PAE properties allow for future formulations, such as films and microspheres, in addition to tunable release rates and thermal properties by changing the dianhydride used.

3.4. Experimental

3.4.1. Materials

Concentrated hydrochloric acid (HCl), 1 N HCl, 1 N sodium hydroxide (NaOH), poly(vinylidine fluoride) and poly(tetrafluoroethylene) syringe filters, and Wheaton glass scintillation vials were purchased from Fisher Scientific (Fair Lawn, NJ). All other reagents, solvents, and fine chemicals were obtained from Aldrich (Milwaukee, WI) and used as received.

3.4.2. Polymer and Precursor Synthesis

[Synthesis initially performed by Ashley L. Carbone-Howell, Department of Chemistry and Chemical Biology, Rutgers University]

3.4.2.1. Diacid (3) Synthesis

Antimicrobial-containing diacids were synthesized using a modified previously developed procedure.[28] Diacids (**3**) were prepared by reaction of ethyleneaminediaminetetraacetic acid (EDTA) dianhydride (**2**) with the appropriate antimicrobial (**1**) in the presence of a base (triethylamine) (**Scheme 3.1**). The full characterization of the thymol-based system is presented as an example. Phenol (**1**) (2 eq) was dissolved in anhydrous THF (7 mL/mmol) and anhydrous triethylamine (Et$_3$N, 4 eq). EDTA dianhydride (**5**) (1 eq) 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 DI water (~500 mL) and acidified to pH 2 using concentrated HCl. The solid diacid (**3**) that formed was isolated via vacuum filtration, washed with water (3x) and dried overnight under vacuum at room temperature.

Thymol-based Diacid (3a). Yield: 76 % (off-white powder). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.21 (d, 2H, Ar-H); 7.02 (d, 2H, Ar-H); 6.87 (s, 2H, Ar-H); 4.15 (s, 4H, CH$_2$); 3.77 (s, 4H, CH$_2$); 3.13 (s, 4H, CH$_2$); 2.92 (m, 2H, CH); 2.23 (s, 6H, CH$_3$); 1.08 (d, 12H, CH$_3$). IR (KBr, cm^{-1}): 3400 (O-H, acid), 1760 (C=O, ester), 1712 (C=O, acid). MS (ESI): m/z = 557.2 [M+1], (C$_{30}$H$_{40}$N$_2$O$_8$)$_n$ (556.7) $_n$: Calcd. C 64.14, H 7.4, N 4.70; Found C 64.73, H 7.24, N 5.03. T$_m$ = 113°C

Carvacrol-based Diacid (3b). Yield: 85 % (off-white powder). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.16 (d, 2H, Ar-H); 7.04 (d, 2H, Ar-H); 6.91 (s, 2H, Ar-H); 4.03 (s, 4H, CH_2); 3.67 (s, 4H, CH_2); 3.03 (s, 4H, CH_2); 2.81 (m, 2H, CH); 2.03 (s, 6H, CH_3); 1.10 (d, 12H, CH_3). IR (KBr, cm^{-1}): 3500 (O-H, acid), 1761 (C=O, ester), 1699 (C=O, acid). MS (ESI): m/z = 557.2 [M+1], $(C_{30}H_{40}N_2O_8)_n$ (556.7) $_n$: Calcd. C 64.73, H 7.24, N 5.03. Found C 63.72, H 7.46, N 4.92. T_m = 158°C.

Eugenol-based Diacid (3c). Yield: 82 % (off-white powder). ^{1}H-NMR (DMSO-d_6, 400 MHz): 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); 4.02 (s, 4H, CH_2); 3.72 (s, 6H, CH_3); 3.68 (s, 4H, CH_2); 3.53 (s, 4H, CH_2); 3.35 (d 4H, CH_2); 3.03 (s, 4H, CH_2). IR (KBr, cm^{-1}): 3300 (O-H, acid), 1769 (C=O, ester), 1703 (C=O, acid). MS (ESI): m/z = 585.2 [M+1], $(C_{30}H_{36}N_2O_{10})_n$ (584.6) $_n$: Calcd. C 61.63, H 6.21, N 4.80. Found C 61.13, H 6.25, N 4.76. T_m = 166°C.

3.4.2.2. Polymer (4) Synthesis

Diacid (**3**) (1 eq) was dissolved 20 % (w/v) anhydrous DCM, and triethylamine (4.4 eq) was added. Then, triphosgene (1.1 eq) dissolved in anhydrous DCM (10 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 was poured over diethyl ether (~400 mL). The solid polymer (**4**) that formed was isolated by vacuum filtration, washed

with acidic water (100 mL) and dried overnight under vacuum at room temperature.

Thymol-based Polymer (4a). Yield: 39 % (mg brown powder). ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.22 (d, 2H, Ar-H); 7.03 (d, 2H, Ar-H); 6.82 (s, 2H, Ar-H); 4.41 (s, 4H, CH$_2$); 3.68 (s, 4H, CH$_2$); 2.92 (m, 5H, CH$_2$, CH); 2.24 (s, 6H, CH$_3$); 1.06 (d, 12H, CH$_3$). IR (KBr, cm^{-1}): 1815 and 1745 (C=O, anhydride), 1730 (C=O, ester). M$_w$ = 23.2 kDa, PDI 1.3. T$_g$ = 77°C, T$_d$ = 223°C

Carvacrol-based Polymer (4b). Yield: 55 % (brown powder). ^{1}H-NMR (DMSO-d_6, 400 MHz): 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 (KBr, cm^{-1}): 1815 and 1740 (C=O, anhydride), 1725 (C=O, ester). M$_w$ = 19.5 kDa, PDI 1.3. T$_g$ = 65°C, T$_d$ = 221°C

Eugenol-based Polymer (4c). Yield: 64% (brown powder). ^{1}H-NMR (DMSO-d_6, 400 MHz): 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 (KBr, cm^{-1}): 1820 and 1740 (C=O, anhydride), 1725 (C=O, ester). M$_w$ = 11.1 kDa, PDI 1.5. T$_g$ = 86°C, T$_d$ = 229°C

3.4.3. Gel Permeation Chromatography

Polymer 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 DCM (10 mg/mL) and filtering through 0.45 µm poly(tetrafluoroethylene) (PTFE) syringe filters. Samples were resolved on a Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm, Alltech Associates, Deerfield, IL) at 25°C, with DCM as eluent at a flow rate of 0.5 mL/min. Molecular weights were calibrated relative to polystyrene standards (Polymer Source Inc., Dorval, Canada).

3.4.4. *In Vitro* Bioactive Release Studies

First, the release of diacid (**3**) from polymer (**4**) was evaluated to determine the amount of time required to hydrolyze anhydride bonds through *in vitro* degradation in phosphate buffered saline (PBS). Polymers were ground into powder using mortar and pestle to obtain particles of ~ 300-500 µm, as determined by standard testing sieves (Aldrich, Milwaukee, WI). Powdered polymer samples (15 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. At predetermined time intervals, the media was replaced with fresh PBS and the spent media analyzed by ultraviolet-visible (UV-Vis) spectrophotometry (Perkin-Elmer Lambda XLS spectrophotometer, Waltham,

MA) at λ = 270 nm. Additionally, the bioactive (**1**) release from diacid (**3**) was elucidated under the same conditions listed above, using powdered diacid samples (15 mg) incubated in 10 mL PBS (pH 7.4) in 15 mL Centrifuge tubes (BD Falcon, Franklin Lakes, NJ). After centrifugation at 6000 rpm for 4 min, the degradation media (5 mL) was collected at pre-determined timepoints and fresh PBS (5 mL) replaced that which was removed. High performance liquid chromatography (HPLC) quantified the amount of free phenol in spent media released by comparison to calibration curves of standard solutions. Media was analyzed via HPLC using an XTerra RP18 3.5 μm 4.6 × 150 mm column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual Absorbance Detector. All samples were filtered using 0.22 μm poly(vinylidine fluoride) syringe filters and subsequently injected (20 μL) using an autosampler. The mobile phase comprised of methanol (55%) and 50 mM KH_2PO_4 with 1% formic acid in DI water at pH 2.5 (45%) run at 0.8 mL/min flow rate at ambient temperature. Absorbance was monitored at λ = 270. The degradation experiments were performed in triplicate.

3.4.5. Antioxidant Activity Via Radical Scavenging

To determine the degradation media antioxidant activity, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used.[36] This was performed by adding a sample (0.1 mL) to a 24 μg/mL DPPH solution in methanol (3.9 mL). Hour-24 polymer degradation media samples (0.1 mL) were incubated with the

DPPH solution (3.9 mL) at room temperature with gentle shaking. After 1 hr, solutions were analyzed by UV/vis spectrophotometry at λ = 517 nm. For comparison, a solution of freshly prepared bioactive (**1**) at the same concentration of the 24-hour degradation media (as determined at HPLC) was prepared and analyzed via the same method. 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 1 hr. All radical quenching assays were performed in triplicate. Student's *t* tests were used to determine the significant difference of the antioxidant activity between degradation media and free antimicrobial (significantly different if $p < 0.05$).

3.4.6. Antibacterial Activity

[Study performed with assistance of Dr. Susan Skelly, Division of Life Sciences, Rutgers University]

Carvacrol, thymol, and eugenol are well-known antimicrobial agents, exhibiting activity against a range of bacteria;[7] thus, the activity of degradation media against Gram-positive and Gram-negative bacteria was tested using the disc diffusion method.[39] First, polymer was completely hydrolyzed with 1N NaOH, then acidified to pH 2 using concentrated HCl after which bioactives were extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Upon solubilization in 1:1 PBS:DMSO, the concentration of all bioactives was 10 mg/mL. The three free bioactives were tested against

equal concentrations of bioactives extracted from the degradation media (10 mg/mL) and tested as follows: Muller-Hinton agar (Becton Dickinson, Sparks, MD) was poured into sterile petri dishes (Fisher, Fair Lawn, NJ) to an even thickness of 4mm. Bacteria inocula (*S. aureus* or *E. coli*) were suspended in nutrient broth (EMD Chemicals, Gibbstown, NJ) and turbidity to give a bacterial count of approximately 10^8 colony forming units per mL. The agar plate was inoculated with bacteria broth culture using a sterile cotton swab (Fisher, Fair Lawn, NJ). Sterile paper discs (6 mm diameter, Becton Dickinson, Franklin Lakes, NJ) were impregnated with 25 μL of test solutions (one for each free phenol, one for each phenol degradation media, one for EDTA, and one for PBS:DMSO). Discs were then placed onto agar plate and gently pressed down. Plates were incubated at 37°C for 24 hours after which zones of inhibition were measured with a ruler and rounded to the nearest half-millimeter.

*Other methods described in Chapter 9.

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4. ENZYMATIC POLYMERIZATION OF AN IBUPROFEN-CONTAINING MONOMER AND SUBSEQUENT DRUG RELEASE

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4.1. Introduction

The two preceding chapters discussed two unique antimicrobial-releasing polymer systems for very different applications. The chapter presents chemical incorporation of NSAIDs into polyesters as a pendant group. Furthermore, the synthetic method herein makes use of greener processes, including solvent-free, catalytic reactions, less toxic solvents, and biorenewable lipase catalysis. Lipase-catalyzed polycondensations have garnered attention due to their chemoselectivity, non-toxicity, recyclability, and derivation from renewable resources.[2] One such lipase, Novozym 435 (N435, lipase B from *Candida antarctica* immobilized on acrylic resin), has been used to synthesize a wide range of polymers with different properties. Aliphatic polyesters of diol/diacid monomers,[3,4] diol/diester monomers,[5,6] hydroxyacids,[7] and terpolymers of diacids and diols[8-10] have been synthesized, in addition to polycarbonates.[11] Silicone-containing polymers[12,13] and polyamides[14] have also been synthesized using N435, indicating it's broad applicability. Additionally, some enzymatically-synthesized polymers contain pendant groups for future modification (i.e., coupling a bioactive or targeting moiety);[7,8,10] however, limited examples of bioactive-containing monomers polymerized via enzymatic methods are known.[15]

Using polymers for delivery of bioactives, such as drugs, has been widely studied;[16] however, the majority of research has focused on physically incorporating drugs into polymers or chemically conjugating drugs to a pre-existing polymer backbone where the polymer may – or may not – be degradable Drug-containing monomers can allow for higher drug loading and tunability of release rates and other polymer properties not possible with using pre-existing polymers.[17] Amongst such researched drugs, ibuprofen is a widely used non-steroidal anti-inflammatory drug (NSAID) to treat pain, inflammation, and fever yet suffers from severe gastrointestinal side effects at higher doses.[18,19] Physical incorporation of ibuprofen into a polymer has been achieved and exhibits limited drug loadings (< 30%) and a burst release profile.[20-27] In systems where the drug is chemically incorporated into the polymer, non-biodegradable polymers are often used, leading to potential adverse effects when used *in vivo* as the polymer may remain.[28-31] In previous work, an ibuprofen-containing polyester was prepared using N435 but had low drug loading (3-13%) and a burst release; 35% of ibuprofen was released after 18 days, but not thereafter.[32] Polymers of sebacic acid, glycerol, poly(ethylene glycol), and ketoprofen have similarly been developed using N435; however; low drug loading (< 25%) and the use of toxic, expensive vinyl moieties remain issues. Between 10-25% of ketoprofen was slowly released over 14 days; however, the release rate after that time was not studied.[15] Additionally, ibuprofen prodrug micelles were developed;[33] drug loading was between 7-41 wt%, but toxic reagents were used for synthesis. To overcome low drug loadings, Rosario-Melendez, *et al*. reported on the synthesis

of ibuprofen and tartaric acid-based polymers using 1,8-octanediol as comonomer and stannous octoate as catalyst to yield polymers with 65 to 67 wt% ibuprofen.[34] In this work the utility of enzyme-mediated polymer synthesis is expanded; specifically, the development of a drug-containing monomer and the utilization of enzymes (N435) to generate three different ibuprofen and malic acid-based polyesters using diols as comonomers is described.

The synthetic methodology herein utilizes "greener", more sustainable processes when compared with more traditional polycondensation methods. The twelve principles of green chemistry include the i) replacement of environmentally hazardous chemicals with safer materials, ii) solvent elimination, iii) product biodegradation, iv) use of catalysts and v) use of renewable resources[35]– the work described herein incorporates many of these principles. A high priority of this research is inclusion of renewable resources, as such, L-malic acid is a naturally occurring dicarboxylic acid found in many fruits such as apples, grapes, and berries[36,37] and serves as the polymer backbone. The diol comonomers chosen are used in cosmetic and pharmaceutical formulations.[38,39] Additionally, 1,3-propanediol is derived from biological sources.[40] In addition to the novelty of a polymer comprised of renewable monomers, the enzymatic polymerization of such a drug-containing monomer with hydrolytically degradable bioactive pendant ester groups has few literature examples.[15] In summary, this paper presents one of the few examples of synthesizing drug-containing monomers to make biocompatible polymers via green methods including enzyme-based polymerization; the only other literature examples exhibit low drug loading (<25

wt%) and utilize toxic reagents/solvents. Further, these completely biodegradable polymers are anticipated to be significant because of the environmentally sustainable synthetic methodology used to make environmentally sensitive polymers.

Polymer and precursor structures were determined by proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) and Fourier transform infrared (FT-IR) spectroscopies. Thermal properties were evaluated by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Mass spectrometry (MS) and gel permeation chromatography (GPC) were used to determine molecular weights (Mw) of precursors and polymers, respectively. To evaluate drug release from polymer, *in vitro* studies were performed under physiological conditions. Polymer cytocompatibility was determined using fibroblasts, and chemical structures of released drugs were confirmed.

4.2. Results and Discussion

4.2.1. Synthesis and Physicochemical Characterization

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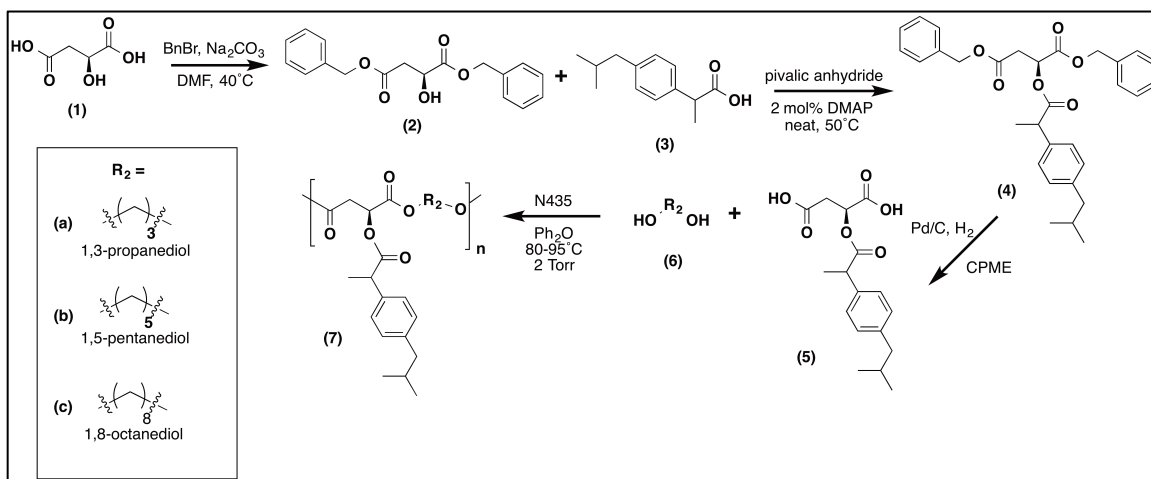
Scheme 4.1: Synthesis of polymer precursors and poly(ibuprofen-*L*-malate) polyesters (**7**) using aliphatic diols of different lengths (a, b, c, in insert)

To prevent unwanted side reactions from occurring, the carboxylic acid groups of *L*-malic acid were protected before ibuprofen was coupled to the malic acid alcohol group (**Scheme 4.1**). Thus, the selective benzyl protection of malic acid carboxylic acid groups was adapted from a previously published procedure using benzyl bromide and sodium carbonate.[41] The appearance of benzylic and aromatic signals in the ^{1}H NMR spectrum (**Figure 4.1**) and the preservation of the FT-IR band (**Figure 4.2**) at 3480 cm^{-1} (O-H) of **2** confirmed that successful reaction occurred at the acid groups without affecting the secondary alcohol. Thereafter, ibuprofen was coupled to the alcohol via a solvent-free esterification using catalytic DMAP and pivalic anhydride to afford **4** in high yield. The significant chemical shift of malic acid backbone protons in the NMR spectra

(**Figure 4.1**) of **4** along with the formation of an IR band (**Figure 4.2**) at 1743 cm^{-1} (ester, C=O) and disappearance of the alcohol band at 3480 cm^{-1} indicated successful coupling of drug to **2**.   Subsequent deprotection via palladium-mediated hydrogenolysis selectively cleaved the benzyl esters while leaving the pendant ester group intact.   During this step, CPME was used as a greener alternative to traditional solvents such as tetrahydrofuran, dioxane, DCM and MeOH, as it is less toxic, less volatile, and less likely to form peroxides.[42] Low volatility is preferable when choosing solvents to minimize exposure to air and laboratory workers, especially in plant-scale labs.[35,42] Diacid **5** was obtained in high yield after filtration through Celite and removal of solvent *in vacuo*.   NMR spectrum (**Figure 4.1**) indicated the disappearance of benzylic protons while IR spectrum (**Figure 4.2**) displayed preserved pendant ester linkage at 1743 cm^{-1} and appearance of an additional carbonyl band at 1719 cm^{-1} (-COOH). At each step, mass spectrometry was used to confirm product molecular weight, and ^{13}C NMR spectroscopy was used to confirm chemical structure.   All polymer precursors were viscous oils or foams that did not display melting temperatures.
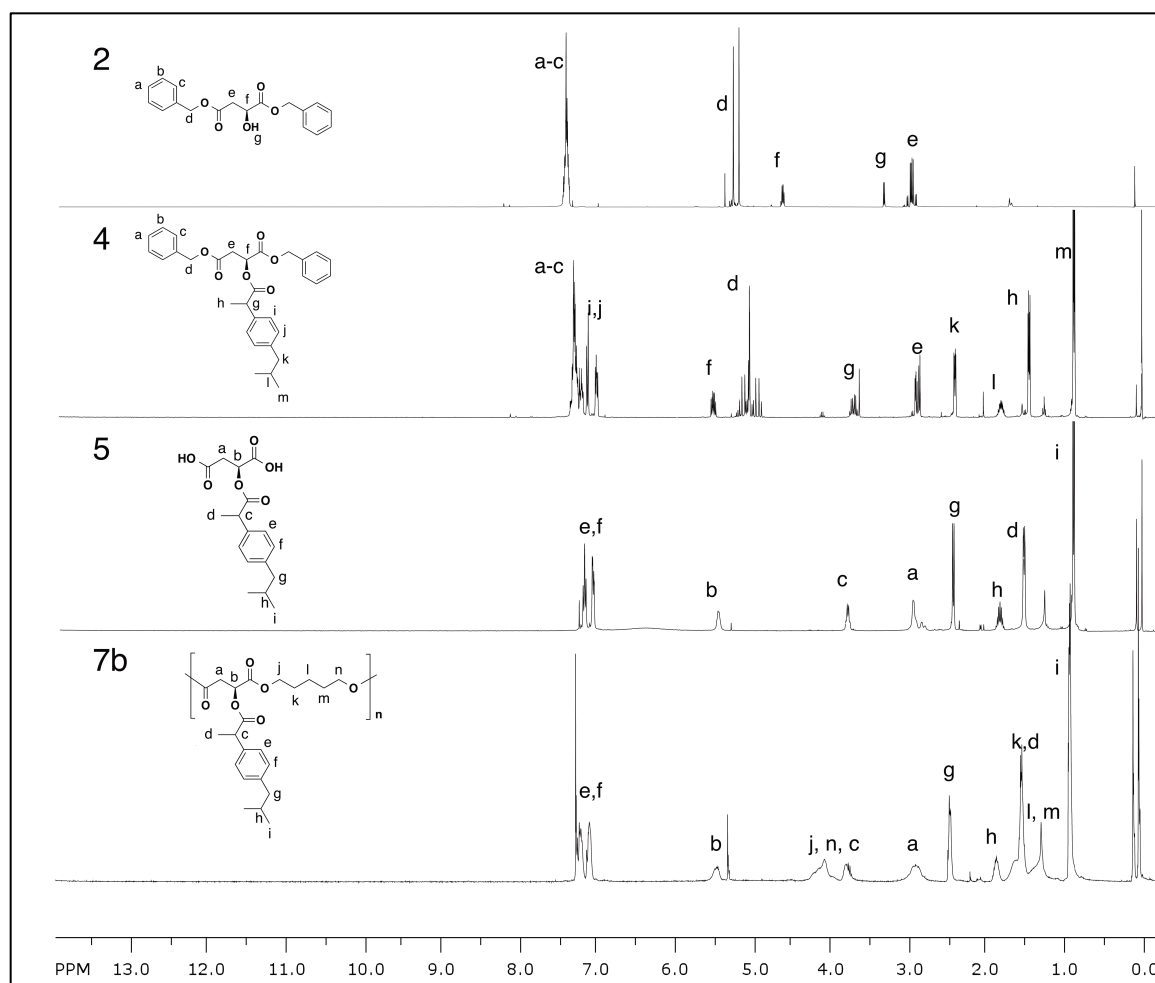
Figure 4.1: ^{1}H NMR spectra of compounds **2**, **4**, **5**, and **7b** showing benzyl protection, drug coupling, deprotection, and subsequent polymerization

Polyester synthesis was performed using a modified polymerization procedure of aliphatic diacids and diols.[3] The polyesterification of equimolar amounts of ibuprofen-L-malic acid and diol were catalyzed by N435. This particular enzyme is readily dispersed in the reaction mixture and known to be activated by heat. In a prior study using a different dicarboxylic acid and diol, the N435 enzyme produced polymers of the same molecular weight and was stable at 70-90°C.[43] Diphenyl ether, a hydrophobic, high-boiling solvent that has been

shown to be effective for such reactions was chosen as solvent. Upon exposure to the initial temperature of 80 °C, the reaction formed a monophasic mixture that remained for the reaction duration. After 48 h of polymerization time, the reaction mixture became solid and product was isolated after dissolution in chloroform, filtration of lipase beads, and solvent removal *in vacuo*. Chloroform was chosen because it is the preferred solvent to terminate the lipase reactions;[44] despite the use of this solvent in this one step, our overall synthetic methods are greener than traditional methods; other methods to achieve similar reactions utilize excess carbodiimides,[45] chlorinated solvents,[32] and metal catalysts often used[46-48] in contrast to solvent-free, catalytic reactions, safer solvents, and enzyme catalyst.

Table 4.1: Summary of thermal properties, molecular weights, and polydispersity indices of polymers **7a-c**

Polymer	T_g (°C)[a]	T_d (°C)[b]	Mw (kDa)[c]	PDI[c]
7a	-5	212	4.8	1.4
7b	-9	215	6.4	1.4
7c	-35	221	8.1	1.3

[a] Determined on second heating cycle of DSC; [b] Determined by TGA as the onset of thermal decomposition; [c] Determined by GPC

GPC analysis indicated that M_w values were moderate and PDI values narrow (**Table 4.1**) substantiating that an additional purification step was unnecessary. The longer chain length diol, 1,8-octanediol, resulted in polymers with slightly higher M_w, as expected based on previously published results.[3,4]

Notably, the M_w of these enzymatically-synthesized polyesters are similar to other malic acid-containing polymers;[10] due to malic acid's high acidity,[49] polymers rarely reach higher than 10 kDa. ^{1}H NMR spectroscopy of polymers **7a-c** displayed all expected peaks; **7b** is provided as an example in **Figure 4.1**. IR spectra display the only carbonyl peak at 1743 cm^{-1}, indicative of an ester in Figure 4.2. Polymer T_g values were low (< 0˚C), and decreased with increasing aliphatic diol chain length; no T_m transitions were exhibited (**Table 4.1**) T_d values were >200˚C, confirming that the polymer should be relatively stable to temperatures at which these polymers would be reacted or processed.
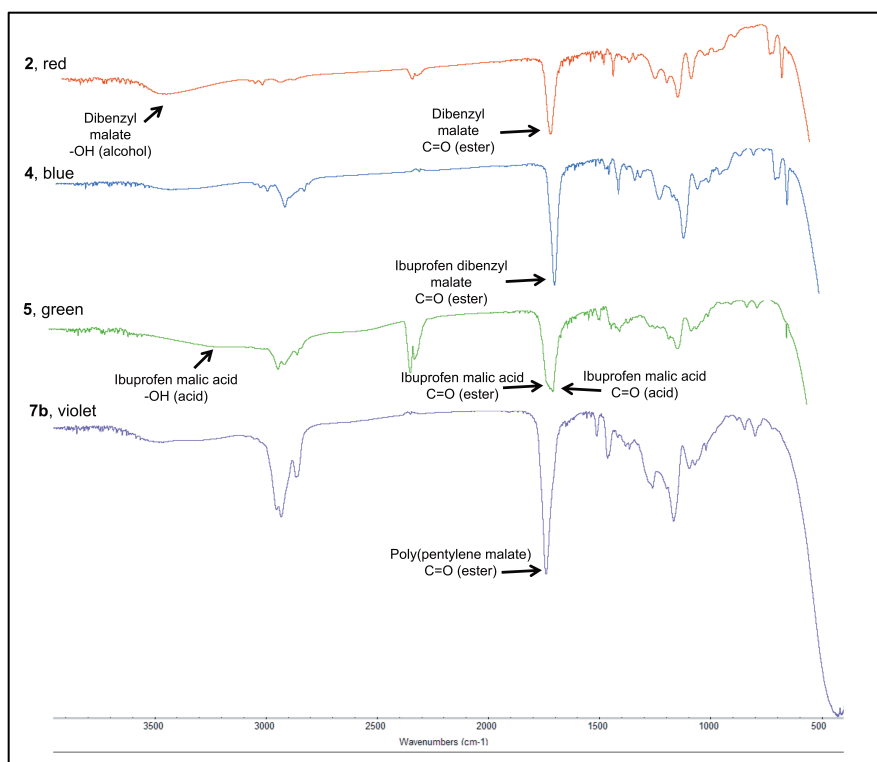
Figure 4.2: IR spectra of precursors **2** (red, upper), **4** (blue, mid-upper), and **5** (green, mid-lower), and polymer **7b** (violet, lower) presented as examples; key IR bands are labeled on each spectrum.

4.2.2. *In Vitro* Bioactive Release Studies

Scheme 4.2: Complete hydrolysis of polymer **7** into diol **(6)**, L-malic acid **(8)**, and ibuprofen **(3)**

In vitro degradation of the polymers was determined by appearance of ibuprofen in degradation media samples in PBS at physiological conditions (37 ˚C, pH 7.4). At predetermined times, an aliquot of release media was collected and analyzed via HPLC. The degradation rate of polymer into bioactive via ester bond hydrolysis (**Scheme 4.2**) is an important factor in obtaining controlled ibuprofen release; during degradation, the ultimate hydrolytic degradation products were the diol (**6**), L-malic acid (**8**), and ibuprofen (**3**).   As an example, ibuprofen (**3**), ibuprofen-malic diacid (**5**), and malic acid (**8**) all exhibited unique retention times at 5.70 min, 4.29 min, and 2.79 min, respectively. Polymers **7a-c** exhibited controlled, sustained ibuprofen release throughout the 30 days of the study. Increased aliphatic chain of the diol used decreased the drug release rate; after 30 days, 42 %, 58 %, and 82 % of total ibuprofen was released from the octylene, pentylene, and propylene polymers, respectively (**Figure 4.3**). The release rate correlates to the water solubility of the diols; the less water-soluble, more hydrophobic 1,8-octanediol (log P = 1.44) displays the slowest release

followed by intermediate 1,5-pentanediol (log P = 0.19), and 1,3-propanediol (log P = -0.68). After 30 days, the polymers were completely hydrolyzed according to the methods described above.
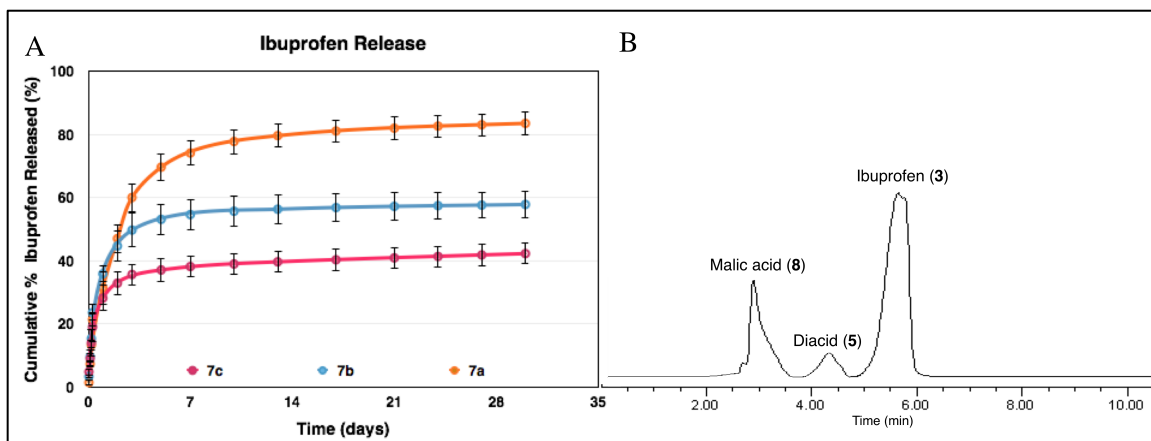
Figure 4.3: A. Cumulative release of ibuprofen as determined by HPLC data; B. Representative HPLC chromatogram depicting the unique retention times of L-malic acid, **8** (R_t 2.79 min), diacid, **5** (R_t 4.29 min), and ibuprofen, **3** (R_t 5.70 min)

Extracted ibuprofen was quantified in all samples to ensure a mass balance of the drug in the remaining polymer residue. Complete polymer degradation and 100% ibuprofen release is expected in 8-15 months for all three polymers. Additionally, to ensure that polymer processing did not affect the drug molecule structure, the ibuprofen extracted from media was dried under vacuum and analyzed. The ^{1}H NMR spectrum of extracted drug (**Figure 4.4**) showed all peaks at the same chemical shifts as free ibuprofen.
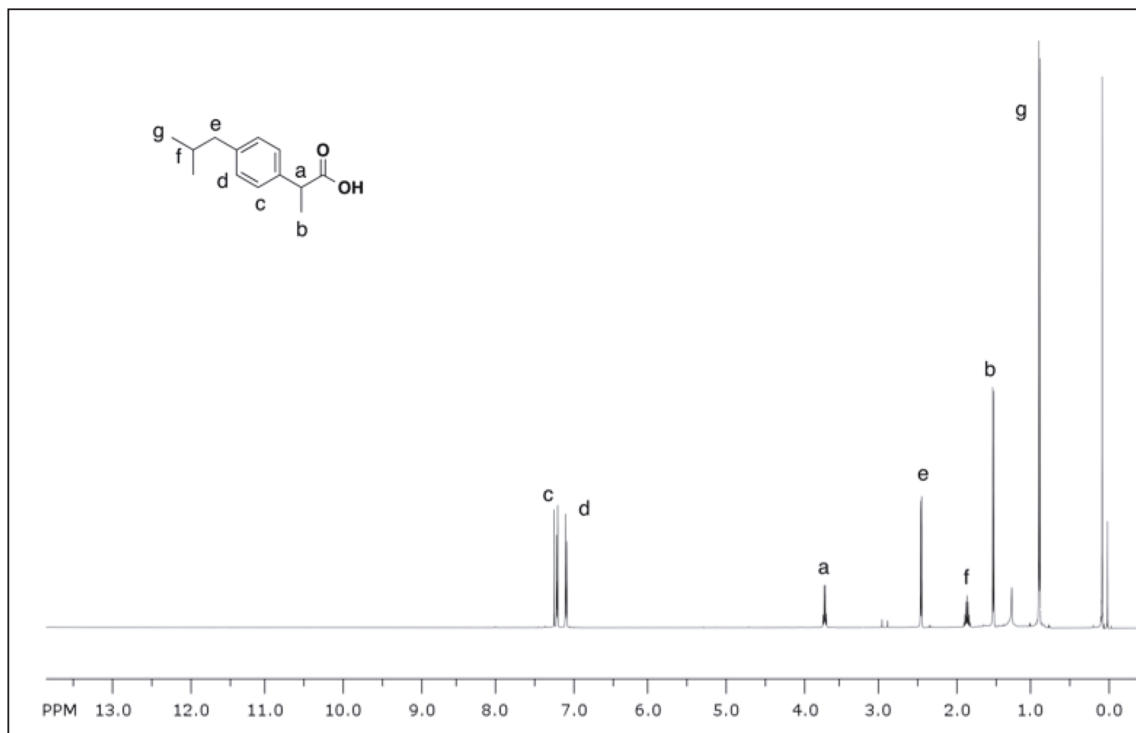
Figure 4.4: ^{1}H NMR of polymer **7b** hydrolysis product in CDCl$_3$ showing all peaks indicative of pure ibuprofen

4.2.3. Cytocompatibility Studies

[Performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University]

*Based on the therapeutic plasma concentration of ibuprofen, two different concentrations of polymer were tested.[50,51] All polymers were cytocompatible at 0.01 mg/mL and 0.001 mg/mL over 72 h (**Figure 4.5**), i.e. no significant difference in cell viability was found between the polymer groups and the media alone control. The 0.01 mg/mL concentration is comparable to the commonly used ibuprofen dosing.[50] This data demonstrated that these ibuprofen-based*

polymers are cytocompatible at clinically relevant concentrations and thus promising candidates for biomedical applications.
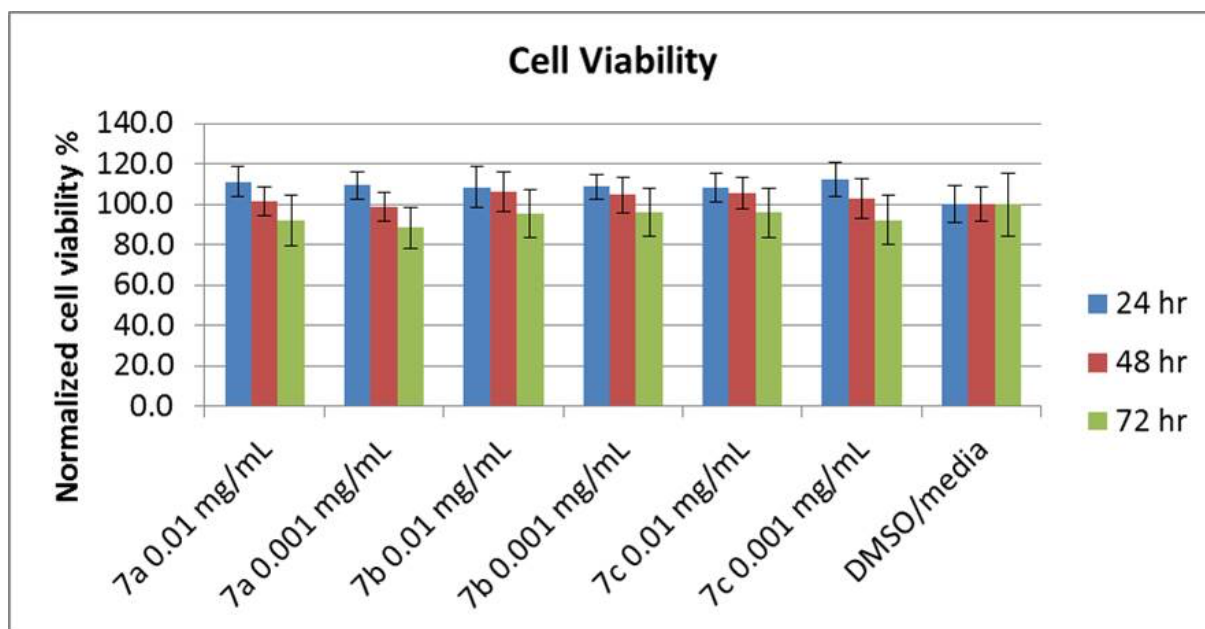
Figure 4.5*: Cytocompatibility of polymers after 24, 48 and 72 h incubation. All groups contained 1% DMSO in cell media and control group has no polymer. Absorbance at 490 nm after MTS treatment is proportional to cell viability. Data presented as mean ± standard deviation. N=6 in each group*

4.3. Conclusion

This polyester synthetic methodology moves toward a more environmentally friendly approach in developing biomaterials for drug delivery. Through the use of naturally occurring resources, safer solvents and/or eliminating solvent altogether, polymer precursors were synthesized in high yield

(85-95%). A lipase-mediated polymerization, as opposed to a metal-mediated polymerization, produced three polyesters with varying aliphatic chain length with varying release characteristics. All polymers released ibuprofen over 30 days under physiological conditions; the release rate can be tuned by varying the diol used. Utilization of a shorter chain, more water-soluble diol (i.e., 1,3-propanediol) results in faster release. Significantly, the polymers are designed for hydrolytic degradation and the ultimate polymer degradation products are deemed 'safe' as they are either bioactive, natural, or non-toxic. Cytocompatibility studies using fibroblasts assured the biocompatible nature of the polymers at relevant therapeutic concentrations, and NMR analysis determined that the ibuprofen structure remained unaffected throughout the synthesis procedure and polymer degradation process. The greener methodology presented here for precursor synthesis can be widely applicable to other hydroxyacids and bioactives with carboxylic acid functionalities, opening the door to other sustainable, enzymatic polymerizations of drug-containing monomers. Future works include investigating other bio-based diols to tune thermal properties and performing *in vitro* anti-inflammatory activity assays and investigating additional formulations (e.g., coatings, films).

4.4. Experimental

4.4.1. Materials

(Poly(vinylidine fluoride) (PVDF) and polytetrafluoroethylene (PTFE) syringe filters, and Wheaton glass scintillation vials were purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals and reagents were purchased from Sigma Aldrich (Milwaukee, WI).

4.4.2. Polymer and Precursor Synthesis

4.4.2.1. Dibenzyl-L-Malate (2) Synthesis

Using the same methodology as Guo, *et al.*,[41] L-malic acid (**1**, 1 equiv) was dissolved in anhydrous DMF and stirred in a round-bottomed flask under nitrogen as shown in **Scheme 4.1**. Sodium carbonate (2.4 eq) was added to the reaction to form a white suspension that was stirred for 30 min. Benzyl bromide (3 eq) was then added and reaction was heated to 40 °C with stirring overnight. The reaction mixture was then concentrated in vacuo, diluted with ethyl acetate (EtOAc), and washed with saturated sodium bicarbonate (3x), deionized (DI) water, and brine. The organic layer was dried over $MgSO_4$, filtered, and the solvent removed *in vacuo* to yield compound **2**.

Yield: 95% (off-white oil). ^1H NMR (400 MHz, $CDCl_3$): δ 7.28-7.38 (m, 10H, Ar-H); 5.10-5.20 (split, 4H, CH_2); 4.54 (s, 1H, CH); 3.23 (s, 1H, OH); 2.82-2.94 (split, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.4, 170.5, 135.4, 135.4, 128.9, 128.6, 68.0, 67.6, 67.0, 39.0. IR (NaCl, cm^{-1}): 3480 (O-H), 1740 (C=O, ester). MS (ESI): *m/z* = 315.1 $[M + 1]^+$. T_d = 248°C

4.4.2.2.   Ibuprofen Dibenzyl-L-Malate (4) Synthesis

A solvent-free esterification reported by Sakakura, *et al.*[52] was used to couple the drug to compound **2**. Ibuprofen (**3**, 1.1 eq), 4-(dimethylamino)pyridine (DMAP; 2 mol%), and dibenzyl-L-malate (**2,** 1 eq) were combined in a round-bottomed flask under nitrogen.   Then, pivalic anhydride (1.1 eq) was added. Reaction was heated to 55 °C and stirred overnight.   Reaction was then diluted with EtOAc and washed with saturated sodium bicarbonate (3x), DI water, and brine.   The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield compound **4**.

Yield: 85% (off-white oil). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.38 (m, 10H, Ar-H); 7.15 (m, 2H, Ar-H); 7.04 (m, 2H, Ar-H); 5.52 (m, 1H, CH); 4.90-5.20 (split m, 4H, CH_2); 3.72 (m, 1H, CH); 2.88 (split m, 2H, CH_2); 2.40 (dd, 2H, CH_2); 1.81 (m, 1H, CH); 1.46 (dd, 3H, CH_3); 0.87 (dd, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9, 170.5, 169.0, 140.8, 137.2, 135.3, 129.5 (2C), 128.8 (6C), 128.6 (6C), 127.5 (2C), 68.7, 67.6, 67.0, 45.1, 38.9, 36.3, 30.4, 22.6, 18.6. IR (NaCl, cm^{-1}): 1743 (C=O, ester). MS: *m/z* = 502.3 [M + 1]$^+$. T_d = 268°C

4.4.2.3.   Ibuprofen-L-Malic Acid (5) Synthesis

Ibuprofen dibenzyl-L-malate (**4**, 1 eq) was dissolved in anhydrous cyclopentylmethyl ether (CPME, 10 mL/g) and 10 % palladium on carbon (Pd/C,

catalytic amount) was added. The reaction flask was evacuated under vacuum and purged with hydrogen gas (3x), then allowed to stir at room temperature under hydrogen overnight. The mixture was filtered through Celite to remove Pd/C. The filtrate was concentrated *in vacuo* to yield pure compound **5**.

Yield: 91% (ight tan paste). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.19 (t, 2H, Ar-H); 7.08 (dd, 2H, Ar-H); 5.47 (split, 1H, CH); 3.78 (m, 1H, CH); 2.75 (split, 2H, CH$_2$); 1.83 (m, 1H, CH); 1.52 (d, 3H, CH$_3$); 0.88 (d, 6H, CH$_3$). ^{13}C NMR (CDCl$_3$, 125 MHz): δ 174.9, 173.9 (2C), 141.0, 136.9, 129.5, 127.5, 67.8, 45.0, 44.8, 35.9, 22.6, 18.6. IR (NaCl, cm^{-1}): v = 3100-3300 (O-H, acid), 1743 (C=O, ester), 1719 (C=O, acid). MS: *m/z* = 321.1 [M - 1]$^-$. T$_d$ = 211˚C.

4.4.2.4.   Ibuprofen-L-Malate Polymer (7) Synthesis

Ibuprofen-L-malic acid (**5**, 1 eq) and diol (**6**, 1 eq) were added to a round-bottomed flask with N435 (10 wt% of total monomers, dried at room temperature and 2 Torr for 24 h). Diphenyl ether (200 wt% of total monomers) was added. Stirring (200 rpm) was initiated and reaction was performed in three sequential steps: 1) reaction stirred 80 ˚C for 4 h at atmospheric pressure under nitrogen, 2) reaction stirred at 80˚C under vacuum (2 Torr) for 24 h, and 3) reaction was stirred at 95 ˚C under vacuum (2 Torr) for an additional 24 h. Upon completion, the reaction was brought to room temperature, dissolved in chloroform (CHCl$_3$), and gravity-filtered to remove lipase. Filtrate was concentrated *in vacuo* to yield compound **7**.

Poly(octylene ibuprofen-L-malate) (**7a**) Yield: 82% (tan paste). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.21 (b, 2H, Ar-H); 7.09 (b, 2H, Ar-H); 5.47 (b, 1H, CH); 3.62-4.22 (b, 5H, CH, CH$_2$); 2.84 (b, 2H, CH$_2$); 2.44 (b, 2H, CH$_2$); 1.84 (b, 1H, CH); 1.17-1.71 (b, 15H, CH$_3$, CH$_2$, CH$_2$, CH$_2$) 0.89 (b, 6H, CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 178.2, 174.5 (2C), 139.9, 137.1, 129.9, 128.1, 68.9, 45.3, 44.7, 35.5, 31.1, 29.6 (2C), 22.3, 18.9, 17.2 (2C). IR (NaCl, cm^{-1}): 1741 (C=O, ester). M_w = 8.1 kDa, PDI = 1.4. T_g = -35°C, T_d = 221°C

Poly(pentylene ibuprofen-L-malate) (**7b**) Yield: 78% (brown paste). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.19 (b, 2H, Ar-H); 7.08 (b, 2H, Ar-H); 5.47 (b, 1H, CH); 3.78 (b, 1H, CH); 2.75 (b, 2H, CH$_2$); 1.83 (b, 1H, CH); 1.52 (b, 3H, CH$_3$); 0.88 (b, 6H, CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 179.1, 172.7 (2C), 139.7, 136.0, 128.4, 126.3, 67.2, 44.0, 43.8, 35.1, 29.1 (2C), 28.7, 27.0, 21.3 (2C), 17.5, 17.1. IR (NaCl, cm^{-1}): 1743 (C=O, ester). M_w = 6.4 kDa, PDI = 1.4. T_g = -8°C, T_d = 215°C

Poly(propylene ibuprofen-L-malate) (**7c**) Yield: 81% (brown paste). ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.20 (b, 2H, Ar-H); 7.08 (b, 2H, Ar-H); 5.47 (b, 5H, CH$_2$, CH); 3.70-4.20 (b, 1H, CH); 2.91 (b, 2H, CH$_2$); 2.44 (b, 2H, CH$_2$); 1.79-2.12 (b, 3H, CH$_2$, CH); 1.51 (b, 3H, CH$_3$); 0.89 (b, 6H, CH$_3$). ^{13}C NMR (125 MHz, CDCl$_3$): δ 174.9, 172.6 (2C), 139.7, 136.0, 128.4, 126.3, 68.2, 45.3, 44.8, 35.8, 30.4 (2C), 22.6 (2C), 18.6, 18.4. IR (NaCl, cm^{-1}): 1743 (C=O, ester). M_w = 4.8 kDa, PDI =1.3 . T_g = -5°C, T_d = 212°C

4.4.3. *In Vitro* Bioactive Release Studies

Drug release from polymers (**7a-c**) was evaluated by *in vitro* degradation in phosphate buffered saline (PBS) under physiological conditions. Polymer samples (30 mgs each, n = 3) 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. At predetermined time intervals throughout the 30 days of the study, media (5 mL) was collected and replaced with fresh PBS (5 mL) and the spent media was analyzed by high performance liquid chromatography (HPLC). Analysis was performed using an XTerra RP18 3.5 μm 4.6 × 150 mm column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual Absorbance Detector. All samples were filtered using 0.22 μm PVDF syringe filters and subsequently injected (20 μL) using an autosampler. The mobile phase, which was developed as a modification of a published procedure,[53] was comprised of acetonitrile (70 %) and 10 mM KH_2PO_4 in DI water at pH 2.5 (30 %) run at 0.5 mL/min flow rate and ambient temperature. Absorbance was monitored at λ = 223, one of the absorption wavelengths for ibuprofen. Amounts were calculated from a calibration curve of known standard solutions.

4.4.4. Structural Characterization of Released Ibuprofen

Polymers (40 mgs) were placed in 20 mL Wheaton scintillation vials, then PBS (10 mL) and 1 N NaOH (2 mL) added to the vials, which were incubated in a

controlled environment incubator-shaker at 37 °C with 60 rpm agitation. After complete polymer degradation, solutions were acidified to pH 2 using concentrated HCl and ibuprofen was extracted from the samples with DCM (10 mL, 3x). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo.

4.4.5. Cytocompatibility Studies

[Performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University]

In vitro cytocompatibility studies were performed by culturing 3T3 mouse fibroblasts in cell media (DMEM supplemented with 10 % FBS, 1 % pen/strep) containing the 3 different polymers. Polymers were first sterilized under UV at λ = 254 nm for 900 s (Spectronics Corporation, Westbury, NY), dissolved in DMSO to yield 10 mg/mL solutions, and then diluted with cell media to reach concentrations of 0.01 mg/mL and 0.001 mg/mL. Cell media containing polymers were then added to allocated wells in a 96-well plate with 2000 cells/well and incubated at 37 °C. DMSO (1 %) in cell media was used as control. Cell viability was determined using CellTiter 96® Aqueous One Solution Cell Proliferation Assay. After 24 h, 48 h, and 72 h incubation with polymers, 20 μL of (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) reagent was added to each well and further incubated for 4 h at 37 °C. The absorbance was then recorded with a microplate reader (Coulter,

Boulevard Brea, CA) at 492 nm.

*Other methods described in Chapter 9.

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5. LINEAR, MANNITOL-BASED POLY(ANHYDRIDE-ESTERS) WITH HIGH TUNABILITY:BIODEGRADABILITY WITH SUSTAINED ANTI-INFLAMMATORY ACTIVITY

5.1. Introduction

As stated in Chapter 1, examples of polymers containing bioactives covalently attached as pendant groups, namely poly(N-(2-hydroxypropyl)methacrylamide) (HPMA), and poly(ethylene glycol) (PEG), have been ubiquitous in the field of drug delivery.[1-6] While a number of these systems have proven effective for targeted and stimuli-responsive delivery, they suffer from a few drawbacks. First, HMPA and acrylate polymers are not fully biodegradable; if used *in vivo*, the nondegraded residue may need to be removed due to immune response.[1] Additionally, HPMA and PEG, as well as polysaccharide systems often suffer from low drug loading (<20%).[4] To improve upon these limitations, novel, fully biodegradable polymers with improved drug loading have been developed; however, the few literature examples that exist have the bioactive bound within the backbone rather than as pendant groups.[7,8] Hydrolytically degradable polymers with pendant bioactives employ sugars[9] or other generally regarded as safe (GRAS) molecules (e.g., EDTA)[10] as backbones, with drug loading approaching 50%. However, drug loading can be further increased by using monomers with multiple pendant attachment sites per

repeat unit. Examples of such monomers can be based on polyols, such as sugar alcohols (**Figure 5.1**).
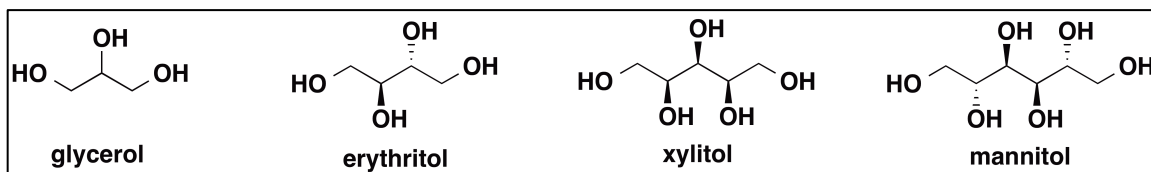
Figure 5.1: Structures of representative sugar alcohols with three, four, five, and six hydroxyl groups

Sugar alcohols are well-studied, nontoxic, biocompatible, and on the GRAS list; however, there exists a dearth of research using these as components of *linear* bioactive delivery systems, as most sugar alcohols are either incorporated into dendrimers, or utilized as cross-linked materials for tissue engineering. Although polyglycerol-based dendrimers have been utilized for targeted and stimuli responsive bioactive delivery,[11-15] their large number of branch points and high molecular weight often limits the drug loading of such systems. Sugar alcohols have also been incorporated into elastomers for soft tissue engineering and other biomedical applications.[16-18] Poly(xylitol sebacate), has been developed Langer *et al.*, has been developed into photocrosslinked gels and elastomers that exhibit *in vivo* biocompatibility better than poly(lactic-*co*-glycolic acid).[19] Similiarly, poly(xylitol carboxylates),[20,21] poly(erithritol carboxylates),[22] poly(mannitol sebacate),[23] and poly(mannitol citrate)[24] have been developed for tissue engineering applications; however, in all cases,

polymers were branched through cross-linking or curing of the secondary hydroxyl groups, often leading to poor solubility in most solvents. Linear polymers containing sugar alcohols include xylitol-based polycarbonates[25] and polyesters,[26] and mannitol-based polyesters.[27] Although these polymers are linear and not cross-linked, the secondary hydroxyl groups were transformed into methoxy groups, thereby preventing any further modification. Additionally, various sugar alcohols were used to efficiently make several different polyesters in one step through enzymatic catalysts; while branching was less than chemical methods, it was not eliminated altogether.[28,29] For drug delivery applications, a polyglycerol dendrimer with ibuprofen pendant groups achieved high drug loading (70%), but did not release ibuprofen in methanol (i.e., non-physiological conditions) after one day; no other quantitative release studies were performed.[30] Linear ketoprofen prodrugs comprised of glycerol, PEG, and sebacate linkages were developed but drug loading was <20% and products were oils, limiting their formulation potential.[31] Cross-linked mannitol-*co*-salicylic acid-*co*-sebacate polyesters were developed for localized delivery, but also exhibited limited solubility due to cross-linking and lower drug loading (~40%).[32] This uncured polymer released 20% bioactive after five days, but only 15% over the next four months; cured polymer was insoluble and released 3.5% bioactive after four months.

The focus of this work is to use a sugar alcohol to synthesize a linear, fully biodegradable polymer with high drug loading (>70%) that exhibits sustained, well-defined bioactive release under physiological conditions. Thus, we

developed a novel poly(anhydride-ester) using a mannitol-*co*-succinate backbone with four pendant ibuprofen groups per repeat unit as a model bioactive-releasing system. The new synthetic methodology presented herein is versatile and can easily be applied to a myriad of systems: sugar alcohols of different size can alter drug loading, release, and thermal properties, chain extenders of varying hydrophobicity can tune release rates, and different classes of bioactives as pendant groups can be utilized.

Polymer and precursor structures were determined by proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and Fourier transform infrared (FT-IR) spectroscopies. Thermal properties were evaluated by differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). Mass spectrometry (MS) and gel permeation chromatography (GPC) were used to determine molecular weights (Mw) of precursors and polymer, respectively. To evaluate drug release from polymer, *in vitro* studies were performed under physiological conditions. Cytocompatability of the polymer was determined using fibroblast cells. A cyclooxygenase-2 inhibition assay was performed comparing the bioactivity of released ibuprofen to that of free ibuprofen.

5.2. Results and Discussion

5.2.1. Synthesis and Physicochemical Characterization

To develop linear PAEs using mannitol as a backbone and ibuprofen as pendant groups, silyl groups were used to first protect the 1,6 positions of mannitol, followed by esterification with ibuprofen. The silyl groups were deprotected, and the diol chain extended with succinate prior to polymerization (Scheme 5.1). Slightly modifying a published procedure, the primary alcohols of mannitol were selectively protected with TBDMS groups to afford **2** after purification via column chromatography. The appearance of TBDMS groups in the ^{13}C-NMR spectrum (**Figure 5.2**) confirmed that successful protection proceeded smoothly; in addition, all characterization matches reported literature values (e.g., chemical shifts, integration). In the next step, ibuprofen was coupled to all four secondary hydroxyls of **2** via carbodiimide coupling to afford pure **4** in high yield.   The appearance of ibuprofen peaks and the significant shift of backbone methines in the NMR spectra (Figure 5.2) along with disappearance of the IR band at 3440 cm^{-1} (O-H) and the formation of an IR band (**Figure 5.3**) at 1740 cm^{-1} (ester, C=O) indicated successful and complete coupling.
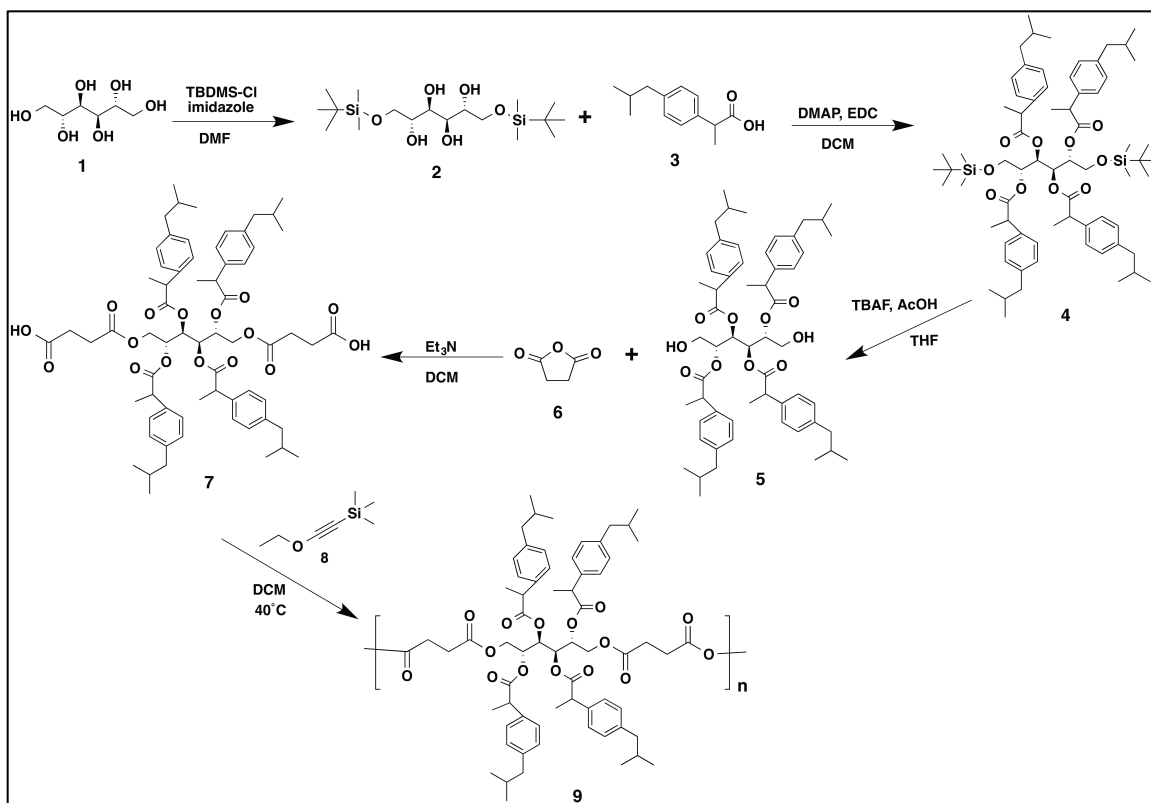
Scheme 5.1: Synthesis of poly(tetraibuprofen mannitol succinate) and precursors

To synthesize **5**, TBDMS protecting groups were selectively removed with TBAF in the presence of an equimolar amount of glacial acetic acid to counteract the basicity of TBAF. The disappearance of TBDMS groups and the preservation of all other peaks in the NMR spectrum indicated the selective deprotection; the formation of the O-H band at 3460 cm^{-1} and preservation of the ester C=O band at 1740 cm^{-1} in the IR spectra additionally confirmed product structure. Next, pure diacid **7** was generated through the ring-opening of succinic anhydride in the presence of triethylamine. The appearance of succinyl linker peaks in the NMR spectrum along with the formation of a new C=O IR band (acid, 1715 cm^{-1})

confirmed product formation. In addition to ^{1}H and ^{13}C NMR spectroscopies, and FT-IR to confirm structure, at each step, mass spectrometry was used to confirm molecular weight. All polymer precursors were also characterized by DSC to elucidate melting temperatures.
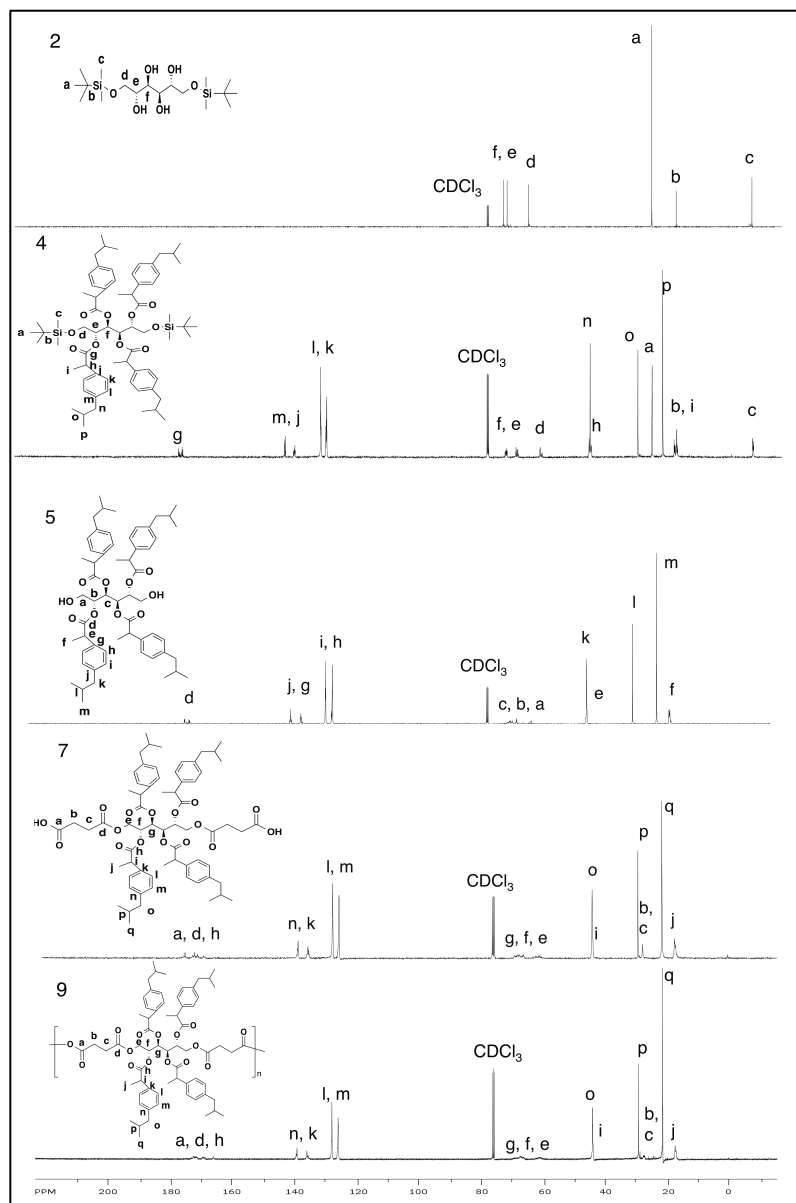
Figure 5.2: ^{13}C NMR spectra of polymer precursors **2**, **4**, **5**, and **7**, and polymer **9** showing successful synthesis

Polyanhydride formation through melt-condensation polymerization under vacuum at elevated temperatures was not viable, as the starting material started to decompose at 100°C. Thus, polymerization was carried out following a procedure by Qian and Mathiowitz[33] in which TMSEA acts as a dehydrating reagent under mild conditions to facilitate polyanhydride synthesis (**Scheme 5.2**). The ^{13}C NMR spectrum of polymer displayed all expected peaks (**Figure 5.2**). IR also indicated the formation of anhydride bands (1784 and 1824 cm^{-1}), preservation of ester band (1746 cm^{-1}), and absence of a carboxylic acid band (**Figure 5.3**). GPC determined that polymer Mw was 22 kDa and PDI was 1.5. Thermal analysis revealed that polymer T_g was 20°C and T_d was 205°C.
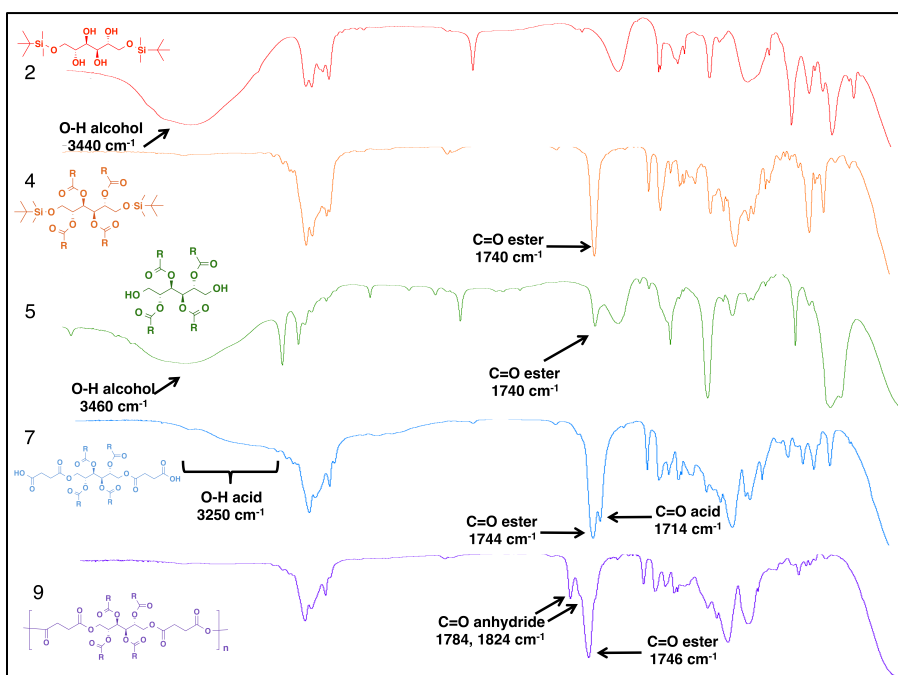
Figure 5.3: IR spectra of precursors **2**, **4**, **5**, and **7** and polymer **9**; key IR bands are labelled on each spectrum. Esterification, selective deprotection, chain extension, and polymerization is confirmed
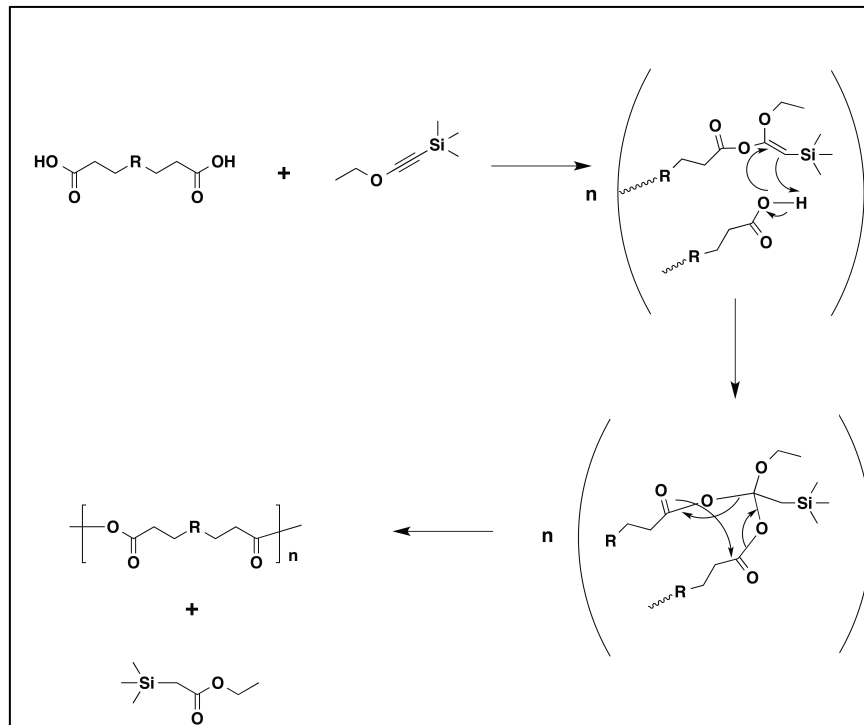
Scheme 5.2: Mechanism of polyanhydride synthesis via the electrophilic addition-elimination reaction of TMSEA. Adapted with permission from Qian H and Mathiowitz E, *Macromolecules* **2007**, 40, 7748-7751. ©2007 American Chemical Society.

5.2.2. *In Vitro* Bioactive Release

In vitro degradation of the polymers was determined by appearance of ibuprofen in degradation media samples in PBS at physiological conditions (37 °C, pH 7.4). At predetermined times, an aliquot of release media was collected and analyzed via HPLC (ibuprofen retention time: 4.08 min). Ibuprofen release is controlled by both anhydride and ester bond hydrolysis. Based upon previous

studies using NSAID-containing PAEs, more labile anhydride bonds cleave first, followed by ester bond hydrolysis.[34] PAEs exhibited controlled, sustained ibuprofen release throughout the 14 days of the study, with approximately 7% of total bioactive released after that time period (**Figure 5.4**). Complete (i.e., 100%) ibuprofen release is expected after 6 months, through extrapolation. After drying *in vacuo*, polymer residue was massed. A mass balance was performed to account for remaining ibuprofen; through HPLC data over 14 days and polymer mass, remaining ibuprofen was accounted for. Remaining polymer sample was then completely hydrolyzed with NaOH, and the ibuprofen extracted for further analysis.
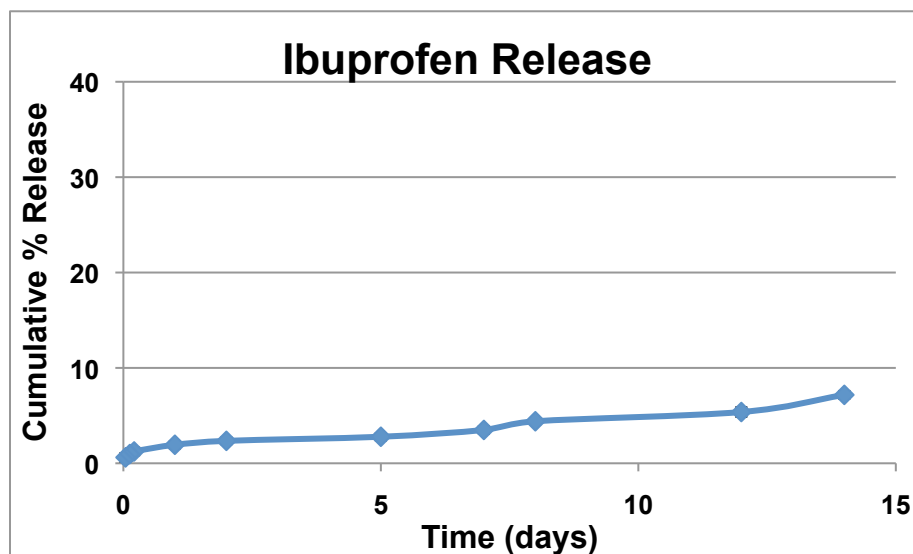
Figure 5.4: Cumulative ibuprofen release as determined by HPLC data

5.2.3. Cytocompatibility Assay

[Performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University]

As in Chapter 4, two different concentrations of polymer were tested. All polymers were cytocompatible at 0.01 mg/mL and 0.001 mg/mL over 72 h), i.e. no significant difference in cell viability was found between the polymer groups and the media alone control (**Figure 5.5**). This data demonstrated that these ibuprofen-based polymers are cytocompatible at clinically relevant concentrations and thus promising candidates for biomedical applications.
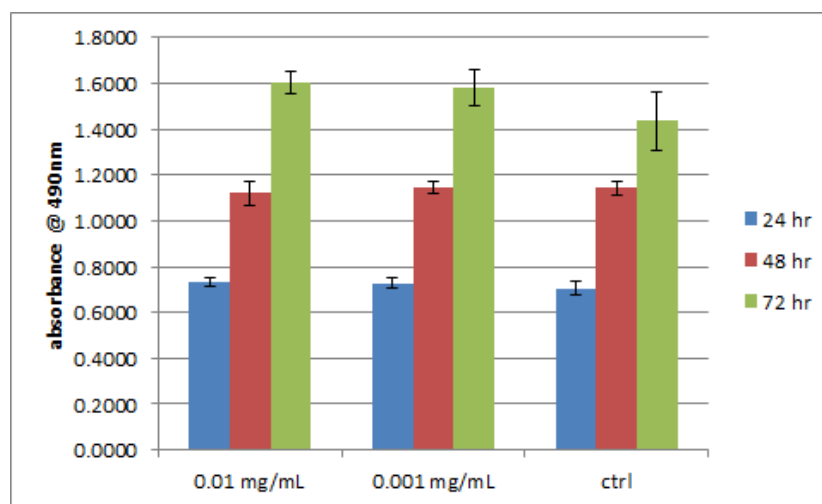
Figure 5.5: Cytocompatibility of polymers after 24, 48 and 72 h incubation. All groups contained 1% DMSO in cell media and control group has no polymer. Absorbance at 490 nm after MTS treatment is proportional to cell viability. Data presented as mean ± standard deviation. N=6 in each group

5.2.4. Anti-inflammatory Activity Via Cyclooxygenase-2 Inhibition Assay

[Performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University]

*The amount of prostaglandin (PG) produced by cyclooxygenase (COX) under various conditions was quantified. The amount of PG is proportional to COX activity; the lower the PG level, the less active is COX and thus more effective COX inhibition. All ibuprofen solutions significantly reduced PG level compared to the DMSO negative control (**Figure 5.6**). The extracted ibuprofen showed slightly higher inhibition activity than the treated or pure ibuprofen solution. DMSO alone yielded much higher PG compared to the inactive enzyme baseline.*

Pure ibuprofen showed comparable activity with treated ibuprofen, showing that the extraction process did not affect ibuprofen activity. The extracted ibuprofen had comparable activity with the treated ibuprofen, suggesting that neither the polymerization process nor the release study impaired ibuprofen activity. The extracted ibuprofen had higher activity than the treated ibuprofen, probably due to the presence of small amounts of intermediate degradation products, which would gradually degrade to yield more ibuprofen. The DMSO alone sample had significantly higher PG level compared to inactive enzyme, proving that the amount of PG detected is indeed produced by COX and any reduction in PG is due to the inhibition of COX activity.
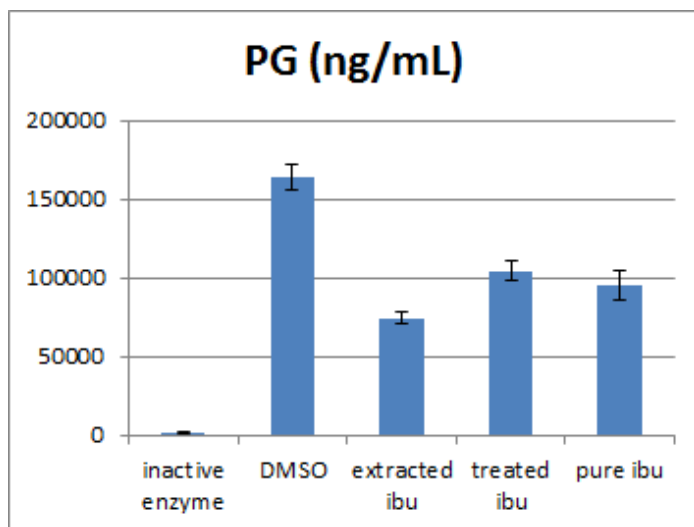
Figure 5.6: *Plot of PG levels of five samples. A lower concentration means higher anti-inflammatory activity. ibuprofen-containing media from polymer degradation exhibited slightly higher activity than ibuprofen alone, likely due to intermediate degradation products*

5.3. Conclusion

Novel PAEs containing high bioactive content via multiple pendant group attachments per repeat unit were successfully synthesized. With ibuprofen and mannitol as model bioactive and backbone, respectively, controlled bioactive release via polymer hydrolytic degradation could be achieved. Furthermore, polymers were non-toxic to fibroblast cells and the released ibuprofen retained anti-inflammatory activity. As the first linear biodegradable polymers using polyols with high bioactive loading, these PAEs can be modified with respect to number of pendant groups, stereochemistry, and chain extender hydrophobicity, thereby altering release rate and thermal properties. With multiple pendant sites,

additional molecules, such as other bioactives to achieve a synergistic effect and targeting moieties to efficient delivery can be incorporated into the polymer. Investigation of sugar alcohol bioactivity

5.4. Experimental

5.4.1. Materials

Silica gel was obtained from VWR (Radnor, PA). Chloroform-d (Acros, 99.8%), Poly(vinylidine fluoride) (PVDF) and poly(tetrafluoroethylene) (PTFE) syringe filters, and Wheaton glass scintillation vials were obtained from Fisher Scientific (Fair Lawn, NJ). EDC was obtained from AK Scientific (Union City, CA). Ethoxyacetylene (50% w/w in hexanes) was obtained from GFS Chemicals (Powell, OH). All other reagents, solvents, and fine chemicals were obtained from Sigma Aldrich (Milwaukee, WI) and used as received.

5.4.2. Polymer and Precursor Synthesis

5.4.2.1. 1,6-Di-(*t*-butyldimethylsilyl) Mannitol (2) Synthesis

Using a slightly modified procedure to synthesize 1,6-di-*O*-(*t*-butyldimethylsilyl)-mannitol (**2**), D-mannitol (**1**, 1 eq) was stirred in anhydrous DMF (15 mL/g) under nitrogen at 0°C as shown in **Scheme 5.1**. Imidazole (2.2

eq) was added followed by addition of *tert*-butyldimethylsilyl chloride (TBDMS-Cl, 2.2 eq). Reaction was stirred at 0°C for 30 min. After reaching room temperature, the reaction was then stirred at 40°C for an additional 3 hrs. Reaction mixture was diluted with DI water and extracted with EtOAc (2x). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting oil was purified on silica gel via column chromatography with a gradient of 4:1 hexanes:EtOAc to 100% EtOAc as eluent to yield pure compound **2**.

Yield: 58% (white waxy solid). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.71 (m, 8H, OH, CH_2); 3.21 (s, 2H, CH); 2.78 (s, 2H, CH); 0.80 (s, 12H, CH3); 0.00 (s, 18H, CH_3). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 72.3 (2C), 71.2 (2C), 64.4 (2C), 25.9 (6C), 18.4 (2C), -5.3 (4C). IR (NaCl, cm^{-1}): 3440 (O-H). MS (ESI): *m/z* = 423.4 [M + Na]. T_m not observed

5.4.2.2. 2,3,4,5-Tetraibuprofen-1,6-Di-(*t*-butyldimethylsilyl) Mannitol (4) Synthesis

Compound **2** (1 eq) was dissolved in anhydrous DCM (10 mL/g). 4-Dimethylaminopyridine (DMAP, 4 eq) was added, followed by ibuprofen (**3**, 4.2 eq) dissolved in anhydrous DCM (5 mL/g). EDC (6 eq) was then added slowly. Reaction was stirred under nitrogen at room temperature overnight. Reaction mixture was diluted with EtOAc and washed with 10% $KHSO_4$ (2x), saturated $NaHCO_3$ (2x), and brine. The organic layer dried was over $MgSO_4$, filtered, and concentrated *in vacuo* to yield pure compound **4**.

Yield: 88% (3.75 g pale yellow paste). ^{1}H-NMR (CDCl$_3$), 500 MHz): δ 7.21 (m, 8H, Ar-H); 7.19 (m, 8H, Ar-H); 5.39-5.60 (m, 2H, CH); 4.55-4.85 (m, 2H, CH); 3.69 (m, 4H, CH); 2.88-3.57 (m, 4H, CH$_2$); 2.47 (m, 8H, CH$_2$); 1.48 (m, 12H, CH$_3$); 0.92 (m, 24H, CH$_3$); 0.72-0.88 (split m, 18H, CH$_3$); 0.34-0.01 (split m, 12H, CH$_3$). ^{13}C-NMR (CDCl$_3$, 125 MHz): δ 171.9 (4C), 139.5 (4C), 139.1 (4C), 128.1 (4C), 126.5 (4C), 70.3 (2C), 66.8 (2C), 59.6 (2C), 43.9 (4C), 44.0 (4C), 29.1 (4C), 24.7 (6C), 21.3 (8C), 16.9 (6C), -6.8 (4C). IR (NaCl), cm^{-1}): 1740 (C=O, ester). MS (ESI): *m/z* = 1185.2 [M + Na]. T$_m$ not observed

5.4.2.3. 2,3,4,5-Tetraibuprofen Mannitol (5) Synthesis

Compound **4** was dissolved in anhydrous THF (5 mL/g) and stirred at 0°C under nitrogen. Glacial acetic acid (8 eq) was added followed by addition of 1.0 M tetrabutylammonium flouride (TBAF, 8 eq) in THF over 5 min. Reaction was stirred overnight, and was allowed to reach room temperature. Reaction mixture was diluted with EtOAc and washed with saturated NaHCO$_3$ (2x), DI water, and brine. The organic layer dried was over MgSO$_4$, filtered, and concentrated *in vacuo.* The resulting oil was purified on silica gel via column chromatography with a gradient of 2:1 hexanes:EtOAc to 100% EtOAc as eluent to yield pure compound **5**.

Yield: 81% (pale yellow paste). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 7.16 (m, 8H, Ar-H); 7.09 (m, 8H, Ar-H); 4.90 (m, 2H, CH); 4.00-4.46 (m, 2H, CH); 3.56-3.97 (broad m, 8H CH$_2$, CH); 2.44 (m, 8H, CH$_2$); 1.83 (m, 4H, CH); 1.44 (m, 12H,

CH_3); 0.89 (m, 24H, CH_3). ^{13}C-NMR (CDCl$_3$, 125 MHz): δ 171.0 (4C), 140.9 (4C), 137.6 (4C), 129.6 (4C), 127.4 (4C), 70.4 (2C), 67.8 (2C), 65.9 (2C), 45.3 (4C), 45.2 (4C), 30.4 (4C), 22.7 (8C), 18.5 (4C). IR (NaCl, cm^{-1}): 3460 (O-H), 1740 (C=O, ester). MS (ESI): *m/z* = 957.2 [M + Na] . T$_m$ not observed.

5.4.2.4. 2,3,4,5-Tetraibuprofen Mannitol Succinyl Diacid (7) Synthesis

Compound **5** (1 eq) was dissolved in anhydrous DCM (5 mL/g). Succinic anhydride (**6**, 2 eq) was added, followed by anhydrous triethylamine (4 eq). Reaction was stirred overnight then washed with acidic H_2O (3x) and brine. The organic layer was dried over MgSO$_4$, filtered, and concentrated *in vacuo* to yield pure compound **7**.

Yield: 86% (off white foam). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 7.16 (m, 8H, Ar-H); 7.09 (m, 8H, Ar-H); 4.86-5.30 (m, 2H, CH); 3.83-4.23 (m, 2H, CH); 3.33-3.63 (broad m, 8H, CH2, CH); 2.59 (m, 8H, CH$_2$); 2.46 (d, 8H, CH$_2$); 1.83 (m, 4H, CH); 1.44 (m, 12H, CH$_3$); 0.89 (m, 24H, CH$_3$). ^{13}C-NMR (CDCl$_3$, 100 MHz): δ 177.5 (2C), 175.0 (4C), 173.3 (2C), 140.8 (4C), 137.5 (4C), 129.4 (4C), 127.6 (4C), 70.4 (2C), 68.8 (2C), 65.9 (2C), 45.3 (4C), 45.2 (4C), 30.6 (4C), 30.0 (4C), 22.7 (8C), 18.5 (4C). IR (NaCl, cm^{-1}): 3250 (O-H, acid), 1744 (C=O, ester), 1714 (C=O, acid). MS (ESI): *m/z* = 1157.0 [M + Na]. T$_m$ not observed

5.4.2.5. (Trimethylsilyl)ethoxyacetylene (8) Synthesis

TMSEA was synthesized using the procedure outlined by Qian and Mathiowitz.[33] Briefly, ethoxyacetylene (EA) solution (1 eq. EA) was dissolved in anhydrous diethyl ether (12 mL/g) at 0°C. Methyllithium (1.6 M in diethyl ether, 1.05 eq) was added dropwise over 1 h. Solution continued to stir for 30 min before trimethylsilyl chloride (1.05 eq) was added dropwise over 30 min. After 30 min stirring at 0°C, reaction was brought to room temperature and stirred overnight. Resulting solid salt was filtered and washed with ether (3x). Ether was removed *in vacuo*, the resulting oil was dissolved in anhydrous pentane (8 mL/g), and then placed in freezer overnight. Resulting solid was filtered off and solution was vacuum distilled (20 Torr, 70°C) to afford TMSEA.

Yield: 70 % (clear liquid). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 4.13 (q, 2H, CH$_2$); 1.37 (t, 3H, CH$_3$); 0.13 (s, 9H, CH$_3$). ^{13}C-NMR (CDCl$_3$, 100 MHz): δ 109.7, 75.0, 36.9, 14.4, 0.8 (3C).

5.4.2.6. Poly(2,3,4,5-tetraibuprofen Mannitol Succinate (9) Synthesis

Using a previously published procedure to synthesize polyanhydrides,[33] diacid **7** (1 eq) was dissolved in anhydrous DCM (1 mL/mmol diacid) in a glass vial. TMSEA (**8**, 1.1 eq) was added. Vial was capped and stirred at 40°C for 4 h. Mixture was concentrated *in vacuo*. Resulting product was dissolved in ACN and

washed with hexanes. ACN layer was concentrated *in vacuo* to afford pure polymer **9**.

Yield: 85% (light tan foam). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 7.16 (b, 8H, Ar-H); 7.09 (b, 8H, Ar-H); 5.01-5.45 (b, 2H, CH); 3.86-4.35 (b, 2H, CH); 3.40-3.73 (broad m, 8H, CH$_2$, CH); 2.43 (b, 8H, CH2); 1.83 (b, 4H, CH); 1.42 (b, 12H, CH$_3$); 0.89 (b, 24H, CH$_3$). ^{13}C-NMR (CDCl$_3$, 125 MHz): δ 170.7 (2C), 147.7 (2C), 137.3 (2C), 137.2 (2C), 128.1 (2C), 126.9 (2C), 122.5 (2C), 72.7 (2C), 27.0 (2C), 23.5 (2C), 23.3 (2C), 21.0 (2C); IR (NaCl, cm^{-1}): 1824 and 1784 (C=O, anhydride), 1746 (C=O, ester). T_g = 20°C, T_d = 205°C

5.4.3. Gel Permeation Chromatography

Polymer weight-averaged molecular weights (M_w) and polydispersity indices (PDI) were determined by gel permeation chromatography (GPC) on a Waters liquid chromatography system consisting of a 2414 refractive index detector, a 1515 isocratic HPLC pump, and a 717plus autosampler. Automation of the samples and collection and processing of the data was done using an IBM Thinkcentre computer running Breeze 2 software. Polymer samples were prepared for autoinjection by dissolving polymer in DCM (10 mg/mL) and filtering through 0.45 µm poly(tetrafluoroethylene) (PTFE) syringe filters. Samples were resolved on a Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm, Alltech Associates, Deerfield, IL) at 25°C, with DCM as eluent at a flow rate of 1.0

mL/min. Molecular weights were calibrated relative to polystyrene standards (Polymer Source Inc., Dorval, Canada).

5.4.4. *In Vitro* Bioactive Release Studies

Drug release from polymers **9** was evaluated by *in vitro* degradation in phosphate buffered saline (PBS) under physiological conditions. Polymer samples (20 mgs each, n = 3) 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. At predetermined time intervals throughout the 14 days of the study, media (10 mL) was collected and replaced with fresh PBS (10 mL) and the spent media analyzed by high performance liquid chromatography (HPLC). Analysis was performed using an XTerra RP18 3.5 µm 4.6 × 150 mm column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual Absorbance Detector. All samples were filtered using 0.22 µm PVDF syringe filters and subsequently injected (20 µL) using an autosampler. The mobile phase was comprised of acetonitrile (60 %) and 10 mM KH_2PO_4 in DI water at pH 2.5 (40 %) run at 1.0 mL/min flow rate and ambient temperature. Absorbance was monitored at λ = 223, one of the absorption wavelengths for ibuprofen. Concentrations were calculated from a calibration curve of known standard solutions. At conclusion of study, remaining sample was hydrolyzed with 1 N NaOH and extracted with EtOAc. Organic layer was dried over $MgSO_4$,

filtered, and concentrated *in vacuo*. This released ibuprofen was used for further analysis.

5.4.5. Cytocompatibility Assay

[Performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University]

In vitro cytocompatibility studies were performed by culturing 3T3 mouse fibroblasts in cell media (DMEM supplemented with 10 % FBS, 1 % pen/strep) containing the 3 different polymers. Polymers were first sterilized under UV at λ = 254 nm for 900 s (Spectronics Corporation, Westbury, NY), dissolved in DMSO to yield 10 mg/mL solutions, and then diluted with cell media to reach concentrations of 0.01 mg/mL and 0.001 mg/mL. Cell media containing polymers were then added to allocated wells in a 96-well plate with 2000 cells/well and incubated at 37 °C. DMSO (1 %) in cell media was used as control. Cell viability was determined using CellTiter 96® Aqueous One Solution Cell Proliferation Assay. After 24 h, 48 h, and 72 h incubation with polymers, 20 µL of (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) reagent was added to each well and further incubated for 4 h at 37 °C. The absorbance was then recorded with a microplate reader (Coulter, Boulevard Brea, CA) at 492 nm.

5.4.6. Anti-inflammatory Activity Via Cyclooxygenase-2 Inhibition Assay

[Performed by Weiling Yu, Department of Biomedical Engineering, Rutgers University]

The in vitro bioactivity of the released ibuprofen was determined using the COX inhibitor screening assay kit (Cayman Chemicals, Ann Arbor, MI) following manufacturer's protocol. The released ibuprofen was extracted with EtOAc (see end of Section 5.4.4.). Ibuprofen treated with EtOAc then dried in vacuo was used as a positive control and the pure, unaltered ibuprofen solution was chosen to ensure that the extraction would not alter ibuprofen activity. The ibuprofen solutions were pre-incubated with COX for 15 minutes at 37 ˚C. DMSO was used as negative control and inactive COX was used to assess the baseline according to the protocol. At the end, the plate was incubated for 60 min and then absorbance at 410 nm was measured.

*Other methods described in Chapter 9.

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6. SUGAR-BASED POLY(ANHYDRIDE-ESTERS) CONTAINING NATURAL ANTIOXIDANTS AND ANTIMICROBIALS: SYNTHESIS AND FORMULATION INTO POLYMER BLENDS

6.1. Introduction

Because of the pervasive issue of foodborne illnesses,[1] active packaging has garnered increasing interest.[2-7] Traditionally, the purpose of food packaging was to prevent direct environmental contamination (e.g., dirt) and delay the inevitable spoilage of many food products.[5] However, the advent of active packaging allows for dynamic package properties; prevention of bacterial spoilage and oxidation through release of antioxidants and antimicrobials, controlled gas permeation, and moisture control have all manifested themselves as attributes of active packaging to increase shelf-life and food safety.[2-7] To prevent bacterial growth and spoilage, one of the most dangerous consequences of poorly-kept food, antimicrobial agents can be added to the food product itself, or to the packaging (e.g., wrap, container). The latter method is preferred, as consumers do not approve of synthetic food additives over concerns about side effects; in addition, spoilage starts on the food-air interface, so antibacterial additives throughout the entire foodstuff is unnecessary.[8] Rather than synthetic additives, naturally-occurring antimicrobial agents have the added benefit of being classified as generally regarded as safe (GRAS) by the FDA; oftentimes, these compounds can be found in essential oils of herbs.[9] Such ubiquitous

examples include carvacrol and thymol, the components of oregano and thyme oils, respectively.[10] Research on adding carvacrol, thymol, and numerous other antimicrobial agents, to existing polymer packaging has been extensively reviewed by Kuorwel, *et al.*;[8] blending with non-biodegradable polymers, such as low density polyethylene (LDPE) and polypropylene (PP), as well as biodegradable polycaprolactone (PCL) and poly(lactic-*co*-glycolic acid) has shown some success against a range of bacteria;[2,8,11-18] however, concerns remain about the effectiveness of adding these small molecules to packaging films. Previous research has shown that processing (i.e., blending at elevated temperatures) many natural antimicrobials reduce their efficacy, primarily owing to their inherent volatility.[12] That is, a portion of the antimicrobial agent is lost during processing, resulting in less active incorporated than needed to be effective.[3,19,20]

Moreover, current active packaging methods do not address the issue of recyclability or sustainability; physical admixture of small molecule antimicrobials can potentially mediate food spoilage, but does not decrease the amount of nondegradable polymer used. Similarly, a biodegradable/nonbiodegradable polymer blend can be more environmentally friendly through reduction of nonbiodegradable polymer content but may not exhibit antimicrobial activity. Thus, a novel biodegradable polymer containing biorenewable compounds *and* antimicrobials could reduce LPDE content whilst exhibiting active packaging capabilities. With unique surface-erosion properties that facilitate near-zero order release and highly tunable chemical structures, poly(anhydride-esters) (PAEs)

could function as the biodegradable component of an active packaging polymer blend.

The focus of this project is developing a novel polymer system that undergoes controlled hydrolysis to release antimicrobial and antioxidant compounds for active packaging applications. Moreover, all degradation products are on the GRAS list. PAEs with thymol, tartaric acid, and succinic acid chemically incorporated into the polymer were synthesized to overcome the issues of current active packaging systems. Polymer and precursor structures were determined by proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and Fourier transform infrared (FT-IR) spectroscopies. Thermal properties were evaluated by differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA). Mass spectrometry (MS) and gel permeation chromatography (GPC) were used to determine molecular weights (Mw) of precursors and polymer, respectively. Novel PAEs were blended with LDPE to ensure miscibility and compressed into films. Bioactive release rate from raw polymer was elucidated at room temperature. Antioxidant and antimicrobial studies were performed on release media from both raw and processed polymer to ensure that formulation at elevated temperature did not impact bioactivity of released thymol.

6.2. Results and Discussion

## 6.2.1. Synthesis and Physicochemical Characterization
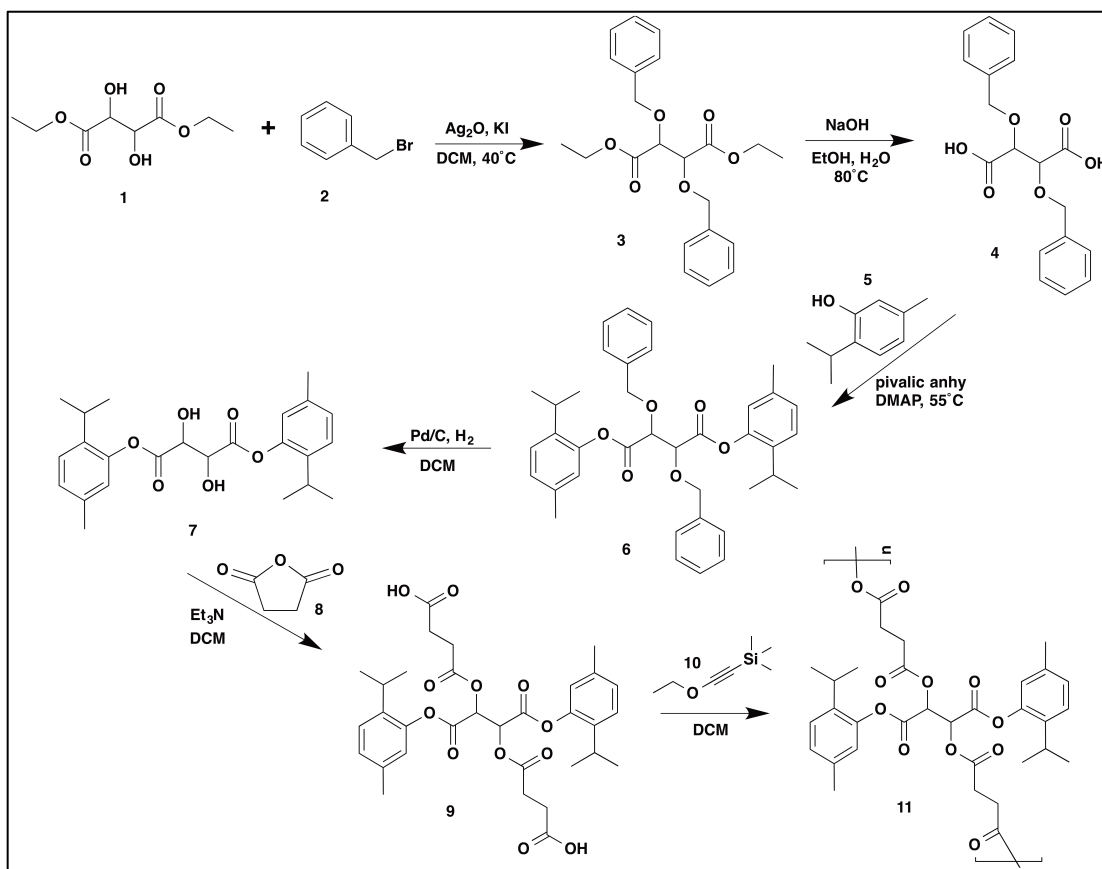
**Scheme 6.1:** Synthesis of thymol-containing poly(anhydride-ester) and polymer precursors

Using a previously published procedure,[21] the hydroxyl groups of diethyl-L-tartrate were benzyl protected using benzyl bromide and silver oxide. The appearance of benzylic and aromatic signals in the ^{1}H-NMR spectrum (**Figure 6.1)** and absence of an alcohol FT-IR band (**Figure 6.2**) of **3** confirmed that successful protection of secondary alcohols proceeded smoothly. Thereafter, the ethyl esters were cleaved under basic conditions to afford free acid **4** in high yield. The disappearance of the ethyl group protons in the NMR spectra (**Figure**

6.1) along with disappearance of the IR band at 1756 cm^{-1} (ester, C=O) and the formation of an IR band (**Figure 6.2**) at 1732 cm^{-1} (acid, C=O) indicated successful deprotection. Thymol was next coupled to the alcohol via a solvent-free esterification using catalytic DMAP and pivalic anhydride to afford crude **6**. Crude compound **6** was reacted in the next step, with palladium-catalyzed hydrogenolysis selectively cleaving the benzyl ethers of **6** while leaving the thymol ester groups intact. After filtration through Celite and removal of solvent *in vacuo*, the crude oil was chromatographed to yield pure **7**. NMR spectra (**Figure 6.1**) indicated the presence of peaks associated with thymol and the disappearance of benzylic protons. IR spectra (**Figure 6.2**) indicated ester bond formation in the previous step by the disappearance of the carboxylic acid IR band (1732 cm^{-1}) and the new pendant ester linkage at 1743 cm^{-1}, in addition to the formation of an alcohol band at 3500 cm^{-1} (O-H). Next, pure diacid **8** was generated through the ring-opening of succinic anhydride in the presence of triethylamine. The appearance of succinyl linker peaks and the significant upfield shift of the tartaric acid methine protons in the NMR spectra (**Figure 6.1**) along with the formation of a new carbonyl IR band (acid, 1715 cm^{-1}) confirmed product formation. MS confirmed all molecular weight of all products, and ^{13}C-NMR was further confirmed structure. DSC was used to elucidate melting temperatures of all polymer precursors.
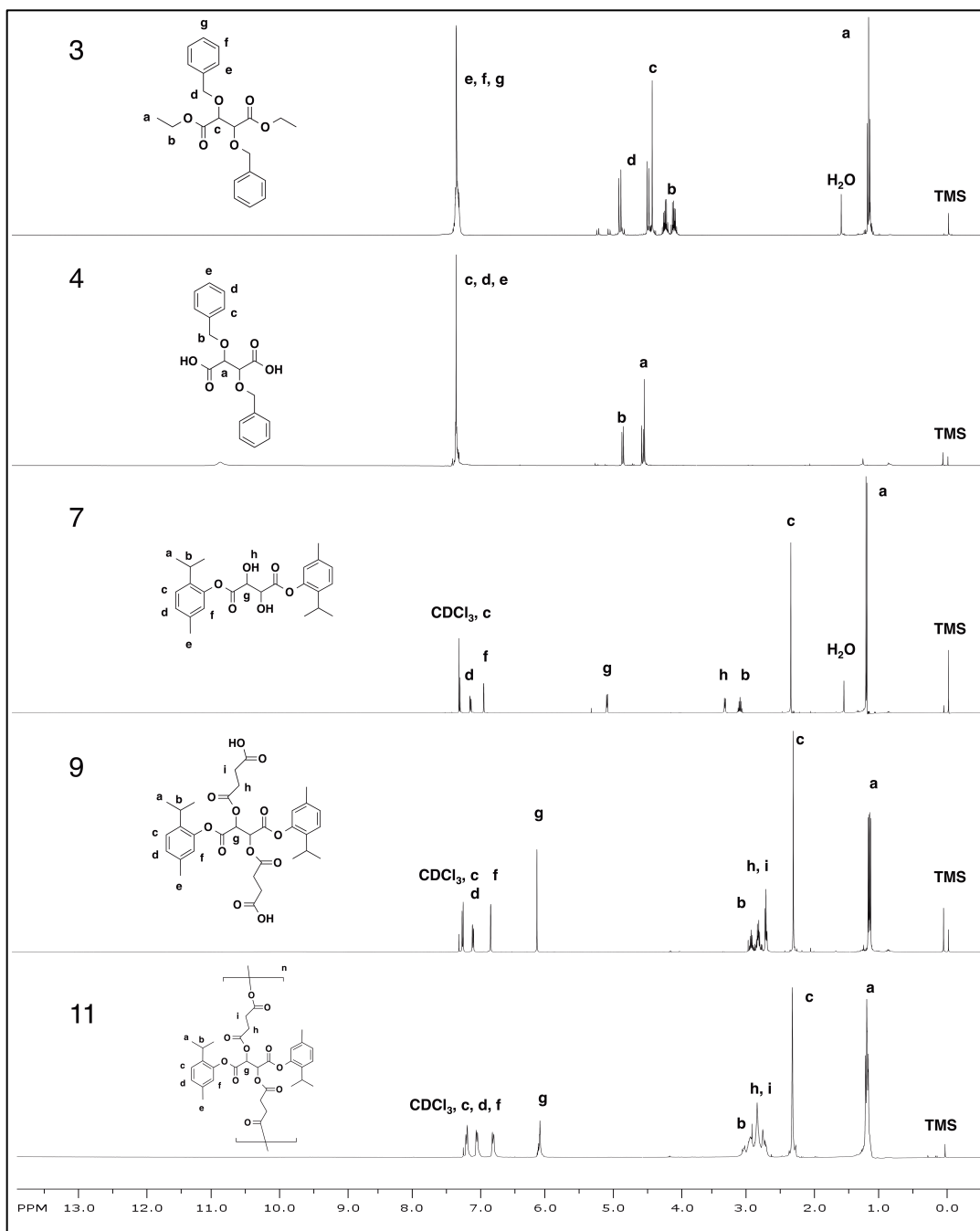
Figure 6.1: ^{1}H NMR spectra of polymer **11** and precursors **3**, **4**, **7**, and **9** with peak assignments showing ethyl deprotection, coupling, benzyl deprotection, chain extension, and polymerization
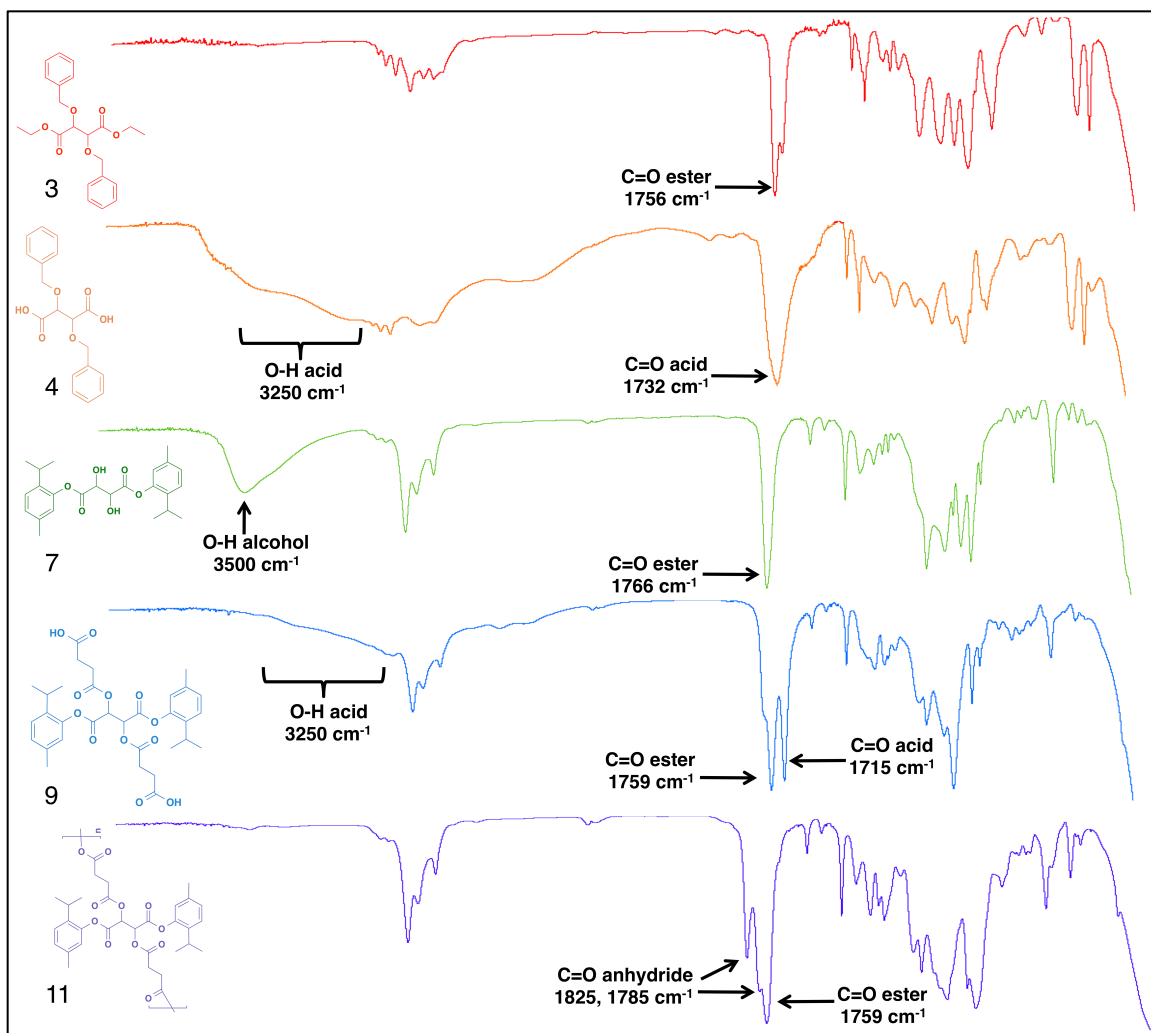
Figure 6.2: FT-IR spectra of polymer **11** and precursors; key IR bands are labelled on each spectrum to highlight the formation of new chemical bonds

Polymerization was carried out following a procedure by Qian and Mathiowitz[22] in which TMSEA acts as a dehydrating reagent under mild conditions to facilitate polyanhydride synthesis. TMSEA was synthesized using a reported procedure.[22] Following polymerization, ^{1}H NMR spectrum of PAE **11** (**Figure 6.1**) displayed all expected peaks. IR spectra (**Figure 6.2**) also indicated the formation of anhydride bands (1825 and 1785 cm^{-1}), preservation of ester band (1759 cm^{-1}), and absence of a carboxylic acid band. GPC determined that

polymer M_w's was 32 kDa and PDI was 1.5. Thermal characterization revealed that polymer T_g was 34 °C; polymers were amorphous and did not exhibit a T_m. Given that T_d was ~200°C, polymers could be processed via melt-blending at elevated temperatures.

6.2.2. *In Vitro* Bioactive Release

Bioactive release was carried out on raw polymer discs in neutral DI water before polymer was processed into a film. Given that **11** is a novel polymer system, thymol release under controlled, neutral conditions was determined before any further processing or formulation. The degradation rate of polymer into bioactive via anhydride and ester bond hydrolysis is an important factor in obtaining controlled antimicrobial release. Based upon studies of PAEs, the anhydride bonds are expected to hydrolyze first, followed by the ester bonds. The studies were conducted for 21 d, after which the remaining polymer disc was assessed to perform a mass balance. The gravimetric mass loss matched the calculated mass loss (from HPLC) to within 5%. While diacid **9** was minimally soluble in the release media, minute amounts of bisthymol tartrate **7** and monothymol tartrate were also observed in degradation media, with the amounts of thymol increasing over time (e.g., thymol retention time of 4.14 min, monothymol tartrate retention time of 8.78 min, bisthymol tartrate retention time of 18.00 min). A small burst of thymol occurred in the first day of release, likely due to the presence of low molecular oligomers, as PAE was not fractionated

prior to formulation. After day one, constant, well-controlled thymol release was observed (**Figure 6.3**). Furthermore, at each timepoint, a bioactive concentration of thymol was released from the polymer disc. While different formats (e.g., films, powders) will alter release rates, this study elucidated that thymol was released from discs in a controlled and sustained manner.
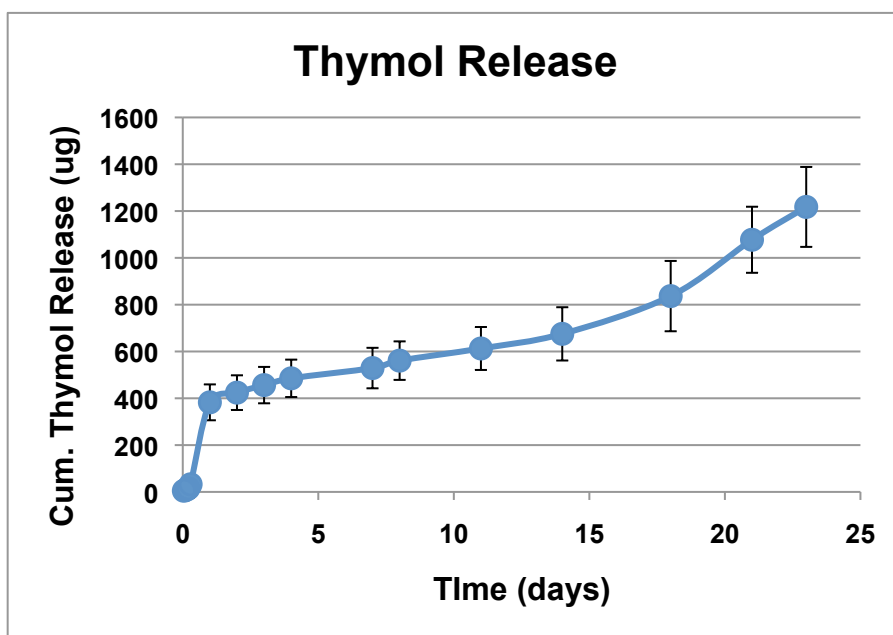

Figure 6.3: Cumulative thymol release from PAE **11** over time as determined by HPLC showing sustained release

6.2.3. LDPE/PAE Blend Formulation

Because the ultimate goal is to formulate PAE polymer into food packaging, PAE **11** was melt-blended with LDPE. Blending the two polymers would impart active packaging capabilities (i.e., antioxidant and antimicrobial activities) while maintaining the flexibility required for the films; in addition,

replacement of a percentage of LDPE with PAE lowers the amount of non-biodegradable polymer in the formulation. Moreover, mechanical properties could be retained when compared with blending thymol with LDPE, as small molecules tend to plasticize blends; because two polymers are blended in this work, the plasticizing effect should be decreased. Thus, LPDE and PAE were melt-blended at 150°C with constant overhead stirring to ensure a homogenous blend was obtained. Upon removal from heat, light tan polymer blend was compression molded into a 200 µm thick film at 130°C. The translucent films that were formulated elucidate LDPE/PAE film potential for packaging applications (Figure 6.4).
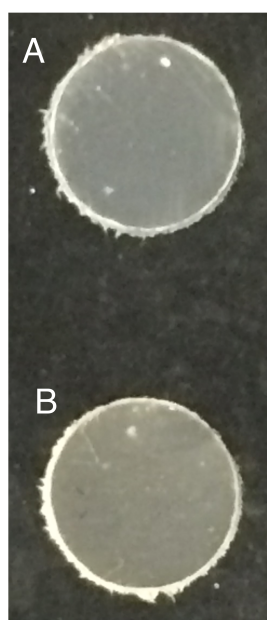
Figure 6.4: Compression molded film of pure LDPE (A) and LDPE with PAE blend at 10 wt% (B) shows transparency of films

6.2.4. Antioxidant Studies

To ensure bioactive released from polymers exhibited the same efficacy as free bioactive, a DPPH radical quenching assay was used.[23] This assay uses UV-vis spectrophotometric analysis at 517 nm to monitor change in absorption due to radical quenching; the solution color turns from deep purple to light yellow upon quenching, thereby reducing absorbance at 517 nm. Because this assay depends on antioxidant concentration,[24] degradation media at 24 h were analyzed and compared to freshly prepared solutions of free thymol of the same concentrations (25 μg/mL). In addition, thymol extracted from polymer blend degradation media was tested at the same concentration. Student's t-tests were performed to ascertain significant differences ($p < 0.05$) between the released and free thymol samples. The observed activities displayed no significant differences between released samples and free bioactive for all samples (**Figure 6.5**); thus neither polymerization nor processing at elevated temperatures had an effect on free radical quenching ability.
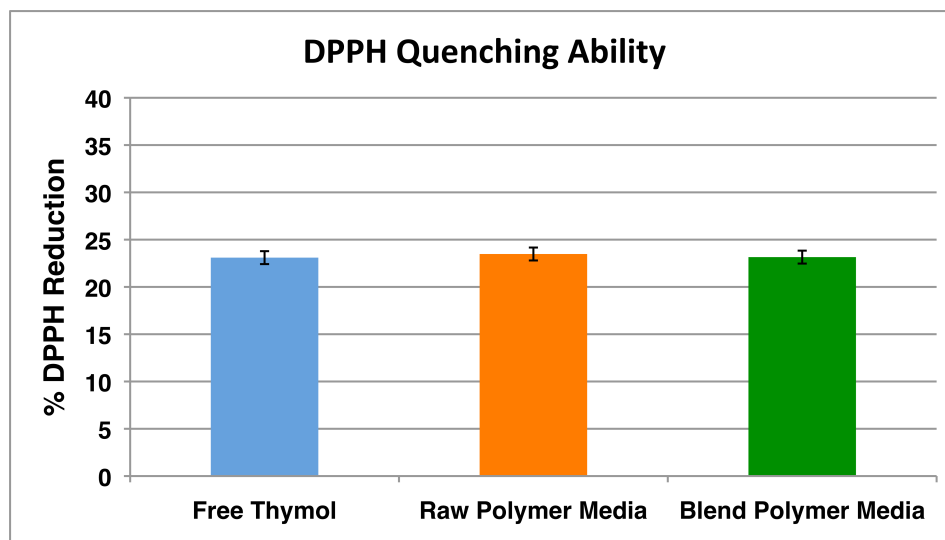
Figure 6.5: DPPH reduction results comparing free thymol to thymol extracted from raw PAE and LDPE/PAE blend

6.2.5. Antibacterial Studies

As thymol is a well-known antimicrobial agent exhibiting activity against a range of bacteria,[9] the activity of thymol released from PAE **11** (i.e., degradation media) against bacteria was tested using the disc diffusion method. Initially, antibacterial studies on hydrolyzed polymer, both raw and processed, was performed to ensure that polymer degradation product (i.e., thymol) retained its bioactivity after polymerization and melt blending. As in **Section 6.2.4**., PAE was completely hydrolyzed and a specific bioactive concentration was prepared from extracted products and compared to that of equivalent concentrations of free bioactives; the concentration for all bioactives was kept at 10 mg/mL (greater than the minimum inhibitory concentrations (MICs) to ensure a clear zone is

observed) in 1:1 PBS:DMSO. *S. aureus*, a Gram-positive bacterium, and *E. coli*, a Gram-negative bacterium, were both evaluated, as both strains are commonly encountered and often responsible for contamination of products leading to spoilage. As depicted in **Figure 6.6**, free thymol diffused from the paper disc into the agar, thereby causing a zone of bacterial growth inhibition, represented by a ring around the disc. In all cases, the zones of inhibition were nearly identical (**Table 6.1**); as with the DPPH results, processing methods did not have an impact on bioactivity.
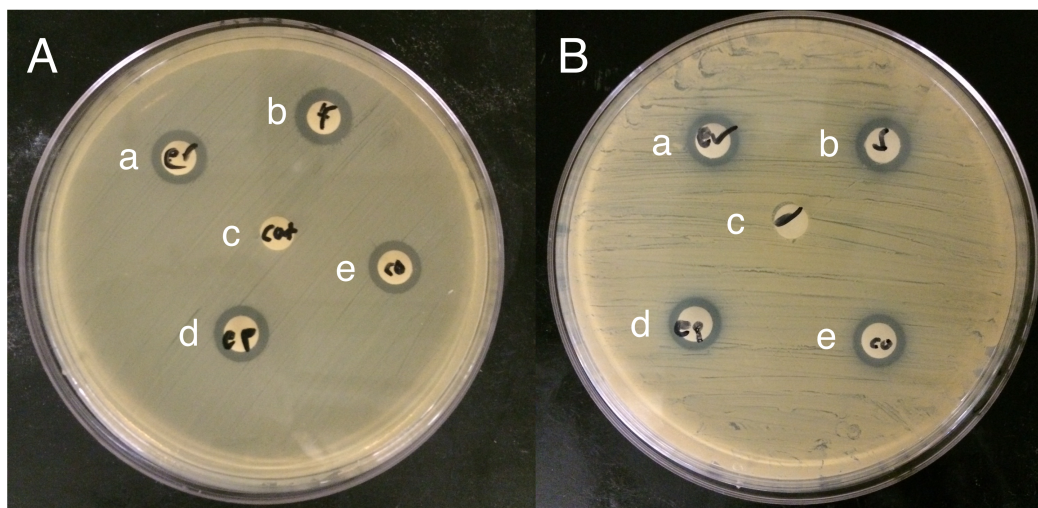
Figure 6.6: Disk diffusion assay results for *E. coli* (**A**) and *S. aureus* (**B**) showing zones of growth inhibition for thymol from raw polymer (a), free thymol (b), 1:1 H₂O:DMSO (c), thymol from polymer blend (d), mixture of a-d (e) showing that activity is retained in all cases

Table 6.1: Sizes of zones of growth inhibition for free thymol and thymol released from polymers

Entry	Compound	E. coli (A) Zone of inhibition (mm)	S. aureus (B) Zone of inhibition (mm)
a	Thymol from raw polymer	10.5	9
b	Free thymol	11	9.5
c	1:1 H$_2$O:DMSO	-	-
d	Thymol from polymer blend	10.5	9
e	Mixture of **a-d**	10.5	9

6.3. Conclusion

PAEs derived from renewable resources, including a natural phenol with antioxidant and antimicrobial activities, were designed and physicochemical properties determined. Raw polymer (**11**) in disc formulation hydrolytically degraded at room temperature in a controlled, near-zero order fashion to consistently release concentrations of thymol above its MIC. Formulation into miscible LDPE/PAE blends elucidated their potential for active packaging applications. Antimicrobials screening techniques and a free radical quenching assay determined that no bioactivity was lost during high temperature processing, in contrast to previous literature reports of thymol/LDPE blends.

Not only can PAEs prevent bacterial spoilage and food oxidation to eradicate foodbourne illnesses, but their completely biodegradability leads to a more sustainable and environmentally friendly type of food packaging.

6.4. Experimental

6.4.1. Materials

Silica gel was obtained from VWR (Radnor, PA). Poly(vinylidine fluoride) (PVDF) and poly(tetrafluoroethylene) (PTFE) syringe filters, and Wheaton glass scintillation vials were obtained from Fisher Scientific (Fair Lawn, NJ). Ethoxyacetylene (50% w/w in hexanes) was obtained from GFS Chemicals (Powell, OH). All other reagents, solvents, and fine chemicals were obtained from Sigma Aldrich (Milwaukee, WI) and used as received.

6.4.2. Polymer and Precursor Synthesis

6.4.2.1. Dibenzyl Diethyl-L-Tartrate (3) Synthesis

Using a modified procedure[21] to benzyl protect tartaric acid alcohols, diethyl-L-tartaric acid (**1**, 1 eq) was dissolved in anhydrous DCM and stirred under nitrogen as shown in **Scheme 6.1**. Silver (I) oxide (Ag_2O, 2.2 eq) was added to the reaction followed by potassium iodide (KI, 20 mol%) to form a dark suspension. Benzyl bromide (3 eq) was then added and reaction was refluxed at

45°C with stirring for 4 h in the dark. The reaction mixture was then filtered through Celite. The filtrate was washed with DI water, the organic layer dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting oil was purified on silica gel via column chromatography with 4:1 hexanes:ethyl acetate as eluent to yield pure compound **3**.

Yield: 88% (colorless oil). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 7.30 (m, 10H, Ar-H); 4.87 (d, *J* = 11.9 Hz, 2H, CH$_2$); 4.46 (d, *J* = 11.9 Hz, 2H, CH$_2$); 4.39 (s, 2H, CH); 4.20 (dt, 2H, CH$_2$); 4.08 (dt, 2H, CH$_2$); 1.18 (t, 6H, CH$_3$). ^{13}C-NMR (CDCl$_3$, 125 MHz): δ 169.3 (2C), 137.3, 128.8, 128.5, 128.2, 78.7, 73.5, 61.5, 14.4. IR (NaCl, cm^{-1}): 1756 and 1732 (C=O, ester). MS: *m/z* = 386.9 [M + 1]. T$_m$ not observed

6.4.2.2.   Dibenzyl-L-Tartaric Acid (4) Synthesis

Compound **3** (1 eq) was dissolved in a 3:1 ethanol:water mixture containing sodium hydroxide (4 eq). Reaction was refluxed at 80°C with stirring for 24 h. Upon cooling to room temperature, reaction was acidified to pH 2 with concentrated HCl. Product was extracted with DCM (3x), the organic layer dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield pure compound **4**.

Yield: 97% (off-white solid). ^{1}H-NMR (CDCl$_3$), 500 MHz): δ 7.30 (m, 10H, Ar-H); 4.79 (d, *J* = 11.6 Hz, 2H, CH$_2$); 4.55 (d, *J* = 11.6 Hz, 2H, CH$_2$); 4.49 (s, 2H, CH). ^{13}C-NMR (CDCl$_3$, 125 MHz): δ 173.8 (2C), 136.2 (2C), 128.8 (8C), 128.7 (2C), 78.1 (2C), 74.2 (2C). IR (NaCl, cm^{-1}): 3250 (O-H, acid), 1732 (C=O, acid). MS: *m/z* = 329.2 [M - 1]. T$_m$ = 94 °C

6.4.2.3.   Dibenzyl Protected Bisthymol-L-Tartrate (6) Synthesis

To couple thymol to diacid **4**, a solventless procedure developed by Sakakura, *et al.* was used. Thymol (**5**, 2.2 eq), DMAP (20 mol%), and dibenzyl-L-tartaric acid (**4,** 1 eq) were combined in a round bottomed flask under nitrogen and pivalic anhydride (2.2 eq) added.   Reaction was heated to 55°C and for stirred for 48 hrs. Reaction was then diluted with ethyl acetate and washed with saturated $NaHCO_3$ (3x), DI water, and brine. After concentration *in vacuo*, crude product **6** was used in the next step without further purification.

6.4.2.4.   Bisthymol-L-Tartrate (7) Synthesis

Crude mixture containing **6** was dissolved in anhydrous DCM (10 mL/g) and 10% palladium on carbon (Pd/C, 30 wt%) was added. The reaction flask was evacuated with vacuum and purged with hydrogen gas, then allowed to stir at room temperature under hydrogen overnight. The mixture was filtered through Celite to remove Pd/C. The filtrate was concentrated *in vacuo* to yield crude grey oil. The product was purified on silica gel via column chromatography with 4:1 hexanes:ethyl acetate as eluent to yield pure compound **7**.

Two-step yield: 60% (white powder). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 7.25 (d, 2H, Ar-H); 7.09 (d, 2H, Ar-H); 6.89 (s, 2H, Ar-H); 5.06 (s, 2H, CH); 3.32 (d, 2H, OH); 3.10 (m, 2H, CH_2; 2.34 (s, 6H, CH_3); 1.21 (d, 6H, CH_3). ^{13}C-NMR ($CDCl_3$, 125

MHz): δ 170.7 (2C), 147.7 (2C), 137.3 (2C), 137.2 (2C), 128.1 (2C), 126.9 (2C), 122.5 (2C), 72.7 (2C), 27.0 (2C), 23.5 (2C), 23.3 (2C), 21.0 (2C); IR (NaCl, cm^{-1}): 3500 (O-H, alcohol), 1766 (C=O, ester). MS: *m/z* = 437.4 [M + 23]. T_m = 128 °C

6.4.2.5. Bisthymol-L-Tartrate Succinyl Diacid (9) Synthesis

Compound **7** (1 eq) was dissolved in anhydrous DCM. Succinic anhydride (**8**, 2 eq) was added, followed by triethylamine (4 eq). Reaction was stirred for 3 h, then washed with acidic H_2O (3x) and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield pure compound **9**.

Yield: 78% (white powder). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 7.20 (d, 2H, Ar-H); 7.05 (d, 2H, Ar-H); 6.79 (s, 2H, Ar-H); 6.10 (s, 2H, CH); 2.92 (m, 2H, CH); 2.82 (m, 4H, CH$_2$); 2.70 (t, 4H, CH$_2$); 2.30 (s, 6H, CH$_3$); 1.17 (dd, 6H, CH$_3$). ^{13}C-NMR (CDCl$_3$, 125 MHz): δ 177.9 (2C), 171.1 (2C), 164.8 (2C), 147.4 (2C), 137.2 (2C), 137.1 (2C), 128.1 (2C), 126.8 (2C), 122.3 (2C), 71.3 (2C), 28.9 (2C), 28.7 (2C), 27.1 (2C), 23.3 (2C), 23.2 (2C), 21.0 (2C) IR (NaCl, cm^{-1}): 3250 (O-H, acid), 1759 (C=O, ester), 1715 (C=O, acid). MS: *m/z* = 637.2 [M + 23]. T_m = 136 °C

6.4.2.6. Poly(Bisthymol Tartrate Succinate) (11) Synthesis

Using a previously published procedure to synthesize polyanhydrides,[22] diacid **9** (1 eq) was dissolved in anhydrous DCM (1 mL/mmol diacid) in a round

bottomed flask. (Trimethylsilyl)ethoxyacetylene (TMSEA, 1.1 eq) was added. Flask was capped and stirred at 40˚C for 4 h. Mixture was concentrated *in vacuo*. Resulting material was dissolved in acetonitrile, washed with hexanes, and concentrated *in vacuo* to afford pure polymer **11**.

Yield: 90% (off-white powder). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 7.20 (d, 2H, Ar-H); 7.04 (d, 2H, Ar-H); 6.80 (s, 2H, Ar-H); 6.10 (s, 2H, CH); 2.92 (m, 2H, CH); 2.83 (m, 4H, CH$_2$); 2.71 (t, 4H, CH$_2$); 2.30 (s, 6H, CH$_3$); 1.17 (dd, 6H, CH$_3$). ^{13}C-NMR (CDCl$_3$, 100 MHz): δ 170.6 (2C), 169.5 (2C), 167.2 (2C), 147.2 (2C), 137.2 (2C), 137.1 (2C), 128.2 (2C), 126.6 (2C), 122.2 (2C), 71.3 (2C), 30.2 (2C), 28.9 (2C), 27.5 (2C), 26.6 (2C), 23.4 (2C), 21.1 (2C). IR (NaCl, cm^{-1}): 1825 and 1785 (C=O, anhydride), 1759 (C=O, ester). T$_g$ = 34 ˚C

6.4.3. *In Vitro* Bioactive Release Studies

[Michelle Moy (Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ) contributed to release studies]

Thymol release from polymer **11** was elucidated by *in vitro* degradation in DI water (pH 7) at room temperature (22˚C). Polymer discs (n=3, 55 mgs per disc, 8 mm diameter x 0.95 mm thickness each) were prepared by pressing polymer **11** using an IR pellet die (International Crystal Laboratories, Garfield, NJ) with a hydraulic press (Carver model M, Wabash, IN) at 10,000 psi for 10 min. Polymer discs were incubated in pH 7 DI water (10 mL) in 20 mL Wheaton glass scintillation vials on controlled shaker at 60 rpm at 22˚C. At pre-determined

time intervals, degradation media (10 mL) was collected and replaced with fresh DI water (10 mL). Spent media was analyzed via high performance liquid chromatography (HPLC). HPLC analysis was performed using an XTerra RP18 3.5 μm 4.6 × 150 mm column (Waters, Milford, MA) on a Waters 2695 Separations Module equipped with a Waters 2487 Dual λ Absorbance Detector. Mobile phase was comprised of 60% acetonitrile and 40% 20 mM KH_2PO_4 with 1% formic acid (pH 2.5) at a flow rate of 1 mL/min and 25°C. All samples were filtered using 0.22 μm PVDF syringe filters and subsequently injected (20 μL) using an autosampler. Thymol release was quantified by comparison to calibration curves of standard solutions. Upon conclusion of study, remaining polymer was dried in a vacuum desiccator to constant mass. Then, remaining polymer was massed.

6.4.4. Formulation of PAE/LDPE Blended Films

LDPE and PAE **11** (10 wt%) were placed in a round bottomed flask and heated to 150 °C. Polymer mixture was stirred (200 rpm, 5 min) with an overhead mechanical stirrer (T-line laboratory stirrer, Talboys Engineering Corp., Montrose, PA) equipped with a PTFE paddle. Polymer blend (3x) was removed from heat and immediately placed in a room temperature beaker. Resulting blend was then compression-molded into a 200μm thick film using a Carver Press (Fred S. Carver Inc.) as follows. Blend (250 mg) was heated at 130°C for 3 min to allow for temperature equilibration within the press. Pressure (5000 psi) was then

applied for 1 min. After that time, the film was removed from heat and cooled to room temperature.

6.4.5. Antioxidant Studies Via Radical Scavenging

To determine the degradation media antioxidant activity, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used:[23] degradation media sample (0.1 mL, 25μg/mL) was added to a 24 μg/mL DPPH solution in methanol (3.9 mL). Additionally, LDPE/PAE blended film was physically cut into smaller pieces and refluxed in 1 N NaOH at 90°C overnight. Upon reaching room temperature, polymer residue was filtered off. Filtrate was neutralized with concentrated HCl and extracted with EtOAc. Organic layer was dried over MgSO$_4$, filtered, and concentrated *in vacuo* to afford thymol extracted from processed polymer. Raw polymer degradation media samples (0.1 mL) and blended polymer degradation media samples (0.1 mL) were incubated with the DPPH solution (3.9 mL) at room temperature with gentle shaking. After 1 h, solutions were analyzed by UV/vis spectrophotometry at λ = 517 nm. For comparison, a solution of free thymol at the same concentration both degradation medias (as determined at HPLC) was prepared and analyzed via the same method. DPPH % radical reduction was calculated by [(Abs$_{t0}$ − Abs$_t$)/Abs$_{t0}$] x 100, where Abs$_{t0}$ is the initial absorbance, and Abs$_t$ is the absorbance after 1 hr. All radical quenching assays were performed in triplicate. Student's *t* tests were used to determine the significant difference of the antioxidant activity between degradation media and free antimicrobial (significantly different if *p* < 0.05).

6.4.6. Antibacterial Studies

[Study performed with assistance of Dr. Susan Skelly, Division of Life Sciences, Rutgers University]

This disc diffusion study[25] was performed to ensure that polymerization and subsequent processing into films at elevated temperatures did not affect thymol bioactivity. Following the procedure in **Section 6.4.5**., first, PAE **11** was completely hydrolyzed with 1N NaOH overnight, then neutralized using concentrated HCl after which bioactives were extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford thymol extracted from raw polymer. Thymol obtained from processed LDPE/PAE polymer blend, as described in **Section 6.4.5**., was also tested. Upon solubilization in 1:1 H_2O:DMSO, the concentration of all bioactives was 10 mg/mL. The thymol analytes were tested as follows: Muller-Hinton agar (Becton Dickinson, Sparks, MD) was poured into sterile petri dishes (Fisher, Fair Lawn, NJ) to an even thickness of 4 mm. Bacteria inocula (*S. aureus* or *E. coli*) were suspended in nutrient broth (EMD Chemicals, Gibbstown, NJ). The agar plate was inoculated with bacteria broth culture using a sterile cotton swab (Fisher, Fair Lawn, NJ). Sterile paper discs (6 mm diameter, Becton Dickinson, Franklin Lakes, NJ) were impregnated with 20 μL of test solutions (one for free thymol, one for raw polymer degradation media, one for processed polymer degradation media, one for a combination, and one for H_2O:DMSO). Discs were then placed

onto agar plate and gently pressed down. Plates were incubated at 37°C for ~18 h after which zones of inhibition were measured with a ruler and rounded to the nearest half-millimeter.

*Other methods described in Chapter 9.

6.5. References

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7. MISCELLANEOUS PROJECTS

7.1. Chemically Incorporating Isosorbide as Poly(ibuprofen-tartrate) Comonomer to Improve Thermal Properties

As mentioned in Chapter 4, NSAID use at high dosages can lead to gastrointestinal side effects such as stomach bleeding and ulcer formation. Controlled NSAID release via polymer degradation could mediate these issues. To address this issue, ibuprofen was successfully coupled to each hydroxyl of tartaric acid, which was subsequently polymerized with 1,8-octanediol in previous work by Rosario-Melendez, *et al.*[1] While sustained ibuprofen release was exhibited, the presence of the long chain linear aliphatic diol resulted in a polyester with a T_g well below 0°C (i.e., -17°C). Rather than linear diols with a long aliphatic chain, an alternative rigid or aromatic diol may improve thermal properties. Thus, isosorbide was investigated as comonomer instead of 1,8-octanediol. Isosorbide is a non-toxic, rigid heterocyclic compound derived from biobased feedstocks.[2] As opposed to 1,8-octanediol use, isosorbide use would result in a more biorenewable polymer system as the degradation products are bioactive and two natural, sugar-based compounds. Moreover, previous literature has shown isosorbide to improve polymer thermal and mechanical properties for use in plastics engineering and biomedical applications[2-6] by imparting rigidity on polymer chains[7,8]

Because of these thermal properties, isosorbide was investigated as a comonomer to tartrate-based polymers. The effect of isosorbide on tartrate polyester thermal properties was elucidated. Ibuprofen tartaric diacid was synthesized according to previously published work.[1] Briefly, ibuprofen was coupled to each hydroxyl group of dibenzyl-L-tartrate via carbodiimide reaction. Benzyl protecting groups were selectively cleaved using palladium-catalyzed hydrogenation to yield diacid. Diacid and isosorbide were then polymerized neat in the presence of tin catalyst. Under the reaction conditions used, reactants melted and formed a monophasic mixture that increased in viscosity as reaction progressed. When compared to poly(octylene ibuprofen tartrate) (**Figure 7.1b**), poly(isosorbide ibuprofen tartrate) (**Figure 7.1a**) exhibits a T_g of 4°C, 21°C higher; the presence of isosorbide had a significant effect on polymer thermal properties.
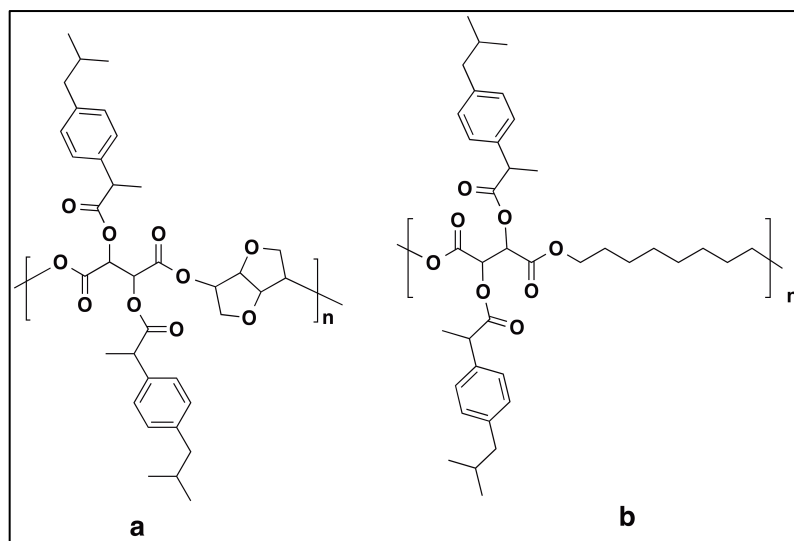
Figure 7.1: Sturctures of poly(isosorbide ibuprofen tartrate) (**a**) and poly(octylene ibuprofen tartrate) (**b**)

7.1.1. Experimental

7.1.1.1. Materials

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

7.1.1.2. Synthesis

Ibuprofen tartaric acid was synthesized using previously developed methods.[1] Diacid (1 eq) was then added to a round bottomed flask with isosorbide (1 eq) and stannous octoate (5 wt% monomers). Vessel was evacuated of air and purged with nitrogen. Reaction was stirred mechanically (120 rpm) under vacuum (2 Torr) at 130°C for 6 h.

Yield: 76 % (pale yellow foam). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 7.20 (b, 4H, Ar-H); 7.09 (b, 4H, Ar-H); 5.63 (b, 2H, CH); 5.12-5.30 (b, 2H, CH); 4.30-4.39 (b, 2H, CH); 3.57-4.02 (b, 6H, CH); 2.45 (b, 4H, CH$_2$); 1.82 (b, 2H, CH); 1.25 (b, 6H, CH$_3$); 0.87 (b, 12H, CH$_3$). M$_w$ = 3.0 kDa, PDI = 1.1. T$_g$ = 4°C

7.2. Glycerol-based Polyesters for Sustained Moisturizer Delivery

Thus far, the bioactives such as antioxidants, antimicrobials, analgesics, and antibiotics that have been discussed all contain one or two reactive moieties that are utilized for precursor synthesis and subsequent polymerization. However, a multitude of bioactives have more than two reactive functional groups. In this section, glycerol, the smallest sugar alcohol, was chemically incorporated into a polyester backbone. While little research on releasing glycerol over a sustained time period is known in the literature; glycerol has often been used to synthesize dendritic polymers,[9-12] owing to its three potential branching points. Hyperbranched polyglycerol is a polyether that has been studied in applications ranging from peptide conjugation and delivery to the developing of new non-fouling polymers.[10] NSAIDS have been conjugated to polyglycerol dendrimers via hydrolytically degradable ester bonds.[13] Polyglycerol conjugates have also been used to treat cancer[11,12]; although drug is released, the polyglycerol itself is not biodegradable.

Developing a completely biodegradable, glycerol-containing polymer with a favorable release rate would be beneficial, as glycerol is ubiquitously used as a moisturizer in many personal care and cosmetic products.[14-16] While glycerol esters have been developed,[13] they have not been studied for glycerol release. Additionally, these previously reported polymers had low glycerol content, as they were evaluated for materials properties rather than sustained release. The

overall goal is to create a hydrolytically degradable polymeric form of glycerol with high bioactive content (>50%) that releases in an appropriate time frame; for skin moisturization, the desired time period is <48 h. In addition, the polymer must be appropriate for formulation into an appropriate skin care product.

Glycerol is relatively low in molecular weight (92 Da); thus to ensure high glycerol content in the polymer, a small molecule diacid comonomer was used. A number of diacid monomers were considered for their potential to impart rigidity. Diglycolic acid (**Scheme 7.1**) was tested because its ether oxygen would prevent free rotation of bonds and its hydrophilicity also increased the likelihood that glycerol would be released faster. Itaconic acid (**Scheme 7.1**) was considered for its short carbon chain length in addition to its olefin that could be cross-linked. Finally, 2,5-furandicarboxylic acid was investigated because it is a small (i.e., low Mw) biocompatible aromatic diacid; the oxygen content will increase hydrophilicity and aromatic content would increase rigidity. As N435 effectively catalyzes both esterification (i.e., diacid-diol reactions) and transesterifications (i.e., diester-diol reactions),[17-20] the diacids, and dimethyl and diethyl esters were all tested (**Scheme 7.1**). For example, dimethyl-2,5-dicarboxylate will not melt or solubilize under the reactions conditions necessary; therefore, diethyl-2,5-dicarboxylate was used (**Scheme 7.2**). Likewise, dimethyl itaconate is known to be more reactive than diethyl itaconate. Properties including melting temperatures, reactivity, and solubility were all taken into account to determine which compounds to use.

Because glycerol has three hydroxyl groups that can react with a diacid, an alternative means of polymerization beyond traditional chemical methods was required. Comparing tin catalysis with enzymatic N435 catalysis, Gross *et al.* found that a significantly lower amount of cross-linking occurred during poly(oleic diacid-*co*-glycerol) synthesis using N435.[21] This observation can be explained by the regioselectivity of N435 for primary alcohols compared to secondary alcohols. With this work as inspiration, a control reaction was performed: stannous octoate catalyzed the reaction between glycerol and itaconic acid at 130 °C under vacuum. After 1 h, the mixture solidified; the primary and secondary alcohols of glycerol and itaconic acid quickly cross-linked because tin catalysts do not exhibit regioselectivity; the olefin may also lead to further cross-linking. Furthermore, the resulting solid was minimally soluble in water or any organic solvents tested (DCM, CHCl$_3$, EtOAc, acetone, THF, DMF, DMSO).
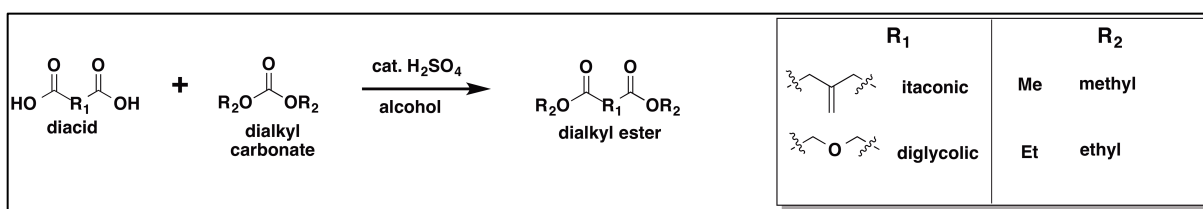
Scheme 7.1: Synthesis of dialkyl esters via acid-catalyzed esterification
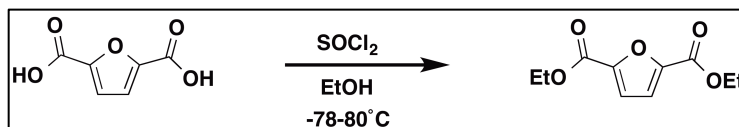
Scheme 7.2: Synthesis of diethylfuran-2,5-dicarboxylate through esterification of acyl chloride

Enzymatic polymerizations were carried out between N435 and the diacids and dialkyl esters in diphenyl ether under vacuum (2 Torr) (**Scheme 7.3**). Reactions with furan dicarboxylate did not progress due to low reactivity, likely from steric hindrance of the aromatic ring. Reactions with diglycolates also did not form, potentially due to the high hydrophilicity of the reaction itself. However, other products, including poly(glycerol itaconate) and poly(glycerol itaconate-*co*-succinate) did form, ranging from light tan paste to white powder. Thermal properties of polymers were determined via DSC; as an example, poly(glycerol itaconate) exhibited a T_g of 84°C and a T_m of 119°C. Molecular weights were low at 5-6 kDa, but were in accordance with polymers of short chain alcohols and itaconates.
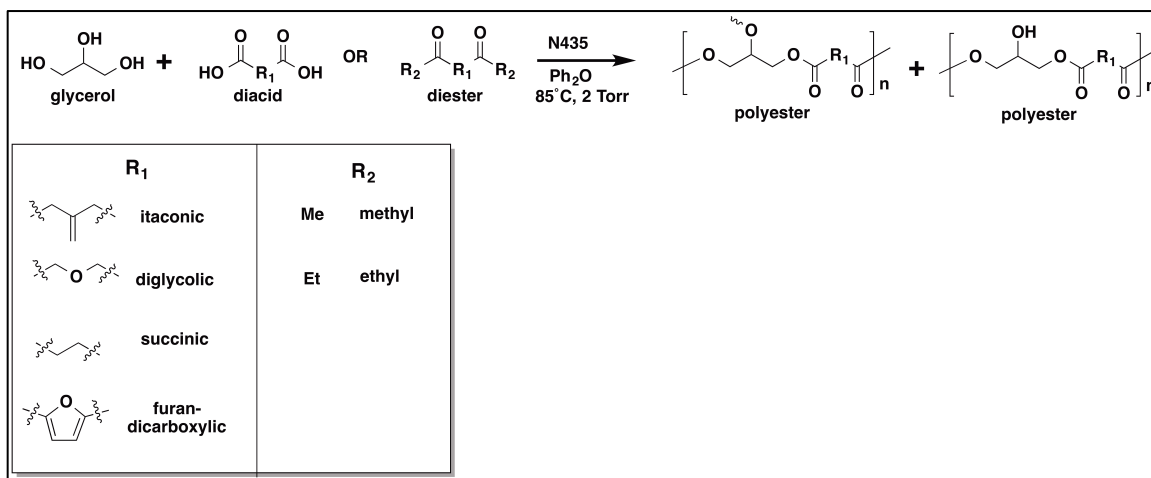
Scheme 7.3: Lipase-mediated synthesis of glycerol-based polyesters using aliphatic, heteroatom-containing, and aromatic comonomers

7.2.1. Experimental

7.2.1.1.   Materials

Dimethyl carbonate and diethyl carbonate were obtained from Acros (Geel, Belgium). 2,5-furandicarboxylic acid was obtained from AK Scientific (Union City, CA). All other reagents, solvents, and fine chemicals were obtained from Aldrich (Milwaukee, WI) and in all cases reagents were used as received.

7.2.1.2.   Stannous Octoate Control Polyesterification

Itaconic acid (1 eq) and glycerol (1 eq) were placed in a round bottomed flask with stannous octoate (5 wt%). Flask was purged with nitrogen and put under vacuum (2 Torr) at 130°C. Reaction stirred (120 rpm) with PTFE paddle and overhead mechanical stirrer (T-line laboratory stirrer, Talboys Engineering Corp., Montrose, PA). Reaction was terminated when mixture completely solidified. Solid was then suspended in DCM and filtered.

7.2.1.3.   Dimethyl Itaconate Synthesis

Itaconic acid (1 eq) was dissolved in anhydrous dimethyl carbonate (3.6 eq). Concentrated sulfuric acid (0.2 eq) was added. Reaction was heated to

reflux at 90°C overnight with stirring. Upon cooling to room temperature, reaction mixture was diluted with EtOAc and washed with saturated NaHCO$_3$ (2x). Organic layer was dried over MgSO$_4$, filtered, and concentrated *in vacuo* to yield pure dimethyl ester.[22]

Yield: 89 % (white solid). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 6.26 (s, 1H, =C-H); 5.65 (s, 1H, =C-H); 3.70 (s, 3H, CH$_3$); 3.62 (s, 3H, CH$_3$); 3.28 (s, 2H, CH$_2$).

7.2.1.4.   Diethyl Itaconate and Dlethyl Diglycolate Synthesis

Diacid (1 eq) was dissolved in anhydrous diethyl carbonate (1.3 eq) and anhydrous ethanol (4.6 eq). Concentrated sulfuric acid (0.2 eq) was added. Reaction was heated to reflux at 100°C with stirring overnight. Upon cooling to room temperature, reaction mixture was diluted with EtOAc and washed with saturated NaHCO$_3$ (2x). Organic layer was dried over MgSO$_4$, filtered, and concentrated *in vacuo* to yield pure dimethyl ester.[22]

Diethyl itaconate. Yield: 78 % (colorless liquid). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 6.17 (s, 1H, =C-H); 5.78 (s, 1H, =C-H); 4.08 (split m, 4H, CH$_2$); 3.32 (s, 2H, CH$_2$); 1.17 (t, 6H, CH$_3$).

Diethyl diglycolate. Yield: 54 % (colorless liquid). ^{1}H-NMR (CDCl$_3$, 500 MHz): δ 4.17 (s, 4H, CH$_2$); 4.10 (m, 4H, CH$_2$); 1.18 (t, 6H, CH$_3$).

7.2.1.5. Diethylfuran-2,5-dicarboxylate Synthesis

2,5-furandicarboxylic acid (1 eq) was stirred in anhydrous ethanol (5 mL/g) to form a white suspension. Mixture was cooled to -78°C using an acetone/dry ice bath. Thionyl chloride (2.3 eq) was slowly added over a few min. The reaction mixture stirred at -78°C for 2 h, then ice bath was removed and reaction heated to reflux at 85°C overnight with stirring. After cooling to room temperature, reaction was placed in freezer overnight. Resultant white crystals were vacuum filtered and washed with cold ethanol to afford pure diethyl ester.

Yield: 77 % (white crystals). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 7.38 (s, 2H, Ar-H); 4.31 (m, 4H, CH_2); 1.29 (t, 6H, CH_3).

7.2.1.6. Polymer Synthesis

Glycerol (1 eq) and diacid, dimethyl ester, or diethyl ester (1 eq) were placed in a round bottomed flask. N435 (10 wt%) was added, followed by Ph_2O (200 wt%). Reaction was brought to 85°C under inert gas, formed a monophasic mixture, and stirred for 2-16 h. Vacuum (2 Torr) was applied for the following 48-72 h.[23] At end of reaction, $CHCl_3$ was added, lipase was filtered off, and filtrate concentrated *in vacuo*. In case of reactions with itaconic acid and glycerol, filtrate was added dropwise over chilled MeOH.

Poly(glycerol itaconate). Yield: 84 % (white solid). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 6.09 (s, 1H, =C-H); 5.70 (s, 1H, =C-H); 4.40 (m, 1H, CH); 3.22-3.89 (br m, 6H, CH$_2$). IR (KBr, cm^{-1}): 1735 (C=O, ester). M$_w$ = 6.0 kDa. T$_g$ = 84°C, T$_m$ = 119°C.

Poly(glycerol succinate-*co*-itaconate). Yield: 88 % (tan paste). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 6.19 (s, 1H, =C-H); 5.82 (s, 1H, =C-H); 4.38 (m, 1H, CH); 3.85-4.10 (br m, 4H, CH$_2$); 3.23-3.62 (br m, 6H, CH$_2$). M$_w$ = 5.7 kDa. T$_m$, T$_g$ not observed

7.3. Alternative Opioid-based Poly(anhydride-esters) and Precursors
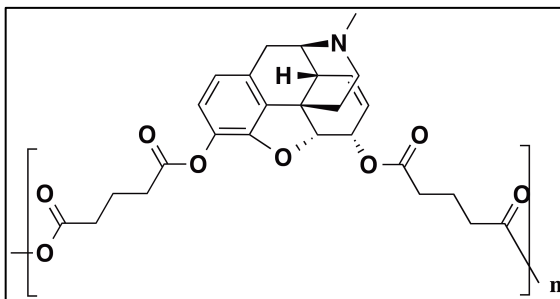
Figure 7.2: Structure of morphine-based polymer with glutaric linker

Morphine belongs to a class of potent analgesic drugs that are the gold standard for treating intensive acute and chronic pain. However, opioids suffer from multiple drawbacks include a short half-life necessitating frequent dosing, side effects, as well as tolerance and addiction issues.[24,25] Thus, a controlled release formulation in which morphine was chemically incorporated into a polymer was developed. Morphine was successfully incorporated into a PAE

backbone (PolyMorphine) by Rosario-Melendez, *et al.* for extended analgesia (~3 days) via sustained opioid release (**Figure 7.2**).[26] While a single dose of PolyMorphine exhibited twenty times the analgesic window of morphine itself, further extending morphine release would lessen the amount of dosing required by patients and raise patient compliance. One such way to prolong morphine release is to increase polymer hydrophobicity, namely by changing the linker molecule from a glutaric acid to 3,3-dimethylglutaric acid (**Scheme 7.4**). In other PAEs, the branched alkyl chains present in the linker significantly slowed bioactive release.[27] This increase in hydrophobicity slows the penetration and uptake of water by the polymer, thereby slowing the degradation rate.

Modifying a previously published procedure[26], morphine was dissolved in excess pyridine, which acted both as base and solvent. Then, excess 3,3-dimethylglutaric anhydride was added and reaction was refluxed overnight to afford diacid. Diacid structure was confirmed by ^{1}H NMR and MS analysis.
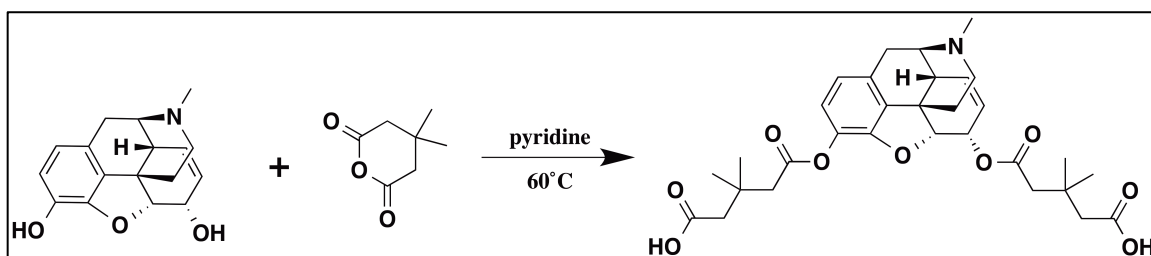
Scheme 7.4: Synthesis of morphine diacid with more hydrophobic 3,3-dimethylglutaric linker

Morphine has been successfully incorporated into a polymer backbone for sustained release. However, in certain cases, other opioids are preferable to

morphine. The incorporation of hydromorphone, an opioid with a single hydroxyl moiety as opposed to morphine's two hydroxyl moieties, was investigated. With one less reactive group than morphine, hydromorphone required an alternate synthetic approach. Use of a cyclic dianhydride (i.e., pyromellitic anhydride) to synthesize diacid via dual ring opening esterifications[28] was attempted (**Scheme 7.5**), but steric hindrance prevented effective dual esterification.
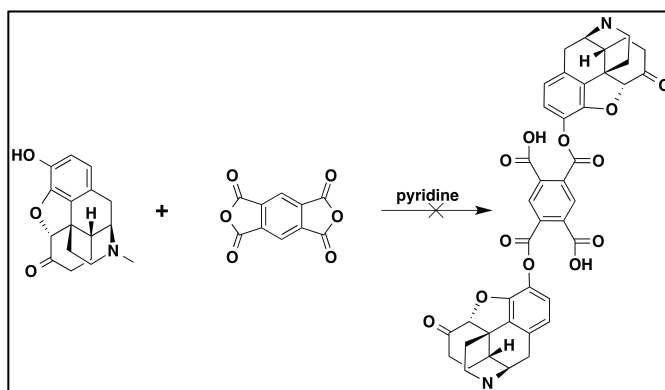
Scheme 7.5: Attempted synthesis of hydromorphone diacid with pyromellitic linker

7.3.1. Experimental

7.3.1.1. Materials

Hydromorphone and morphine were kindly provided by Noramco (Athens, GA). All other reagents, solvents, and fine chemicals were obtained from Aldrich (Milwaukee, WI) and used as received.

7.3.1.2.   Morphine 3,3-Dimethylglutaric Diacid Synthesis

Morphine (1 eq) was dissolved in anhydrous pyridine (20 mL/g) under nitrogen. 3,3-Dimethylglutaric anhydride (10 eq) was slowly added. Reaction stirred at 60°C overnight. Pyridine was azeotropically removed with toluene. The resulting brown paste was washed with DCM (5x) and isolated via decantation. Diacid was dried *in vacuo*.

Yield: 74% (light tan paste). ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.72 (d, 1H, Ar-H); 6.56 (d, H, Ar-H); 5.48 (dq, 2H, CH); 5.17 (s, 1H, CH); 5.05 (d, 1H, CH); 3.35 (s, 1H, CH$_2$); 3.00 (d, 1H, CH); 2.78 (s, 1H, CH); 2.20-2.58 (comp, 14H, CH$_2$, CH$_3$); 2.07 (t, 1H, CH$_2$); 0.9-1.2 (comp, 10H, CH$_2$, CH$_3$). MS: 570.3 [M + 1]

7.3.1.3.   Hydromorphone Pyromellitic Diacid Synthesis

Hydromorphone (2 eq) was dissolved in pyridine (20 mL/g). Pyromellitic anhydride (1 eq) was slowly added. Reaction was heated to reflux at 60°C overnight. Pyridine was azeotropically removed with toluene. The resulting brown paste was dried in vacuo. Characterization methods determined that reaction would not go to completion; monoacid was synthesized. This observation is likely due to the high level of steric hindrance.

7.4. Levofloxacin-containing Malates

Ampicillin-containing polymers previously discussed in Chapter 2 utilized beta-lactam antibiotics. While beta-lactams are proven effective against a range of Gram-negative and some Gram-positive bacteria, in some cases, a stronger, more resistant antibiotic may be required. Fluoroquinolones are a class of synthetic broad-spectrum antibiotics[29] that do not suffer from the same mechanism of bacterial resistance that affects beta-lactams (**Figure 7.3**). Fluoroquinolones have been incorporated into polymethacrylate polymer backbones and exhibited antibacterial activity;[30,31] however, nonbiodegradable methacrylates can lead to potential issues *in vivo*. Hydrolytically or enzymatically degradable polymers containing fluoroquinolones solved this issue, but the few examples that exist exhibit low (<10%) drug loading.[32,33] Thus, incorporation of levofloxacin, a fluoroquinolone antibiotic, into a polyester backbone with high drug loading was investigated.
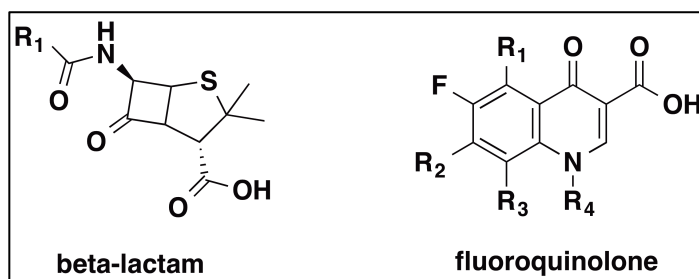
Figure 7.3: Representative structures of two antibiotic classes: beta-lactams (left) and fluoroquinolones (right)

Levofloxacin contains a carboxylic acid as a reactive moiety; thus, the methodology presented in Chapter 4, in which ibuprofen was coupled to malic acid and polymerized, was adapted. The esterification reaction between levofloxacin and dibenzyl-L-malate was investigating using several approaches (**Scheme 7.6**). Initially, traditional carbodiimde coupling utilizing EDC with DMAP was used; however, these reactions did not progress to any great extent. Thus, (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) a newer, more efficient coupling agent was used along with bulky bases.[34,35] Both diisopropylethylamine (DIPEA) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were investigated as bases and COMU as coupling reagent.[36] Although starting material was consumed, the desired product was not obtained; other methods would need to be investigated for this reaction to succeed.

Rather than esterification via coupling reagents, utilizing the reaction of acyl chlorides with alcohols was attempted. Because levofloxacin is unstable to thionyl and oxalyl chlorides,[37] milder reaction conditions were required; triphenylphosphine (PPh$_3$) and trichloroacetonitrile (CCl$_3$CN) generate acyl chlorides from acids *in situ* under mild conditions.[38] Thus, this method was attempted for esterification, but product was not acquired. Alternate reactions and work up methods are necessary to obtain desired product.
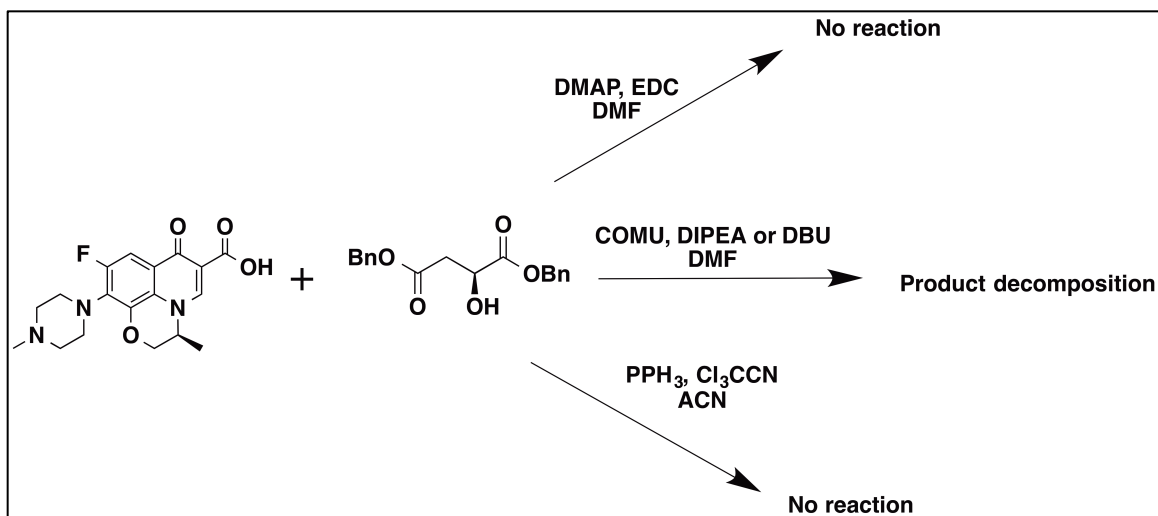
Scheme 7.6: Attempted synthetic methods to make levofloxacin dibenzyl malate and their outcomes

7.4.1. Experimental

7.4.1.1. Materials

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

7.4.1.2. Synthesis Via EDC Coupling

Dibenzyl-L-malate was synthesized as previously discussed in Chapter 4. Levofloxacin (1.1 eq) was dissolved in anhydrous DMF (10 mL/g). DMAP (1.1 eq) was added, followed by dibenzyl-L-malate (1 eq). EDC (2.2 eq) was slowly added and the reaction was monitored over 48 h before concentrated *in vacuo*.

Resulting orange paste was dissolved in EtOAc and washed with acidic water (2x) and brine. NMR characterization revealed that the reaction did not proceed to desired product.

7.4.1.3. Synthesis Via COMU Coupling

Levofloxacin (1.1 eq) was dissolved in anhydrous DMF (10 mL/g). Base (DIPEA or DBU) (2 eq) was added, followed by COMU (1.5 eq). Dibenzyl-L-malate (1 eq) was added and the reaction was monitored over 48 hrs before concentrated *in vacuo*. Resulting brown paste was dissolved in EtOAc and washed with 10% $KHSO_4$ (2x), saturated $NaHCO_3$, and brine. Characterization revealed that the drug did not tolerate reaction conditions based on the NMR spectrum.

7.4.1.4. Synthesis Via PPh$_3$/CCl$_3$CN

Dibenzyl-L-malate (1 eq) was dissolved in anhydrous DCM (20 mL/g) along with PPh$_3$ (2 eq) and DMAP (3 eq). CCl$_3$CN (2 eq) was added. Reaction was refluxed at 40°C overnight. DCM was concentrated *in vacuo*. Resulting brown paste was dissolved in ACN and washed with DI water (3x). NMR characterization revealed that a significant amount of starting material was present and product formation was minimal.

*Other methods described in Chapter 9.

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8. CONCLUDING REMARKS AND FUTURE WORKS

Sustained and controlled bioactive release via polymer degradation offers significant improvements compared to traditional bioactive administration. Although physical incorporation of bioactives into polymers often leads to low drug loading and short release timeframes, the chemical incorporation of bioactives into biodegradable polymer backbones presented in this dissertation can resolve these issues. This chapter summarizes the dissertation and offers future research directions based upon the work presented herein.

Chapter 2 discussed the development of ampicillin-containing poly(anhydride-amides) as a means to prevent local implant-related infections. Biodegradation under physiological conditions and exhibition of antibacterial activity elucidated potential for *in vivo* use. The chemical incorporation of other antibiotic classes necessitates further research to combat multi-drug resistant infections via localized, as opposed to systemic, delivery.

The development of PAEs that biodegraded into naturally-occurring bioactives and EDTA, all compounds used in consumer products, was presented in Chapter 3. Released compounds retained their antioxidant and antimicrobial activities. Not only can these polymers can act as preservatives in a number of products to prolong shelf-life, but all degradation products are active, leading to a highly atom-efficient system.

Green chemistry methods were utilized in Chapter 4 to make polyesters that contain malic acid and ibuprofen. Through using less toxic solvent, reducing

solvent use, and using biocatalysts and renewable feedstocks, novel polymers that release ibuprofen over an extended timeframe were prepared. Because of the synthetic success of these polyesters, green chemical methods warrant further investigation for other systems.

In Chapter 5, sugar alcohol-based PAEs with multiple pendant groups per repeat unit were designed as delivery systems with high bioactive loading, using ibuprofen and mannitol as model compounds. The methodology presented can be applied to all sugar alcohols, thereby allowing control over drug loading, number of pendant groups, and pendant group stereochemistry. With several sites for facile attachment, up to four unique molecules can be bound to a single biodegradable polymer backbone. These tunable polymer systems can take advantage of bioactive synergism or be used as dual delivery systems; as an example, an antibiotic and an NSAID can be used together for localized delivery to fight both inflammation and infection, two major issues that arise with surgery. Moreover, targeting groups such as folic acid, biotin, and selectin be bound to the mannitol backbone for targeted and efficient delivery to specific areas *in vivo*.

The development of novel thymol-containing PAEs was discussed in Chapter 6. Quantitative release studies were performed to elucidate thymol release rate from raw polymer. PAEs were successfully blended with LDPE to be used as food packaging wraps. Both raw PAEs and blends released the naturally-occurring phenol, which exhibited unchanged antimicrobial activity. Antioxidant activity of released thymol was also the same as free thymol. As PAE/LDPE blends would be used for active packaging applications, thymol

release under real-world packaging conditions (i.e., storage temperature, humidity, etc.) should be performed using representative food samples and compared to LDPE alone. Once release from blend has been assessed, biodegradation studies should be performed to evaluate the effect of blending non-biodegradable LDPE with completely biodegradable PAE.

This dissertation elucidates the efficacy of bioactive-conjugated polymers in regard to controlled and sustained release. Through green chemistry and alternative polymerization methods, polymers were designed to delivery antimicrobial, antioxidant, and anti-inflammatory compounds.

9. GENERAL EXPERIMENTAL METHODS

9.1. Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy

^{1}H-NMR spectra were acquired on a Varian 400 or 500 MHz spectrophotometer. Samples (5-10 mgs) were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) that also acted as internal reference or deuterated chloroform (CDCl$_3$) with tetramethylsilane as internal reference. Each spectrum was an average of 16 scans.

9.2. Carbon Nuclear Magnetic Resonance (^{13}C-NMR) Spectroscopy

^{13}C-NMR spectra were acquired on a Varian 400 or 500 MHz spectrophotometer. Samples (40-50 mgs) were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) that also acted as internal reference or deuterated chloroform (CDCl$_3$) with tetramethylsilane as internal reference. Each spectrum was an average of 256 scans.

9.3. Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectra were acquired on a Thermo Nicolet/Avatar 360 FT-IR spectrometer. Samples (1-3 wt%) were ground with potassium bromide (KBr) and compressed into a disk (13 mm diameter x 0.5 mm thickness) using a

hydraulic press (Carver model M) by applying pressure (~10,000 psi) for 1 min or by solvent-casting sample (1-3 wt%) onto sodium chloride (NaCl) plates using dichloromethane (DCM). Each spectrum was an average of 32 scans.

9.4. Mass Spectrometry (MS)

MS was used to determine molecular weight of polymer precursors. Analysis was performed on a Finnigan LCQ-DUO running Xcalibur software and an adjustable atmospheric pressure ionization electrospray source (API-ESI Ion Source). Samples were dissolved in methanol (10 µg/mL) and injected with a glass syringe. During the experiment, the pressure was 0.8×10^{-5} Torr and the API temperature was 150°C.

9.5. Thermogravimetric Analysis (TGA)

TGA was used to determine decomposition temperature (T_d). TGA was performed on a Perkin-Elmer Pyris 1 system with TAC 7/DX instrument controller and Perkin-Elmer Pyris software for data collection and processing. Samples (5-10 mg) were heated under dry nitrogen gas from 25 °C to 600 °C at a heating rate of 10 °C/min. T_d was measured at the onset of thermal decomposition.

9.6. Differential Scanning Calorimetry (DSC)

DSC was used to determine melting temperature (T_m) of polymer precursors and glass transition temperature (T_g) of polymers. A TA DSC Q200 running TA Universal Analysis 2000 software was used for data acquisition. Samples (5-10 mg) were heated under dry nitrogen gas from −50 ˚C to 200 ˚C at a rate of 10 ˚C/min with at least two heating/cooling cycles per sample. T_m was calculated at the peak of melting and T_g was defined as the midpoint of the curve.

A. APPENDIX

A.1.　　　Copyright Permissions

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A.1.2. Biomacromolecules Permissions[2]

A.1.3. Macromolecular Bioscience Permissions[3]

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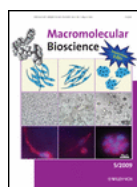
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A.1.4. Macromolecules Permissions[4]

A.2.   References

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