Synthesis of polyanhydrides for controlled drug delivery

by

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# TABLE OF CONTENTS

**LIST OF FIGURES**

**LIST OF TABLES**

**LIST OF SYMBOLS**

**ABSTRACT**

**CHAPTER 1. INTRODUCTION**

1.1 Introduction to Controlled Release  
1.2 References

**CHAPTER 2. LITERATURE REVIEW**

2.1 Early Polyanhydrides  
2.2 Erosion and Degradation  
2.3 High Molecular Weight Polyanhydrides: Melt Condensation  
2.4 Coordination Catalysts  
2.5 Solution Polymerization  
2.6 Poly(ethylene glycol) Containing Polyanhydrides  
2.7 Block Copolymer Synthesis  
2.7.1 Tin(II) Octoate  
2.7.2 Living Polymerization  
2.7.3 Synthesis of Anhydride Rings  
2.7.4 Covalent Coupling of Blocks  
2.8 Ester Amide Synthesis  
2.9 Microwave Synthesis and Polymerization  
2.10 Project Objectives  
2.11 References

**CHAPTER 3. MATERIALS AND METHODS**

3.1 Materials  
3.2 Instrumentation  
3.3 Experimental Methods  
3.3.1 General Synthesis of Homopolymers and Random Copolymers of Polyanhydrides From Diacids  
3.3.2 Synthesis of Polyanhydride-Polyanhydride Block Copolymers  
3.3.2.1 Ring Opening Polymerization  
3.3.2.1 End-Group Modification of Polyanhydrides  
3.3.4 Microwave Polymerization of Polyanhydrides  
3.3.5 Degradation and Dissolution Testing  
3.3.5.1 Dissolution testing  
3.3.5.2 Degradation
### CHAPTER 4. RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Introduction to Results and Discussion</td>
<td>52</td>
</tr>
<tr>
<td>4.2 Ring Opening Polymerization of Sebacic Acid Anhydride</td>
<td>52</td>
</tr>
<tr>
<td>4.3 Synthesis of Polyanhydride Block Copolymers via an Ester Amide Linker</td>
<td>62</td>
</tr>
<tr>
<td>4.4 Synthesis and Characterization of PEG Containing Aromatic Polyanhydrides</td>
<td>70</td>
</tr>
<tr>
<td>4.4.1 Synthesis of PEG Containing Aromatic Polyanhydrides</td>
<td>70</td>
</tr>
<tr>
<td>4.4.2 Release and Degradation Studies of PEG Containing Aromatic Polyanhydrides</td>
<td>80</td>
</tr>
<tr>
<td>4.5 High Throughput Microwave Synthesis of Polyanhydrides</td>
<td>90</td>
</tr>
<tr>
<td>4.6 References</td>
<td>99</td>
</tr>
</tbody>
</table>

### CHAPTER 5. CONCLUSIONS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Introduction to Conclusions</td>
<td>104</td>
</tr>
<tr>
<td>5.2 Attempts at Block Copolymer Synthesis</td>
<td>104</td>
</tr>
<tr>
<td>5.3 Synthesis and Characterization of PEG Containing Aromatic Polyanhydrides</td>
<td>105</td>
</tr>
<tr>
<td>5.4 Microwave Polymerization of Polyanhydrides</td>
<td>105</td>
</tr>
</tbody>
</table>

### CHAPTER 6. FUTURE WORK

### APPENDIX

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>109</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1.1  Sample drug in plasma concentration profile (A) bolus injection (B) controlled release .......................................................... 2

Figure 1.2  Biodegradable polymer degradation mechanisms (A) bulk erosion (B) surface erosion .................................................. 3

Figure 2.1  Synthesis of bis(p-carboxyphenoxy)alkanes ........................................... 9

Figure 2.2  Polycondensation of Prepolymers .......................................................... 13

Figure 2.3  Synthesis of Aromatic-Aliphatic Monomers ........................................... 14

Figure 2.4  Mechanism of melt polyanhydride formation .......................... 17

Figure 2.5  Aromatic diacid monomer containing an internal amide ................. 19

Figure 2.6  Aromatic diacid monomer containing an internal ester ................. 19

Figure 2.7  Mechanism of ester polymerization with tin(II) octoate ................. 24

Figure 2.8  Synthesis of functionalized (PEO-b-PLA) ..................................... 29

Figure 2.9  Mechanism of the Amidation of a Carboxylic Acid with DMT-MM .... 31

Figure 4.1  Formation of acetylated diacid prepolymer and subsequent polymerization under vacuum ...................................................... 53

Figure 4.2  Cyclization of sebacic acid to the anhydride ........................................... 54

Figure 4.3  Solid state carbon NMR of sebacic acid anhydride ring .................. 54

Figure 4.4  Ring opening polymerization product of sebacic acid anhydride with tin(II) octoate at 130°C under vacuum .......................... 56

Figure 4.5  Ring opening polymerization product of sebacic acid anhydride with tin(II) octoate at 130°C under vacuum, fraction 2 .......... 56

Figure 4.6  Soluble fraction of the reaction between poly(CHP), tin(II) octoate and sebacic acid anhydride ring at 180°C under vacuum for 1.5 hr .... 58

Figure 4.7  Tin(II) octoate in CDCl₃ ................................................................. 58
Figure 4.8 Tin(II) octoate reaction with DMF at 60°C in CDCl3 .................................. 60
Figure 4.9 Tri-block copolymer synthesis of polyanhydrides by ester-amide linker .......... 63
Figure 4.10 Reaction product of poly(νννννννν), ethanol amine and DMTMM end-group modification reaction ......................................................... 64
Figure 4.11 CPH prepolymer with an unknown peak at δ = 4.3 ppm ......................... 66
Figure 4.12 Pure CPH diacid recrystallized from NMP ......................................... 67
Figure 4.13 Purified CPH prepolymer made from pure CPH diacid ......................... 68
Figure 4.14 Synthetic route to PEG containing aromatic diacids .............................. 70
Figure 4.15 Halogenation of penta-ethylene glycol .............................................. 71
Figure 4.16 Purified dibromo penta-ethylene glycol ............................................ 72
Figure 4.17 Purified dichloro penta-ethylene glycol ............................................ 73
Figure 4.18 Synthesis of aromatic PEG diacid .................................................... 73
Figure 4.19 Impure CPPEG-3 diacid ............................................................... 75
Figure 4.20 Impure CPPEG-5 diacid ............................................................... 76
Figure 4.21 NMR of pure CPPEG-3 diacid ....................................................... 77
Figure 4.22 NMR of pure CPPEG-5 diacid ....................................................... 77
Figure 4.23 Crude CPPEG-3 prepolymer ......................................................... 78
Figure 4.24 NMR of poly(CPPEG-3) polymerized at 180°C under vacuum for 2 hrs ... 79
Figure 4.25 NMR of poly(CPPEG-5) polymerized at 180°C under vacuum for 2 hrs ... 79
Figure 4.26 Erosion of 100 mg tablet of poly(CPPEG-3) in 900ml of 0.1 M PBS solution at 37°C ................................................................. 81
Figure 4.27 Erosion of 100 mg sphere of poly(CPPEG-5) in 900ml of 0.1 M PBS solution at 37°C ................................................................. 81
Figure 4.28 Rhodamine B Base a hydrophobic model drug used in the release study ... 83
Figure 4.29 Acid Orange 8 a hydrophilic dye used in the release study.........................83

Figure 4.30 Release of Rhodamine B Base, Acid Orange 8 and mass fraction of water swollen in the polymer (without drug) from poly(CPPEG-3) .........................84

Figure 4.31 SEM micrographs of Acid Orange 8 loaded (5 wt%) poly(CPPEG-3). Outer surface (left) and cross section (right).....................................................85

Figure 4.32 SEM micrographs of Rhodamine B Base loaded (5 wt%) poly(CPPEG-3). Outer surface (left) and cross section (right).....................................................85

Figure 4.33 Release of Rhodamine B Base and mass fraction of water swollen in polymer (without drug) from poly(CPPEG-5) .................................................88

Figure 4.34 Release of Acid Orange 8 and mass fraction of water swollen in polymer from poly(CPPEG-5)........................................................................88

Figure 4.35 Calibration of microwave hot spots using water moistened filter paper........91

Figure 4.36 Conventionally prepared prepolymer of sebacic acid...............................92

Figure 4.37 NMR of a sebacic acid anhydride prepolymer microwave polymerized for 30 min at 1100 Watts..............................................................93

Figure 4.38 NMR of conventional polymerization of sebacic acid with in situ prepolymer formation.................................................................97

Figure 4.39 NMR of microwave polymerization of sebacic acid with in situ prepolymer formation, 15 drops.................................................................97
LIST OF TABLES

Table 4.1 Summary physical properties of CPPEG-3 and CPPEG-5 polymers as compared to poly(CPH) and poly(SA) .................................................. 80

Table 4.2 Number average molecular weight of all experiments polymerized in the microwave using sebacic anhydride prepolymer. ........................................ 93

Table 4.3 Number average molecular weight of all experiments polymerized in the microwave with in situ sebacic acid prepolymer preparation ......................... 95

Table 4.4 Number average molecular weight of experiments polymerized in the microwave with in situ sebacic acid prepolymer preparation compared to conventional oil bath heating without vacuum ................................. 96
LIST OF SYMBOLS

CDMT  2-chloro-4,6-dimethoxy-1,3,5-triazine
CPH   1,6-bis(p-carboxyphenoxy) hexane
CPPEG-3  1,8-bis(p-carboxyphenoxy)-3,6-dioaoctane
CPPEG-5  1,14-bis(p-carboxyphenoxy)-3,6,9,12-tertaoxatetradecane
CPP   1,3-bis(p-carboxyphenoxy) propane
DCC   dicyclocexylecarbodiimide
DMAC  dimethyl acetamide
DMF   dimethyl formamide
DMT-MM 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-ethylmorpholiniumchloride
DSC   differential scanning calorimetry
GPC   gel permeation chromatography
MALDI-TOF  matrix assisted laser desorption/ionization time of flight mass spectroscopy
\( \overline{M}_n \)  number average molecular weight
\( \overline{M}_w \)  weight average molecular weight
NMM   n-methyl morpholine
PDI   polydispersity index
PEG   poly(ethylene glycol)
SA    sebacic anhydride
Sn(Oct)\(_2\) tin(II) ethyl hexanoate
ABSTRACT

This thesis is divided into three sections, all related to polyanhydride synthesis: first, attempts at the synthesis of polyanhydride-polyanhydride block copolymers, second, synthesis and characterization of polyethylene glycol containing aromatic polyanhydrides, and third, microwave polymerization of polyanhydrides.

A strategy was developed to produce block copolymers of polyanhydrides using a hydroxyamide linker for the controlled release of proteins and higher molecular weight drugs. Block copolymers offer more control over microstructure than random copolymers, which allows for improved controlled release of proteins. The condensation polymerization of anhydrides typically yields random copolymers, therefore making the synthesis of block copolymers difficult. To synthesize the block copolymers, homopolymers were prepared from acetylated diacids via melt polymerization under vacuum to a number average molecular weight of ~5000. Methods explored consisted of end-functionalizing a short polyanhydride chain with hydroxylamine and ring opening polymerization through a macromonomer initiator. A discussion of the various methods explored is presented.

Adding ethylene glycol segments to the monomers allows one to tailor the hydrophobicity of the polyanhydride, which in turn dictates the solubility of molecules within the polymer matrix and degradation rate of the polymer. Aromatic diacids were synthesized from chlorinated triethylene and pentaethylene glycols and hydroxy benzoic acid. The diacids were acetylated with acetic anhydride to produce homopolymers of polyanhydrides for the stabilization of proteins and drugs. The glycol containing polyanhydrides were characterized by NMR, GPC and DSC and dissolution testing. The release characteristics of the new polyanhydrides were evaluated with a hydrophilic (Acid
Orange) and hydrophobic dye (Rhodamine B Base) and the release mechanisms were investigated.

Microwave radiation was used to explore the high throughput synthesis of polyanhydrides for materials library characterization. Aliphatic diacids were acetylated \textit{in situ} for two minutes in a closed reaction vessel before removal of excess acetic anhydride and subsequent polymerization, all in the microwave. Reaction conditions such as acetic anhydride equivalents, duration of microwave exposure and comparison to conventional polycondensation were explored. Microwave polymerization times of 6 to 8 minutes yielded optimum number average molecular weights in contrast to conventional polycondensation, which takes between 1.5 to 2 hours under high vacuum.

In conclusion, this thesis explored the synthesis and characterization of various polyanhydride-based homopolymers and copolymers by conventional as well as microwave techniques for controlled drug delivery applications.
CHAPTER 1
INTRODUCTION

1.1 Introduction to Controlled Delivery

Over the past twenty-five years controlled release has blossomed from an idea into a legitimate method of therapeutic delivery and drug stabilization. We have seen a revolution from developing polymers that are just biodegradable and biocompatible to targeting specific organs and providing stealth capabilities \textit{in vivo} [1].

The explosion of new macromolecular therapies being developed by pharmaceutical companies and a need for the ability to stabilize and control release profiles has led to intense research in controlled release technology.

The traditional method of delivering proteins \textit{in vivo} is parenteral injection of liquid formulations, mostly due to the low expense of delivery incurred by pharmaceutical companies. Unfortunately, parenteral injection is often poorly accepted by young children, the elderly and the underfed. Furthermore, therapy regimens often require multiple injections which can be an inconvenience and difficult for patient compliance. Additionally, parenteral injection requires high systemic levels of protein to achieve an appropriate dosage at the target organ or other non-localized delivery, resulting in unwanted systemic toxicity and increased cost resulting from the excess injected drug [2]. Preferably, protein concentration should be kept at a level that does not cause toxic effects, but in a range that optimizes the therapeutic results for a chosen time frame (Figure 1.1).
Another issue complicating matters further results from the injection of proteins systemically. Injection leads to significant loss of protein due to the biology of the delivery route and/or degradation of the protein [2]. Proteins can undergo degradation or oxidation reactions in the presence of heat, water, extreme pH and in some instances, aggregation in the presence of hydrophobic surfaces [3]. The result is a loss of the secondary, tertiary or in some cases quaternary structure that leads to inactivation [1]. Therefore, a suitable delivery system that encapsulates macromolecules with defined conformation and protects them from denaturing would be a major advantage over injection of unshielded proteins \textit{in vivo} for both therapeutic and economic reasons. The development of polyanhydrides for the longer term controlled release of molecules offers a unique method of addressing many of the aforementioned problems with direct parenteral injections. For injectable devices, drug loaded microspheres may be fabricated from polyanhydrides using an oil/water emulsion [3-16]. Polyanhydrides are surface eroding polymers that release molecules such as proteins or small drugs. Release
is governed by the hydrolysis of anhydride bonds and subsequent erosion. Polyanhydride devices differ from bulk eroding systems in their mechanism of degradation. Specifically, the mechanism of release is due to the highly labile anhydride bond breaking thus releasing molecules as it erodes from the edge of the device. Water is prevented from swelling the device due to the hydrophobic nature of the polymer and the highly labile anhydride bonds breaking. In contrast, bulk eroding devices deliver therapies by swelling in water, followed by hydrolysis of bonds throughout the polymer matrix; resulting in release of the desired molecules. (Figure 1.2).

![Figure 1.2 Biodegradable polymer degradation mechanisms (A) bulk erosion (B) surface erosion. Bulk erosion is caused by water inclusion throughout the device and then bond scission. Surface erosion releases molecules by degradation of bonds at the outer-edge of the device. Water is unable to penetrate the device due to the hydrophobicity of the polymers used.](image)

Since polyanhydrides are surface eroding polymers, they have a distinct advantage for protein stabilization because they prohibit water penetration that prevents proteins from
denaturing due to physiological conditions. Polyanhydrides are usually made from acetylated diacids that consist of aliphatic and/or aromatic moieties. The rate of release is dependent not only on the rate of polymer degradation but also on the solubility of the monomer in the polymer [10, 17]. Increasing the aromatic content decreases the release rate due to the prevention of water penetration [13-16, 18-23]. However, in homopolymers of poly(1,6-bis(carboxyphenoxy)hexane) p(CPH) loaded with a poorly soluble drug, the release data shows an initial burst which accounts for most of the release. This burst effect was attributed to the concentrating of drug at the polymer-solvent interface during removal of methylene chloride in the preparation of microspheres, which caused the drug to load at the outer edges of the polymeric device [10]. By synthesizing copolymers of aliphatic and aromatic monomers, precise control over the degradation rate can be achieved, from days to years. Random copolymers of poly(1,6-bis(carboxyphenoxy)hexane-co-sebacic anhydride) p(CPH-SA) display microphase separation at compositions when one monomer is in excess in the feed [10, 17]. Previous studies have shown partitioning of low molecular weight drugs into thermodynamically favorable phases in the p(CPH-SA) system [10, 17]. Unfortunately, increasing the aromatic content increases the hydrophobicity of the polymer, which may lead to protein aggregation along the hydrophobic domains inside the polymer matrix [1, 2, 5, 9]. As mentioned previously, protein aggregation leads to inactivation due to conformational changes in its secondary structure.

One method of preventing protein denaturing is to increase the hydrophilicity of the polyanhydride. Unfortunately, adding aliphatic diacids does not reduce the hydrophobic interactions enough for stabilization. Therefore, we believe incorporating short polyethylene glycol (PEG) chains into polyanhydrides may reduce the hydrophobic polymer-protein
interactions within the polymer matrix. Previous research has shown the beneficial effect of PEG inclusion into block copolymers of polyesters such as caprolactone and lactide [1, 5, 9, 24, 25]. Polyanhydride copolymers of sebacic anhydride and PEG segments have been synthesized for pulsatile release and inhalation devices [26-29]. These polymer systems have very fast degradation times ca. 6 hrs to 5 days depending on the composition.

The aim of this project is to synthesize families of polyanhydrides suitable for drug and protein delivery. The first part of this thesis covers the attempts at synthesizing polyanhydride-polyanhydride block copolymers. By synthesizing blocks of polyanhydrides we may be able to control the morphology of phase separation in polyanhydride copolymer systems. Control of the polymer morphology will provide a method of studying the effect of morphology on the release of model drugs and proteins. Furthermore, no researchers have been able to synthesize block copolymers of just polyanhydrides. Therefore, a technique to synthesize block copolymers of polyanhydrides would provide a novel and useful synthetic route to undiscovered polymer properties, release profiles and further knowledge of these release systems for the rational design of new systems. The second part of the thesis deals with synthesis of polyanhydrides that contain PEG chains for the stabilization of proteins while providing controlled release for longer periods of time (months). By end-group modification of PEG diols with p-hydroxy benzoic acid, polyanhydrides can be synthesized with glycol residues while retaining some hydrophobicity to decrease the degradation rate. In contrast to previous research, our polymers will be designed to degrade for a period of months and stabilize proteins within their hydrophilic domains. Thirdly, preliminary experiments into the synthesis of polyanhydrides from diacids using microwaves has been investigated as a high throughput materials library synthesis technique to enable the
screening of copolymers for desired properties such as the glass transition temperature and hydrophobicity.
1.2 References


CHAPTER 2
BACKGROUND AND LITERATURE REVIEW

2.1 Early Polyanhydrides

Bucher and Slade synthesized the first polyanhydrides in 1910. The polyanhydrides resulted from the heating of aromatic diacids in acetic anhydride [1]. Aliphatic anhydrides were first synthesized in 1932 by Hill and Carothers through melt polycondensation in the presence of acetylchlordride or acetic anhydride in order to develop synthetic fibers [2-6]. The polymers had molecular weights of about 5000 and when subjected to a molecular still, two structures resulted. The first structure was a high molecular weight polymer and the second was depolymerization product that included cyclic anhydrides. In the case of sebacic anhydride, a dimer cyclic anhydride structure resulted. The polyanhydrides synthesized were found to be hydrolytically unstable, and therefore not favorable for the production of fibers or textiles. In the late nineteen-fifties and early sixties, Conix and Yoda produced polyanhydrides and polyesters from an array of diacids in an effort to produce more hydrolytically stable polymers [7].

Since the stability of anhydride bonds varies with the monomer chosen, Langer began looking into the use of polyanhydrides for controlled drug delivery [8]. Of interest to controlled release is the synthesis of bis diacids, specifically 1,3-bis(p-carboxyphenoxy)propane or simply (CPP) (Figure 2.1) where R = CH₂CH₂CH₂.

![Chemical structure of bis(p-carboxyphenoxy)alkanes](image)

**Figure 2.1** Synthesis of bis(p-carboxyphenoxy)alkanes
Another diacid of importance to the author's project is bis(carboxyphenoxy)alkane, which contains triethylene glycol in the center of two hydroxybenzoic acid moieties[9-12]. The diacids were synthesized by reaction of a dibromo alkane with two equivalents of hydroxybenzoic acid in the presence of sodium hydroxide. The resulting diacid was then acetylated by refluxing in acetic anhydride, yielding a prepolymer. Next, the prepolymer was polymerized at 282°C for thirty minutes and then subjected to vacuum, resulting in a viscous solution. The resulting polymer, in the case of poly(1,3-bis(p-carboxyphenoxy)propane) (poly(CPP)), was a hard block with a crystalline melting point of 267°C. With the ability to synthesize polyanhydrides from an array of diacids, further research was needed to understand the physical properties and mechanism of degradation from this class of polymer.

2.2 Erosion and Degradation

In order to optimize degradation for controlled release of pharmaceuticals, Langer and Rosen suggested the use of polyanhydrides because of the highly labile anhydride bond and the near zero order erosion kinetics [13]. A series of polyanhydrides were synthesized by Leong in order to understand the erosion rate based on the architecture of the monomer [8]. The polymers synthesized were: poly(CPP), random copolymer poly(1,3-bis(p-carboxyphenoxy)propane-co-sebacic acid anhydride) (poly(CPP-r-SA)), random copolymer poly(1,6-bis(p-carboxyphenoxy)hexane) (poly(CPH)) and poly(terephthalic acid anhydride) (poly(TA)) using the method of Conix [7]. The average molecular weights of the polymers synthesized were between 9000 and 15000 Daltons. The degradation results showed that the erosion rate decreased as the hydrophobic character in the backbone of the polymer increased. Increasing the percentage of sebacic acid caused an increase in the hydrophilicity
of the random copolymer of poly(CPP-r-SA). This in turn increased the erosion rate of the polymer, as might be expected. The most hydrophobic polymer studied, poly(CPP), degraded completely in over 3 years. A 20:80 poly(CPP-r-SA) copolymer began to crumble in the later stages of degradation, resulting in loss of the device shape and an increase in surface area exposed to the buffer solution. The physical breakdown of high percentage polySA random copolymers was attributed to the low molecular weight of the polymers studied. Moreover, the pH of the buffer solution also affected the rate of polymer erosion. At more acidic conditions, a decrease in the degradation rate of the polyanhydrides was observed. When the polyanhydride bond breaks during degradation, a monomer based acid and acetic acid result. Presumably, at lower pH the scission of an anhydride bond is less favorable because the acetic acid present is less likely to catalyze the degradation reaction due to the lack of acetic acid protonation. Albertsson and Lundmark studied the degradation of poly(dodecanoic anhydride) (poly(DA)) with a $M_w \sim 33,000$ in phosphate buffer solution at 37°C [14, 15]. The molecular weight, both $M_w$ and $M_n$, decreased rapidly in the first 24 hours the strips were subjected to buffer solution. Scanning electron micrographs revealed a gradual surface degradation of the initially smooth poly(DA) strips, with deep cracks appearing. The researchers noted that the pH of the buffer solution dropped after the first day the poly(DA) was in the buffer solution. This was attributed to the release of dodecanoic acid from the amorphous regions of the poly(DA) strips caused by hydrolytic degradation of poly(dodecanoic anhydride). In the remaining 8 days of the experiment further degradation took place in the crystalline regions of the polymer, until the poly(DA) began to disintegrate. Apparently, in the first stage of degradation, the amorphous regions of the polymer degrade
and release monomer or oligomers resulting in rapid loss of tensile strength. Next, the degraded polymer (monomer) increases in crystallinity due to the increased mobility of the chains. The higher crystallinity slows the degradation until the polymer disintegrates. Langer and Gopferich found that the solubility of anhydride monomer is dependent upon the pH inside the pores of the degrading polymer, which in turn was dependent on the pKₐ of the monomer acids [16, 17]. The results showed that SA is five times more soluble than CPP at pH values below 7.4. Further, in the case of poly(SA-r-CPP) (80:20), when all the SA monomer was released from the pores an increase in the degradation rate of poly(CPP) was observed. This was due to the change in pH inside the pores of the polymer. The CPP monomer saturated the solution in the pores resulting in a faster release. To suppress the loss of device integrity during degradation, Langer and Leong postulated that increasing the molecular weight of the polymer might resolve this issue [8]. High molecular weight polyanhydrides were an intense area of research.

2.3 High Molecular Weight Polyanhydrides: Melt Condensation

Langer and Domb further refined the method of prepolymer preparation and melt condensation to obtain higher molecular weight polyanhydride random copolymers than had previously been prepared [18-24]. The most convenient method of synthesizing high molecular weight polyanhydride copolymers is by the melt polycondensation of anhydride prepolymer similar to the method of Conix [7]. Briefly, diacetylated prepolymer was prepared by refluxing a dicarboxylic acid in acetic anhydride for half an hour. Next, two prepolymer were reacted in a melt condensation reaction at 180°C under high vacuum to yield high molecular weight random copolymers of polyanhydrides (Figure 2.2).
The difference between the Conix method and the Langer method was mainly the purification of the prepolymer and care to produce as monomeric prepolymer as possible. A monomeric prepolymer is necessary to produce a more random copolymer, know the feed composition of monomer and increase statistical randomness in the polymer chain. The other differences were the reaction time, temperature and application of high vacuum from the beginning of polymerization. Prepolymers of CPP, CPH and isophthalic acid had degrees of polymerization of between 1.4 and 1.6. Prepolymers of sebacic acid, dodecanoic acid and adipic acid had degrees of polymerization ranging from 5-9. Ideally, the degree of polymerization should be one, representing a completely monomeric prepolymer. Oligomerization of the prepolymer resulted from both reaction with acetic anhydride and purification. As the acetic anhydride was removed from the reaction flask under vacuum, temperatures above 70°C were used to increase the rate of evaporation, which lead to oligomerization. Therefore, mild temperatures were used by Domb during the solvent removal process in order to reduce the chance of increased oligomers. Monomeric prepolymers were used to produce polymers of poly(SA) with a molecular weight (\(M_w\)) of \(~140,000\) and poly(CPH) of \(~30,000\). This is a vast improvement in molecular weight over the polymers Hill and Carothers were able to obtain, \(M_w \sim 15,000\) for poly(SA). The molecular weight of the polymer formed is dependent upon the ability to remove acetic acid.
formed during the condensation reaction. This is because the formation of polyanhydride from prepolymer is reversible, and therefore the presence of an acid byproduct shifts the reaction equilibrium in the reverse direction, favoring monomer formation.

Another factor contributing to the formation of high molecular weight polyanhydrides was the reaction temperature. A melt polymerization temperature of 180°C was determined to be the most favorable for production of high molecular weight polymers [18-20]. Increasing or decreasing the reaction temperature reduced the molecular weight of polymer formed under constant vacuum. When the reaction time was above 3 hours, the molecular weight was increased but the polymer formed lacked enough solubility in chloroform for immediate purification resulting in extensive depolymerization, which led to lower molecular weight polymers in the end.

In an effort to synthesize homopolymers of aromatic-aliphatic diacids (i.e. non-bis diacids), polyanhydrides that polymerize head to tail were prepared [25]. Since random copolymers of polyanhydrides degrade based on their architecture (i.e. SA-SA bonds degrade at a different rate then CPP-CPP bonds) polyanhydrides having both aliphatic and aromatic domains were studied without the problem of heterogeneous degradation rates of liable bonds. Monomers were synthesized using brominated methyl esters and hydroxy methyl benzoic ester. Free diacid were obtained by deprotection of an ester with sodium (Figure 2.3).

![Figure 2.3 Synthesis of Aromatic-Aliphatic Monomers](image)

**Figure 2.3** Synthesis of Aromatic-Aliphatic Monomers
The diacids were polymerized using the method of melt polycondensation of prepolymers to give polyanhydrides of molecular weights ranging from 13,000 to 45,000. The homopolymers of aromatic-aliphatic monomers had low melting points and good solubility in common organic solvents. Further studies were performed to determine the degradation characteristics of the synthesized polymers. Degradation was studied in phosphate buffer solution at 37°C, and all polymers displayed zero-order degradation kinetics with the rate of degradation depending on the length of aliphatic chain. A longer chain resulted in slower degradation, from 10 days for methane to approximately 70 days for octane.

A major concern when polymerizing monomers, particularly in condensation polymerization, is the polydispersity index (PDI) of the polymer formed. Polydispersity affects properties such as viscosity and mechanical strength of a polymer. Polydispersity refers to the ratio of the weight average molecular weight ($M_w$) to the number average molecular weight ($M_n$), which measures the amount of variation in chain lengths. Therefore, a monodisperse polymer would have a PDI of unity, which is characteristic of controlled polymerization methods such as anionic and cationic polymerizations. These methods attain molecular weight by an active ion attacking or accepting a monomer individually. Polycondensations are not controlled but rather two stage processes that occur during the growth of high molecular weight chains. During the first two thirds of polymerization, oligomers of anhydrides are formed and then these oligomers form larger and larger chains by condensing together resulting in high molecular weight polymers [19]. Hill and Carothers speculated that when the medium to high molecular weight chains are
forming, cyclic macromolecules are formed by transesterification reactions [3, 4]. The formation of cyclic macromolecules, also called depolymerization, was suggested by Langer and Domb as the cause of the large polydispersities (2.5-9.72) involved in the synthesis of the polyanhydrides. Depolymerization also takes place when polyanhydrides were allowed to remain in solution for extended periods of time. Langer and Domb studied the depolymerization of polyanhydrides in chloroform and tetrahydrofuran (THF) along with the effect of the addition of metals to these solutions [25]. The researchers noted that depolymerization only affects high molecular weight polymers. Further, a more polar solvent, such as THF over chloroform, increased the depolymerization rate. Additionally, the inclusion of zinc and copper metals at room temperature caused a noticeable increase in the rate of depolymerization while aluminum, tin, nickel and palladium showed little effect. No reason was attributed to the difference in depolymerization rate from metal to metal.

2.4 Coordination Catalysts

Coordination catalysts such as cadmium acetate, earth metal oxides and diethyl zinc (ZnEt₂-H₂O) were studied in the polymerization of a wide range of polyanhydrides by Langer and Domb [19]. The catalysts yielded high molecular weight (\(M_w \sim 116,000-199,000\)) polymers in shorter reaction times than traditional melt polymerization methods but did not reduce the polydispersity of polymers formed (PDI 5.8-7.0). The coordination catalysts coordinate to the carbonyl of the prepolymer causing the carbonyl carbon to become more electron deficient allowing a nucleophilic attack of this carbon by an acid anion. Albertsson and Lundmark melt polymerized poly(adipic anhydride) poly(AA), poly(SA) and poly(dodecanoic anhydride) poly(DA) in the presence of diethyl zinc [15]. They found that
increasing the chain length of the diacid increased the molecular weight and the reaction rate. Further, they suggested a four site anhydride exchange intermediate as the mechanism of the polyanhydride formation (Figure 2.4).

![Figure 2.4 Mechanism of melt polyanhydride formation](image)

2.5 Solution Polymerization

Since polyanhydrides are being developed for use in vivo, contaminants in the polymer from the polymerization process may be harmful to patients. Further, many diacids that are sensitive to decomposition at elevated temperatures are excluded from melt polycondensation. Therefore, Langer and Domb developed a method to polymerize polyanhydrides that negates the need for a further purification step to wash away water soluble contaminates and readily polymerized diacids at room temperature [19]. Either phosgene or diphosgene was reacted with sebacoyl chloride and an acid acceptor. The acid acceptor was added to remove the condensation byproduct in order to minimize the degradation of polyanhydride formed. The best results were obtained using diphosgene and poly(4-vinylpyridine) poly(VP) as an acid acceptor in dimethylformamide (DMF). The
poly(VP) was insoluble in DMF as a free amine, enabling an easy separation of the contaminant from the polymer solution. Unfortunately, homo and random copolymers of molecular weights between 4800 and 16,000 were obtained, leaving room for improved methods to obtain higher molecular weights.

2.6 Poly(ethylene glycol) Containing Polyanhydrides

The incorporation of poly(ethylene glycol) (PEG) into polyanhydrides as either blocks or monomers for copolymerization is motivated by the need for faster eroding polymeric delivery devices. Moreover, the more hydrophilic PEG could prevent build-up of unwanted polymer in the host tissue [26]. The advantages of using PEG are as follows: the hydrophilicity of the chain shortens degradation times [27-29], it has "stealth" like effect that increases circulation time in vivo along with high biocompatibility and can increase stability by forming complexes with macromolecules [26, 30-32].

Synthesis of PEG containing polyanhydrides consists of functionalizing PEG in order to obtain complementary endgroups appropriate for reaction with anhydride monomers or polymers. One approach to incorporation of PEG into polyanhydrides was shown by Hartmann et al., which involved insertion of acid chloride functionalized PEG in between aminobenzoic acids to create amide bonds (Figure 2.5) [33]. They synthesized diacid chloride polyols by addition of acrylonitrile and then hydrolysis to the dicarboxylic acid. Next, the diacid was converted to the diacid chloride by reaction with thionyl chloride.
Figure 2.5 Aromatic diacid monomer containing an internal amide

Upon reaction with p-aminobenzoic acid, an amide dicarboxylic acid was formed. Subsequently, the diacid was converted to the anhydride and melt polymerized. The polymers had PDI’s ranging from 2.12 to 3.08 and number average molecular weight ranging from 2,500 to 13,000. The researchers studied the polymerization conditions (time, temperature, acetic anhydride) effect on the number average molecular weight. The highest molecular weights were obtained when the acetylated prepolymer was polymerized without any further addition of acetic anhydride after isolation. Moreover, temperatures of 165-170°C for an hour and a half provided the highest molecular weigh polymers. Hartmann et al. also incorporated PEG into aromatic diacids with the insertion of ester groups into the backbone (Figure 2.6) [34].

Figure 2.6 Aromatic diacid monomer containing an internal ester

A PEG diol was converted to the corresponding diacid by hydrolysis of the nitrile as mentioned above. Next, the diacids were added to thionyl chloride and then p-hydroxybenzoic acid to produce diacids containing ester bonds. The polymers had melting points between 98°C and 180°C depending on the rigidity of the aliphatic backbone. No
biocompatibility or drug delivery studies were preformed for any of the amide or ester containing polyanhydrides in these two papers. This differs from the work described in this Thesis because of the ester bond used to connect the aromatic moieties with the aliphatic chain. The aromatic acid was linked to the aliphatic chain with an ether bond in this Thesis.

Copolymerization of functionalized PEG is another means of incorporating the segments into polyanhydrides. The most common method of functionalizing PEG to convert it for use as a mixed anhydrides is through reaction of the diol with succinic anhydride to produce a diacid [26]. The PEG diacid ($M_n \sim 600$) was acetylated with acetic anhydride and then copolymerized with sebacic anhydride in various ratios. The resulting copolymers, when subjected to phosphate buffer solution at 37°C, degraded in 3 to 10 hours depending on the PEG content. The greater the PEG content, the more hydrophilic the polymer. Therefore, the more the polymer swelled, increasing the erosion rate. Notably, even though the polymers degraded in a period of hours, release of DNA lasted for six days \textit{in vitro} [26]. Since PEG can produce erosion profiles in a period of hours as opposed to weeks or years, Zhu et al. [28] fabricated pulsatile release devices composed of layered polymers. The laminated devices consisted of protein loaded layers of poly(methacrylic acid-ethoxazoline) (PMAA-PEOx), with isolating layers of poly(SA)-b-PEG. The polyanhydride copolymers were used as an acid generator for the pH sensitive pMAA-PEOx layers. As the anhydride bonds degraded they produce acetic acid, which in turn protonated and swelled the PMAA-PEOx layer releasing the loaded protein. Therefore, the number of layers of PMAA-PEOx controlled the number of pulses that released protein.
2.7 Block Copolymer Synthesis

Since Bucher and Slade synthesized the first polyanhydrides in 1910, there has been no mention in the literature of polyanhydride-polyanhydride block copolymers. However, block copolymerization of poly(ester)-poly(anhydrides) and poly(ether)-poly(anhydrides) have been reported. This section describes block copolymerization reactions and other catalysts used in synthesis related to the production of block copolymers.

The main reason that polyanhydride-polyanhydride block copolymers have not been synthesized is because the reactivity ratio of acetylated prepolymers is equal to unity. The reactivity ratio is defined as the reactivity of a monomer reacting with itself divided by the reactivity of a monomer reacting with a different monomer [35, 36]. Monomers with reactivity ratios of one yield random copolymers when they are allowed to react together [37]. A reactivity ratio of less than one implies that a monomer will preferentially react with a different monomer rather than itself. This scenario leads to alternating copolymers. When the reactivity ratios are greater than one, a monomer prefers to react with itself, thus producing block sequences. If both reactivity ratios are much larger than one simultaneous homopolymerizations result due to the overwhelming tendency for the monomers to react with themselves.

2.7.1 Tin(II) Octoate

Block copolymers of poly(ester-anhydrides) or poly(ether-anhydrides) are typically synthesized by a ring opening polymerization, often initiated with a macromonomer in the presence of a catalyst. Although methods exist to couple two polymers together with an appropriate agent, they are not feasible in the case of polyanhydride-polyanhydride block
copolymer synthesis due to the reactivity/degradability of the anhydride bond. No methods are currently available to end-functionalize polyanhydride chains without substantial degradation.

Albertsson and coworkers have successfully polymerized adipic anhydride, a six carbon cyclic anhydride, using stannous 2-ethylhexanoate (Sn(Oct)$_2$ or Tin(II) octoate) as a nonionic ring opening polymerization catalyst [15]. Tin(II) octoate is a polymerization catalyst used in polymerizing lactones industrially. The reaction was carried out at 80, 100 and 120°C for five hours in the melt and yielded low molecular weight (M$_w$ ~1500-5000) homopolymers. The stannous 2-ethylhexanoate acts by a nonionic insertion polymerization mechanism. This was determined by varying the monomer to initiator ratio and plotting it against the molecular weight obtained from the polymerization. There was a tendency for the molecular weight to increase as the monomer to initiator ratio was increased, which was explained by the living character of the initiating species. In the case of an anionic polymerization mechanism, one would expect a linear trend of the ratio of monomer to feed when plotted against the molecular weight. After an hour or more at elevated temperatures, 120°C, the stannous 2-ethylhexanoate promotes anhydride exchange between the chains, to a point where cyclic macromonomers are formed resulting in a high degree of polymerization (~5-10) similar to results seen by Langer and Domb and Hill and Carothers [2-6, 18-24]. Further studies on the mechanism of ring opening polymerization using tin(II) octoate in the presence of lactones and lactides have been done by Albertsson and coworkers [38], Dubois and coworkers [39-41] and Penczek and coworkers [42, 43]. The mechanism was elucidated by studying the polymerization products formed using $^1$H-NMR, MALDI-TOF and computer simulations. The researchers found that tin(II) octoate is not the initiator. A tin alkoxide
formed from trace amounts of impurities in the reaction serves as the initiator. Solution polymerizations in THF were performed in the presence of alcohol and MALDI-TOF and revealed structures with an alkoxide at one end group and an octoate at the other end for long reaction times ($t > 120$ min). For shorter times, the propagating end group was a protonated alcoholate. When the polymerizations were conducted in the presence of amines, amide end groups formed as shown by MALDI-TOF analysis. The researchers also determined that addition of octanoic acid to the polymerization resulted in a reduction in the rate of reaction and no increase in $M_n$. When purified Sn(Oct)$_2$ was used, the polymerization was very slow with a linear increase in $M_n$ and a first order kinetic plot suggesting a living polymerization. Further, as the initial composition of tin(II) octoate was varied with fixed alcohol, the rate of polymerization increased linearly and then leveled off. This result suggests that the polymerization is not initiated by tin(II) octoate but rather a tin(II) alkoxide. The MALDI-TOF also revealed cyclic macrocycles containing $\text{-OC(C}_2\text{H}_5\text{)}_n\text{-O-Sn-}$. The proposed mechanism of the polymerization of cyclic esters is as follows. Sn(Oct)$_2$ in the presence of alcohol (ROH) or water (R=H) is converted to (Oct)Sn(OR) in a reversible reaction. Additionally, the Sn mono-alkoxide can convert to the Sn di-alkoxide in presence of an alcohol. The polymerization propagates at the $\text{-Sn-O-}$ bond (i.e. $\text{-Sn-O-polymer-R}$). The end group results from the RO$^-$ esterification of the open monomer carbonyl. The resulting anion of the monomer inserts into the Sn-Oct bond releasing octanoic acid (Figure 2.7).
Sn(Oct)$_2$ + ROH $\rightarrow$ OctSnOR + OctH

OctSnOR + ROH $\rightarrow$ Sn(OR)$_2$ + OctH

SnOR + nLa $\rightarrow$ SnO-(La)$_n$-R

SnO-(La)$_n$-R + OctH $\rightarrow$ SnOct + HO-(La)$_n$-R

Figure 2.7 Mechanism of ester polymerization with tin(II) octoate [43]

Tin(II) octoate has also been used to prepare block copolymers of ε-caprolactone and L-lactide by Feijen and Coworkers [42]. Block copolymers were prepared by sequentially polymerizing ε-caprolactone and L-lactide with tin(II) octoate in the presence of ethanol at 110°C. The L-lactide was initiated by the macromonomer of poly(ε-caprolactone) P(CL) and Sn(OCH$_2$CH$_2$)$_2$. The reaction did not provide block copolymers when the order of polymerization was reversed, only random copolymers were obtained. Apparently, transesterification reactions resulted from side reactions of ε-caprolactone and the L-lactide macroinitiator. Block copolymers obtained had $M_n$ between 16,000 and 39,000 with a PDI of 1.16-1.27.

Solution polymerization of adipic anhydride was tested at 0, 20 and 40°C in methylene chloride for 24 hrs using anionic, cationic and coordination polymerization catalysts by Albertsson and Lundmark [15]. The results showed, in all cases, that polymerization using stannous 2-ethylhexanoate gave the highest molecular weight polymers ($M_w \sim 3200-4700$). Initiation with the potassium salt of acetic acid and tin(II) octoate were determined to be very fast, completing polymerization in 15 min at 40°C. Aluminum
trichloride displayed a higher conversion at 20°C than the salt/octoate complex mentioned previously. The end groups of the resulting polymers were used to verify the proposed mechanism. The cationic ring opening resulted in an acid end group, the anionic initiation produced a ketone or aldehyde and the coordination polymerization resulted in an anhydride end group. Aluminum isopropoxide, an anionic catalyst, was used to copolymerize adipic anhydride with ε-caprolactone to yield a block copolymer of the two monomers with a molecular weight of $\overline{M}_w \sim 58,000$. The ε-caprolactone was melt polymerized at 90°C first, and then adipic anhydride was initiated by the living aluminum isopropoxide end. The PDI of the copolymer was about 2 to 3, most likely from transesterification and anhydride exchange due to the presence of the catalyst.

2.7.2 Living Polymerization

The living anionic polymerization of ε-caprolactone was carried out using aluminum isopropoxide as an initiator in toluene and methylene chloride by Dubois and coworkers [41]. Homopolymerizations of adipic anhydride and ε-caprolactone were successful in toluene at 20°C but at a molecular weight (above $\overline{M}_n \sim 5000$) chains of poly(adipic anhydride) begin to precipitate out of solution as a result of insolubility in toluene. Therefore, methylene chloride was used as a solvent. Unfortunately, as the dielectric constant of the solvent increases, the reactivity of the catalyst decreases resulting in lower molecular weight polymers ($\overline{M}_n \sim 1000-1200$). The addition of a Lewis base (pyridine or nicotine) enhanced the rate of reaction and suppressed transacylation reactions from occurring. The bases enhanced the rate of polymerization by polarizing the aluminum oxygen bond. The melt
homopolymerization of poly(AA) in the presence of pyridine produced polymers of $M_n \sim 9,500$-58,000 with PDI of 1.25-1.5. Both ε-caprolactone and adipic anhydride polymerize in toluene according to an insertion coordination mechanism. In the case of adipic anhydride, the aluminum isopropoxide forms an active aluminum carboxylate, which is inactive towards the initiation of ε-caprolactone. Therefore, block copolymers were synthesized by polymerizing ε-caprolactone to a desired molecular weight and using the living end of the polymer formed as a macroinitiator to initiate the polymerization of adipic anhydride, yielding block copolymers. Addition of pyridine produced diblock copolymers of poly(ε-caprolactone-b-adipic anhydride) with narrow polydispersities (~1.25-2.00) and $M_n$ of 19,000 P(CL) and 27,000 poly(AA) block.

2.7.3 Synthesis of Anhydride Rings

Since diacids can be cyclized into rings of anhydrides, substitution of diacids may lead to increased functionality, better solubility and improved synthetic control. In order to perform ring opening polymerizations, a ring of the selected monomer must be formed.

Before Bucher and Slade first synthesized polyanhydrides in 1910, Andrelini made anhydride rings of suberic, azaleic and sebacic acids in 1894 [44, 45]. The diacids were refluxed in an excess of acetyl chloride for 8-10 hrs. The acetyl chloride was removed under vacuum and the resulting solid was recrystallized in a benzene/petroleum ether solution. The anhydride rings of suberic, azaleic and sebacic melted at 62-63°C, 52-53°C and 78-79°C respectively. Hill and Carothers studied the ring formation of several diacids including sebacic acid [3-5]. The rings were formed by refluxing the diacids in acetyl chloride or
acetic anhydride and then heating the remaining residue above 200°C. The molecular distillation produced a ring of the diacid, in the case of the 8, 10 and 12 carbon diacids the ring was determined to be dimeric by reaction with aniline. A monomeric ring reacts with aniline to produce a monoamide whereas a dimeric ring will produce a diamide. The sebacic anhydride ring had a melting point of 62-63°C. Albertsson and coworkers synthesized the anhydride ring of adipic acid by reacting the diacid with acetic anhydride and then vacuum depolymerization in the presence of zinc acetate [15]. The ring was obtained in 70% yield in the presence of the catalyst and 50% without catalyst.

Ballini et al have shown that α-nitrocycloalkanones can be cleaved open at the α-nitrocarbon to yield α,ω-dicarboxylic acids, α,ω-dicarboxylic monomethyl and dimethyl esters [46]. Substituted (hydrogen, methyl, isopropyl, phenyl) α-nitrocycloalkanones of ring size between 1 and 10 were subjected to potassium hydrogen persulfate at room temperature yielding the corresponding diacid in 84-99% yield. The esters were produced by treating the α-nitrocycloalkanones with methanol, sodium hydroxide and Na₂HPO₄. There are a number of ways to produce α-nitrocycloalkanone rings, many of which lead to side products and low yields. Pivawer et al. synthesized α-nitrocyclododecanone by reacting cyclododecene with dinitrogen tetroxide/oxygen N₂O₄/O₂ (1:30) in DMF at 0°C for 5hrs. The resulting nitroketone precipitated out of solution in an 86% yield [47, 48]. Synthesis of an array of α-nitrocycloalkanones and α-nitroalkanones from their corresponding cyclic and linear alkenes by reacting the alkene with trimethylnitrate and chromium trioxide at 0°C for 24 hrs, has been reported [49]. The resulting nitrated ketones were purified by silica gel column and produced in yields of 27-94%. The remainder of the methods to synthesize α-
nitrocycloalkanones use an enol trimethylsilyl (TMS) ether and a nitrating agent to produce the said compound [49-52]. This method of substitution of cycloalkanone-1-ene compounds may also be used to substitute alkanes on the ring.

2.7.4 Covalent Coupling of Blocks

Living polymerization is a convenient method of producing monodisperse block copolymers of a variety of polymers but not all monomers can undergo this type of polymerization. Another simpler method of producing block copolymer is by coupling two homopolymers together with the appropriate end groups. For instance, an acid and an alcohol can condense to produce an ester. Storey et al. synthesized poly(ε-caprolactone) polymers with an anhydride linkage between two poly(ε-caprolactone) blocks [53]. The poly(ε-caprolactone) was synthesized by polymerizing ε-caprolactone in the presence of tin(II) octoate and ethanol for 21 hrs. Then the free hydroxyl end of the poly(ε-caprolactone) was reacted with succinic anhydride and 1-methylimidazol (NMIM), yielding a succinic acid end group. Next, diphenyl chlorophosphate was used to couple two succinic acid terminated poly(ε-caprolactone) polymers resulting in the formation of one anhydride linkage. These polymers were formulated to produce release rates outside of the traditional constant release profiles. Gopferich et al. synthesized amine terminated poly(ethylene oxide-b-D,L-lactic acid) poly(EO-b-LA) ($M_n \sim 2000$ PEO, $M_w \sim 20,000$ poly(LA) for the development of biomimetic polymers in tissue engineering and the covalent attachment of peptides and proteins via amine reactive moieties [54]. The amine terminated PEO was prepared by subjecting EO to potassium bis(trimethylsilyl)amide in tetrahydrofuran (THF). The presence
of amine was verified by $^1$H-NMR and the amine proton exchange with D$_2$O. When D$_2$O was added as a co-NMR solvent, protons from the amine were exchanged with deuterium resulting in the loss of the amine proton peak in the $^1$H-NMR spectrum. Next, block copolymer was synthesized by initiating the polymerization of LA with the amine terminated PEO ($M_n \sim 2000$) in the presence of tin(II) octoate. The tin(II) octoate preferentially reacted with the free hydroxyl group instead of the free amine. The resulting block copolymer was subjected to disuccinimidyl tartrate (DST) in triethylamine and acetonitrile (Figure 2.8).

Figure 2.8 Synthesis of functionalized (PEO-b-PLA)

The free amine of the block copolymer formed an amide with the DST yielding an amine reactive polymer for the covalent attachment of free amine containing bioactive molecules. Langer et al. produced block copolymers of poly(D,L-lactic-co-glycolic acid) (PLGA) and poly(lysine) (poly(Lys)) for the covalent attachment of carboxylic acid containing molecules [55]. A copolymer of PLGA (50:50) ($M_n \sim 17,000$) was coupled with poly(ε-carbobenzoxy-L-lysine) ($M_n \sim 6300$) using dicyclohexyl carbodiimide (DCC) in
dimethyl formamide (DMF) and dimethylamino pyridine (DMAP). DCC is a common coupling agent for the production of amides in the synthesis of peptides. The reaction was complete in 36 hrs and yielded the block copolymer in 25% yield as calculated from elemental analysis. The poly(Lys) segment of the block copolymer was deprotected from the carbobenzoxy groups by reacting it with 30% hydrobromic acid/acetic acid. The deprotection reaction had little effect on the degradation of the PLGA segment, as was verified with gel permeation chromatography (GPC).

2.8 Ester Amide Synthesis

A simple way to produce block copolymers is through the coupling of suitable functionalities (amines, acids, alcohols). Presently, dicyclohexylcarbodiimide (DCC) is a popular method of producing peptides from amines and carboxylic acids. But in the presence of an amino alcohol, DCC tends to give lower yields and even ester products. Kunishima et al. have developed an effective coupling agent, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-ethylmorpholiniumchloride (DMT-MM), for the aminolysis of activated carboxylic acids in the presence of water and polar solvents [42, 56-62]. It should be noted, that this method has yet to be used for the synthesis or modification of block copolymers or polymers in general. Therefore, this method not only provides a novel method of synthesizing block copolymers through the coupling of complementary functionalities (hydroxyl/amine) but also may be effective in the synthesis of homopolymers.

The coupling agent DMT-MM was synthesized be reacting 2-chloro-4,6-dimethoxy-1,3,5-triazine(CDMT) with N-methylmorpholine (NMM) in THF for 30 minutes at room temperature giving DMT-MM in 100% yield. A diacid was added to a solution of methanol,
ethanolamine and DMT-MM at room temperature for 3hrs. The amide was isolated with an aqueous work-up with an 82–98 % depending on the carboxylic acid used. The ratio of amide yield to ester yield was 98:1 in methanol, 99:0.7 in ethanol for DMT-MM and 27:4 for DCC in methanol. DMT-MM was also used to produce esters by condensation of a carboxylic acid with an alcohol in very high yields. By adding 1.2 equivalents of NMM along with the acid and DMT-MM in methyl alcohol esters were product in yields ranging from 34-99 % depending on the steric effects of the alcohol and the carboxylic acid used. Methanol produced the highest yields while with all acids while t-butyl acid and 1-hydroxy toluene gave a 34 % yield. It should be noted that in solvents such as chloroform, methylene chloride and THF, demethylation of DMT-MM takes place to give an inactive product 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-morpholine (DMTM). The mechanism of esterification/amidation is as follows (Figure 2.6); the acid anion attacks the DMT-MM and NMM is lost resulting in a carboxy dimethoxy-triazine salt. The carbonyl of the salt is attacked by the corresponding amine and the amide is formed with 2-hydroxy-4,6-dimethoxy-1,3,5-triazine formed as the other product.

\[ \text{R} \text{CO} + \text{DMT-MM} \rightarrow \text{RCONHR} \]

\[ \text{R} \text{CO} + \text{R}_2\text{NH} \rightarrow \text{RNH}_2 \text{CO} + \text{HO-} \]

**Figure 2.9** Mechanism of the Amidation of a Carboxylic Acid with DMT-MM
2.9 Microwave Synthesis and Polymerization

Methods of synthesizing polymeric materials in a high throughput manner are usually restricted to very clean solution polymerization reactions that can stir overnight at room temperature or involve setting up large pieces of equipment that can perform reactions in tandem on a lab to pilot plant scale [63-66]. While chemists have known about the usefulness of microwave energy for the synthesis of small molecules, polymer chemists have just recently begun to investigate microwave polymerizations in the last seven years.

Microwave heating works on the principle of dielectric heating. When a molecule is subjected to an electromagnetic field it will align, and depending on the wavelength of the field the molecule is in, will oscillate as a function of this wavelength. For a commercial microwave, the standard frequency it operates at is 2.45 GHz which corresponds to 12.2 cm wavelength of electromagnetic radiation. At this wavelength, the oscillations occur at $4.9 \times 10^9$ times per second [42]. Since a molecule is aligning and agitated by this field, molecular friction occurs causing internal heating of a sample. The dependence of the effect of heating is dictated by the strength of frequency of the microwave and the dipole moment of the molecules being subjected to the radiation. The larger the dipole (more polar) in the molecule, the greater the heating rate. Therefore solvents such as water, dimethyl formamide and methylene chloride exhibit microwave heating effects while solvents such as toluene and carbon tetrachloride are microwave inactive but may be heated by adding a microwave active substance to the solution[42, 67-72]. Moreover, liquids with similar dipole moments can convert the field radiation energy into thermal energy differently depending on their ability to align with the current field, which is defined as the loss factor. Some materials exhibit
special tendencies at 2.45 GHz, which cause their loss factor to increase with temperature causing an increase in the heating rate and leading to a super heating effect[42].

The microwave heating of reactions tends to speed up the reaction time for conversion and may obtain better yields than in the conventional analogue. It is thought that the increase in reaction rate is due to the increased molecular vibrations, which in turn increases the molecular mobility of the species. Others have proposed that the increase in reaction rate occurs due to a lowering of the activation energy. But most likely, the increased reaction rate is due to the increased rate of heating and the ability for some systems to super heat.

A number of polymerizations have been conducted in the microwave to date, they include ring opening, free radial, Atom Transfer Radial Polymerization, Reverse Atom Transfer Radial Polymerization, Ni (0) mediated coupling, condensation and cationic [49, 69, 72-98]. In most cases, decreases in the time of reaction were from days down to a to minutes/hours with a high conversion and in some cases improved molecular weights. Moreover, reactions have been conducted that were conventionally impossible. For instance, the polymerization of caprolactone was initiated by benzoic acid in the microwave. This is conventionally unlikely to result in high molecular weight polymers because a carboxylic acid isn’t a strong enough nucleophile to initiate the ring opening polymerization of caprolactone. The polymerization lasted 240 minutes to yield a polymer with a molecular weight similar to that of a conventional caprolactone polymerization initiated with a tin octoate reacted for 48 hours[95, 96].

After a thorough investigation of the literature, it was realized that no polymerizations of polyanhydrides have been conducted through microwave polymerization. Microwave
chemistry may be able to decrease the polymerization time necessary to conduct a condensation reaction of acetylated diacid prepolymers into polyanhydrides. This may lead to a novel method of synthesizing polyanhydrides which may further have utility in high throughput screening of polymer matrices (in general) for stabilizing drug delivery candidates.

2.10 Project Objectives

The overall goal of this project is to synthesize new polymers based on polyanhydrides that have the potential to stabilize macromolecules within a controlled microstructure of polyanhydride copolymers. A thorough literature review was conducted and indicates lack of research on the synthesis of polyanhydride-polyanhydride block copolymers due to the reactivity ratios of polyanhydride monomers and prepolymers. Furthermore, the reactivity of the anhydride bond complicates any synthetic endeavor due to the lack of stability in solution and transesterification reactions. The objectives of this project are two pronged: Synthesize poly(anhydride-anhydride) block copolymers; synthesize and characterize polyethylene glycol (PEG) containing diacids and integrate them into polyanhydrides for improved control of polymer hydrophobicity and degradation as well as potentially improved macromolecular stabilization.

Contained within the synthesis of PEG containing aromatic diacids is the halogenation of triethylene and penta ethylene glycols and further etherification with hydroxy benzoic acid. The PEG integrated polyanhydrides were made by polycondensation of acetylated diacids and further characterized by GPC, DSC and NMR. Release
experiments were preformed with hydrophobic and hydrophilic model compounds, and compared to degradation experiments.

Also included in this thesis are experiments that were attempted to synthesize polyanhydride-polyanhydride block copolymers. Work was done to explore the use of tin(II) octoate as a macrorinitiator of polyanhydride chains for the ring opening of cyclized anhydrides. Moreover, a ring of sebacic acid was synthesized for use in the ring opening polymerization. End functionalization of poly(CPH) with ethanol amine and N-methyl ethanol amine was attempted to produce block copolymers via a block-block linking strategy. And lastly, preliminary experiments were conducted to assess the feasibility of producing polyanhydrides in the microwave as a method of high throughput materials synthesis.
2.11 References


89. Mallakpour, S.E., A.-R. Hajipour, and S. Habibi, *Microwave-assisted synthesis of new optically active poly(ester-imide)s containing N,N'-(pyromellitoyl)-bis-L-


CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) with the exception of deuterated chloroform, which was purchased from Cambridge Isotope Laboratories, Inc. Dimethyl formamide (DMF) and chloroform were dried over calcium hydride and freshly distilled before use. Pentaethylene glycol and triethylene glycol were dried by heating at 70°C under high vacuum overnight. All solvents were of analytical grade and used as received.

3.2 Instrumentation

Ultraviolet (UV) spectroscopy was performed using a Shimadzu 1601 UV-Visible Spectrophotometer (Columbia, MD). Thermal analysis studies were conducted using a Perkin Elmer DSC-7 (Shelton, CT) differential scanning calorimeter (DSC), calibrated with Zn and In standards, at a heating rate of 10°C per minute under a nitrogen atmosphere. The DSC sample weights were between 5-8 mg. Molecular weights were estimated by a Waters gel permeation chromatography (GPC) system (Milford, MA). The samples were eluted with chloroform through PL Gel columns from Polymer Laboratories (Amherst, MA). \(^{1}\)H nuclear magnetic resonance (NMR) was used to verify chemical structure and determine the degree of polymerization for each of the polymer and prepolymer. NMR spectra were obtained on a Varian VXR 400 MHz spectrometer (Varian Inc. Palo Alto, CA). The compounds were characterized by \(^{1}\)H NMR in deuterated chloroform (99.8% atom-\(d\)) or deuterated dimethyl
sulfoxide (DMSO) (99.9% atom-\textit{d}). Chemical shifts were calibrated to the chloroform ($\delta = 7.26$ ppm) or DMSO ($\delta = 2.50$ ppm) peak. Microwave polymerizations were conducted in a General Electric 1.1 cu. Ft. microwave of oven (JE1160WC) with a maximum power of 1100 watts. Dissolution experiments were conducted in a USP approved dissolution testing apparatus (SR6, Hanson Research, Chatsworth, CA) maintained at 37°C under 100 RPM. Samples were drawn at different times from the dissolution test chambers and measured using a visible spectrophotometer (Shimadzu 1601 UV/VIS, Columbia, MD).

3.3 Experimental Methods

The experimental methods section of this thesis is broken up into three parts with sub-categories: general synthesis of homopolymers and random copolymers of polyanhydrides, synthesis of polyanhydride block copolymers, polyethylene glycol containing polyanhydrides, microwave polymerization of polyanhydrides and dissolution testing and degradation studies. Within the block synthesis section are subsections of Ring Opening Methods and End-group Modification.

3.3.1 General Synthesis of Homopolymers and Random Copolymers of Polyanhydrides From Diacids

3.3.1.1 General Synthesis of bis(p-carboxyphenoxy) alkanes. Ex: Synthesis of 1,6-bis(p-carboxyphenoxy)hexane diacid (CPH)

In a single-necked, 1 L round bottom flask equipped with a reflux condenser and magnet stir bar was placed a solution of \( p \)-hydroxy benzoic acid (55.4 g, 0.401 mol) and
potassium hydroxide (KOH) (56.0 g, 1 mol) in 750 ml of 75% DMSO in water. Added slowly, over the period of an hour was 1,6-dibromohexane (18.73 ml, 0.201 mol). The reaction was allowed to react over night at 120°C with stirring. Upon completion of the reaction, the solution was poured into a 2 L Erlenmeyer flask along with 200 ml of water. The solution was precipitated with the addition of sulfuric acid (100 ml). The off white precipitate was isolated by filtration and then washed with acetone (2 x 30 ml). The collected solid was dried before recrystallization twice in N-methylpyrrolidinone (NMP). Briefly, crude CPH was dissolved in NMP (200 ml) at 110°C. Next, the solution was filtered hot before crystallization overnight in the freezer. After the final crystallization step, the wet, pure diacid was washed twice with acetone (2 x 30 ml) before drying under vacuum overnight.

**Synthesis of 1,6-bis(p-acetoxycarboxyphenoxy)hexane (CPH prepolymer)**

To a flame dried 250 ml single-necked round bottom flask equipped with a reflux condenser was added CPH diacid (30 g, 0.0837 mol) and acetic anhydride (99.71 ml, 1.047 mol). The mixture was heated at reflux until all the CPH diacid was dissolved in acetic anhydride (~ 30 min). The solution was filtered through a fine sintered glass frit to remove any unreacted diacid before concentration *in vacuo*. The solution was concentrated to about a quarter of the total volume before placement in the refrigerator overnight to precipitate. The solid was filtered and washed with diethyl ether (3 x 40 ml) before drying under vacuum overnight. The prepolymer was recrystallized from hot dimethyl formamide (50 ml) with the addition of diethyl ether (400 ml). The pure prepolymer was isolated and dried overnight before polymerization.
**Synthesis of Poly(1,6-bis(p-carboxyphenoxy)hexane) (poly(CPH))**

To a flame dried 50 ml single-necked round bottom flask equipped with a vacuum line was added CPH prepolymer (10 g, 0.0225 mol). The solid prepolymer was heated to 190°C under high vacuum for 90 minutes. After polymerization, the viscous solid was dissolved in chloroform (20 ml) and precipitated with a cold 50/50 mixture of petroleum ether/diethyl ether (400 ml). The white solid was filtered and dried under vacuum overnight before use.

3.3.2 Synthesis of Polyanhydride-Polyanhydride Block Copolymers

3.3.2.1 Ring Opening Polymerization

**Ring Closure of Sebacic Acid**

To a flame dried 250 ml single-necked round bottom flask equipped with a reflux condenser was added sebacic acid (10 g, 0.0494 mol) and acetyl chloride (28.13 ml, 0.395 mol). The reactants were heated at 125°C and allowed to react for approximately 10 hrs. The reaction mixture was a dark red color after heating overnight. The flask was allowed to cool and the remaining solvent was removed under vacuum leaving dark oil. The dark oil was dissolved in boiling benzene before addition of diethyl ether and precipitation over night in the refrigerator. The white, solid precipitate was filtered and dried under vacuum overnight.
Synthesis of Poly(1,6-bis(p-carboxyphenoxy)hexane-b-sebacic anhydride) 

(poly(CPH-b-SA))

To a flame dried 50 ml single-necked round bottom flask equipped with a vacuum line was added poly(CPH) (1 g, 0.0002 mol) and tin(II) octoate (0.5 g, 0.002 mol). The polymer and initiator were melted together at 180°C under high vacuum for 15 minutes. Next, the ring of sebacic anhydride (2.0 g, 0.02 mol) was added and allowed to react for 2 hrs. After polymerization, the viscous solid was dissolved in chloroform (20 ml) (but it was not soluble in any common solvents) and precipitated with a cold 50/50 mixture of petroleum ether/diethyl ether (400 ml). The white solid was filtered and dried under vacuum overnight before characterization.

Solution Polymerization of Poly(sebacic anhydride)

To a flame dried 250 ml single-necked round bottom flask equipped was dissolved the ring of sebacic anhydride (2.0 g, 0.02 mol) and tin(II) octoate (0.5 g, 0.002 mol) in a 150 ml of dry tetrahydrofuran. The ring and initiator were reacted at room temperature for 3 hrs under a blanket of argon. A white solid begins to precipitate approximately 45 min into the reaction. After polymerization, the white solid was dried under vacuum overnight before characterization.

3.3.2.1 End-Group Modification of Polyanhydrides

Hydroxy Amidation of Poly(1,6-bis(p-carboxyphenoxy)hexane)

To a flame dried 50 ml single-necked round bottom flask was added poly(CPH) ($\overline{M_n}$ = 116,000 g/mol) (0.25 g, 2.15 µmol) and 40 ml of dimethyl formamide (or dimethyl
acetamide depending on the reaction). The contents of the round bottom were allowed to
dissolve at 80°C (approximately 15 min) and cool to room temperature before addition of 2-
chloro-4,6-dimethoxy triazine (CDMT) (1.89 mg, 8.60 µmol) and N-methyl morpholine
(1.31 mg, 12.9 µmol). The solution was allowed to stir for 15 min before addition of ethanol
amine (0.328 mg, 5.38 µmol). The contents of the round bottom were allowed to react for an
additional 15 min before the solution was concentrated to approximately 15 ml and
precipitated with a 50/50 mixture of cold diethyl ether/petroleum ether (200 ml). The solid
was filtered and dried under vacuum overnight.

3.3.3 The Synthesis of Polyethylene Glycol Containing Polyanhydrides

The general synthetic scheme to produce PEG containing polyanhydrides was as
follows: Synthesize halogenated short chain PEG segments such as triethylene and penta
ethylene glycol, then react them with p-hydroxy benzoic acid in the presence of base to yield
a diacid. This diacid can then be acetylated with acetic anhydride and further heated under
vacuum to produce polymer.

_Synthesis of Brominated Polyethylene Glycols (PEG)_

Before reaction all the glycols were dried overnight under high vacuum at 70°C. In a
single-necked, 500 ml round bottom flask equipped magnet stir bar was placed a solution of
pentaethylene glycol ($M_n \sim 238.28$ g/mol) (50 g, 0.21 mol) in toluene (250 ml) and pyridine
(36 ml, 0.44 mol). The round bottom was placed in a dry ice/acetone bath before slow
addition of phosphorous tribromide (PBr$_3$) (15 ml, 0.15 mol). Care was taken to keep the
temperature between -5 and 5°C and keep the gas evolution to a minimum during the addition of PBr₃. A reflux condenser replaced the rubber septum and the reaction was allowed to react over night at 100°C. After the reaction was complete, a two phase system results with an orange precipitate and a clear toluene solution. The toluene solution was filtered through celite (4 mm) twice and clarified with activated charcoal (5 g) twice to remove most of the orange precipitate before extraction with brine (2 x 50 ml), sodium bicarbonate (1 x 50 ml) and water (2 x 50 ml). After extraction, the clear toluene solution was dried over sodium sulfate before removal of the solvent under vacuum. The brominated PEG results as a pale yellow viscous liquid that was used without further purification.

Synthesis of Chlorinated Polyethylene Glycols (PEG)

Before reaction, all the glycols were dried overnight under high vacuum at 70°C. In a two-necked, 500 ml round bottom flask equipped magnet stir bar, a reflux condenser and rubber septum was placed pentaethylene glycol (\(M_n \sim 238.28\)) (50 g, 0.21 mol), n-hexane (90 ml) and dimethyl formamide (DMF) (0.1 ml). Next, thionyl chloride (74 g, 0.63 mol) was slowly added by syringe and allowed to react for 2 hrs at reflux. The now homogenous yellow solution was concentrated, resulting a yellow oil. The oil was distilled under vacuum at 230°C with the chlorinated fraction distilling at 180-185°C resulting in a clear oil.

Synthesis of 1,14-[bis(p-carboxyphenoxy)]-3,6,9,12-tertaoxatetradecane (CPPEG-5)

In a single-necked, 1 L round bottom flask equipped with a reflux condenser and magnet stir bar was placed a solution of p-hydroxy benzoic acid (55 g, 0.398 mol) and
potassium hydroxide (KOH) (56.0 g, 1 mol) in 750 ml of 50% dimethyl acetamide (DMAc) in water. Added slowly, over the period of an hour was dichloro pentaethylene glycol (\( M_n \sim 274.26 \text{ g/mol} \)) (50.0 g, 0.1809 mol). The reaction was allowed to react over night at 120°C with stirring. Upon completion of the reaction, the solution was poured into a 2 L Erlenmeyer flask along with 200 ml of water. Next, the solution was precipitated with the addition of hydrochloric acid (~ 100 ml). The off white precipitate was isolated by filtration and then washed with hot water (2 x 30 ml). The collected solid was air-dried overnight before recrystallization twice in warm isopropyl alcohol. Briefly, crude CPPEG-5 was stirred in isopropyl (200 ml) at 50°C. Next, the solution was cooled in an ice bath and the precipitate filtered and washed with chloroform (2 x 100 ml). After the final washing step, the wet, pure diacid was dried under vacuum overnight.

**Synthesis of poly(1,14-bis(p-carboxyphenoxy)-3,6,9,12-tertaoxatetradecane)**

**poly(CPPEG-5)**

To a flame dried 50 ml single-necked round bottom flask equipped with a reflux condenser was added CPPEG-5 diacid (3.3 g, 0.00686 mol) and acetic anhydride (40 ml, 0.39 mol). The mixture was heated at reflux until all the CPPEG-5 diacid was dissolved in acetic anhydride (~ 30 min). The solution was filtered through a fine sintered glass frit to remove any unreacted diacid before concentration in vacuo. All the unreacted acetic anhydride was removed before attaching a vacuum line and heating to 190°C under high vacuum for 90 minutes. After polymerization, the viscous solid was dissolved in chloroform (20 ml) and placed on an evaporation dish to evaporate and allow collection of the polymer.
The pale brown viscous solid was dried under vacuum overnight before use. The synthesis of (1,8-bis(p-carboxyphenoxy)-3,6-dioxaoctane) (poly(CPPEG-3)) was identical to the procedure for poly(CPPEG-5).

3.3.4 Microwave Polymerization of Polyanhydrides

Before polymerization reactions were run in the microwave, the vessel was calibrated to determine hot spots caused by constructive microwave interference within the chamber. The calibration was achieved by fitting a damp 33 cm piece of filter paper to the size of the interior glass plate and heating on high (1100 watts) for 15 minutes. A ring of charred filter paper results approximately 14 cm from the center of the paper. All samples were kept inside this ring to ensure no reactions occurred in the hot spots.

Sebacic acid (0.100 g, 0.494 mmol) and acetic anhydride (0.3003 g, 2.95 mmol) were placed in a borosilicate vial with cap and subjected to microwave radiation (1100 watts) for 2 minutes. Next, the vial was removed from the microwave (care should be taken because the glass is extremely hot) and the acetic anhydride was evaporated (while hot) by an inert gas (Argon) before replacing in the microwave sans cap and irradiated for another 6 minutes. The resulting polymer was dried under vacuum overnight before characterization.

3.3.5 Degradation and Dissolution Testing

3.3.5.1 Dissolution testing

Polymer, approximately 100 mg, containing 5 weight percent Rhodamine B Base (544 nm) or Acid Orange 8 (490 nm), were rolled into small pellets with an approximate diameter of 4.75 mm or pressed into tablets of 1.25 mm thick by 10 mm diameter by 2.5
metric tons at room temperature. The pellets were then placed within a 40 mesh basket in a USP approved dissolution testing apparatus (SR6, Hanson Research, Chatsworth, CA) containing 900 ml of 0.1 M phosphate buffer solution (pH = 7.4) maintained at 37°C under 100 RPM agitation. Samples were drawn at different times from dissolution test chambers and measured using a visible spectrophotometer. Mass of drug released was determined from a calibration curve of absorbance to concentration of dye. Buffer was replaced as needed. All studies were run in triplicate.

3.3.5.2 Degradation

Polymer tablets of 100 mg were placed in 900 ml of 0.1 M phosphate buffer solution (pH 7.4) maintained at 37°C under 100 RPM agitation in a 1 L beaker equipped with a fine mesh screen to keep the tablets submerged and Saran wrap to prevent evaporation. The tablets were removed at time intervals and patted dry with a Kim wipe to remove excess water from the surface before weighing and drying in a vacuum oven for 48 hrs. The dry weight was measured and used to determine mass of polymer degraded for that time interval. The study was run in triplicate.
CHAPTER 4
RESULTS AND DISCUSSION

4.1 Introduction to Results and Discussion

Contained within this section are the results and discussion of the three research aspects of this thesis. First, a detailed analysis of the attempts at the synthesis of polyanhydride-polyanhydride block copolymers is provided with an emphasis on two main areas: macromonomer ring opening polymerization of sebacic anhydride and (Sec. 4.2) end-functionalization of polyanhydrides with a di-functional ethanol amine to link blocks of anhydride polymers through an ester amide bond (Sec. 4.3). Second, the synthesis and polymerization of tri and penta ethylene glycol containing diacids that were further investigated for their release kinetics, and degradation profiles is described (Sec. 4.4). Third, results of preliminary experiments, involving the synthesis of polyanhydrides from diacids in the microwave as a novel high throughput materials library synthesis method are presented (Sec. 4.5). The underlying theme of the research conducted as part of this Master’s thesis is the synthesis of polyanhydrides with unique properties and applications for controlled drug delivery.

4.2 Ring Opening Polymerization of Sebacic Acid Anhydride

Polyanhydride homopolymers and their copolymers are typically synthesized through melt polycondensation of a di-acetyl activated diacid at high temperatures and low pressures. The polycondensation reaction is an equilibrium reaction that depends on the
purity of the prepolymer and ability of the system to remove acetic acid to drive the reaction progress forward (Figure 4.1). If copolymers are desired, placing ratios of the

\[
\text{HO} \quad \text{R} \quad \text{COOH} \quad \xrightarrow{\text{Acetic Anhydride}} \quad \text{Vacuum} \quad \xrightarrow{\text{Acetic Anhydride}} \quad \text{Vacuum} \quad + \quad \text{AOH}
\]

**Figure 4.1** Formation of acetylated diacid prepolymer and subsequent polymerization under vacuum

prepolymer into the reaction flask at the desired copolymer compositions results in statistically random copolymers due to equal an reactivity ratio of acetylated diacids [1]. Therefore, in order to get other types of copolymers, such as block copolymers, efforts must be made to make the polymerization reaction kinetically more favorable to producing block like sequences[2, 3]. One such method of controlling the polymerization kinetics is through ring opening polymerization with a suitable initiator. Albertsson and coworkers were able to obtain polymer from the ring opening polymerization of adipic anhydride, a six carbon cyclic anhydride, in the presence of tin(II) octoate, a coordination insertion type catalyst commonly used to polymerized lactones industrially [4].

With the knowledge that past researchers had polymerized an anhydride ring in the presence of tin(II) octoate, a goal was set to synthesize block copolymers of polyanhydrides through this method. The underlying assumption for this method to work is that short polyanhydride chains allowed to react with tin(II) octoate would act as a macro-initiator in the ring opening polymerization of sebacic acid anhydride, a ten carbon diacid that may be cyclized into a cyclic anhydride. The desired synthetic route to tri-block copolymers of
poly(sebacic anhydride –б-1,6-bis-(p-acetoxycarboxyphenoxy) hexane-б-sebacic anhydride) is by ring opening polymerization of sebacic acid anhydride initiated by the di-functional tin(II) poly(1,6-bis-(p-carboxyphenoxy) hexane macro-initiator.

![Cyclization of sebacic acid to the anhydride](image)

**Figure 4.2** Cyclization of sebacic acid to the anhydride

Before synthesis of the polymer, the sebacic acid diacid had to be cyclized by reflux in acetyl chloride overnight (Figure 4.2) [5]. Confirmation of the anhydride ring was obtained by gas chromatography/mass spectral analysis (GC/MS) of the isolated reaction product along with solid state carbon NMR (Figure 4.3).

![Solid state carbon NMR of sebacic acid anhydride ring](image)

**Figure 4.3** Solid state carbon NMR of sebacic acid anhydride ring
Solution proton or carbon NMR was not conducted due to the low solubility of the ring of SA in d6-DMSO, CDCl₃ or other common NMR solvents. Therefore, GC/MS was used to determine the mass of the molecule, which was confirmed by m/z 184.9 calc 184.2 g/mol for the anhydride ring and a lack of peak at 202.15 g/mol the mass of sebacic acid.

After confirmation of the synthesis of sebacic acid anhydride, work began on the initiation of the ring opening polymerization reaction mentioned previously. To determine the necessary conditions for polymerization, experiments were attempted with just the ring of sebacic acid and tin(II) octoate (2% monomer/initiator) under vacuum at 180°C for 30 min. The reaction resulted in a dark brown oil, that was precipitated in petroleum ether. The material had no melting peak in the DSC trace and was only soluble in warm tertahydrofuran (THF). And since there was no solubility in common deuterated solvents, no NMR spectrum could be obtained to verify the structure of the product isolated. The reaction was repeated again at the temperature of 130°C to reduce thermal degradation that may have contributed to the dark color of the reaction product at 180°C. The reaction yielded a light yellow oil that was dissolved in THF and precipitated twice, resulting in two fractions of product (Figures 4.4 and 4.5). The NMR spectra of the two fractions of product looked identical, and revealed that the ring of sebacic acid anhydride had indeed opened but no polymerization had taken place, resulting in the formation of the diacid. Interestingly, there was no semblance of the tin(II) octoate catalyst in either of the NMR spectra, most likely due to the fact that no reaction was taking place and thermal effects were facilitating the ring opening. The ring opening reaction was also tried with succinic anhydride and maleic anhydride (two commercially available anhydride rings) at 120°C under vacuum but yielded only black insoluble residue.
Figure 4.4 Ring opening polymerization product of sebacic acid anhydride with tin(II) octoate at 130°C under vacuum.

Figure 4.5 Ring opening polymerization product of sebacic acid anhydride with tin(II) octoate at 130°C under vacuum, fraction 2
Since there was no reaction taking place, another macromonomer was added to see if this would resolve the issue of obtaining mostly insoluble material and just opening the SA ring without polymerizing it. One possible hypothesis was that the ring was not opening because there was no nucleophile to start the ring opening and propagate the reaction.

A macromonomer of poly(1,6-bis-(p-carboxyphenoxy) (poly(CPH)) (\(M_n\sim5000\) g/mol) was prepared by making acetylated prepolymer of the corresponding diacid and was further polymerized by polycondensation. The polymer was isolated and dried before use in the ring opening reaction. Poly(CPH) was chosen as a macromonomer because it is commonly used as a polymer matrix for controlled drug delivery and the prepolymer is used in random copolymerizations with linear sebacic anhydride prepolymer. A macromonomer of molecular weight of about 5000 g/mol was chosen because it was thought that would provide a large enough block to obtain phase separation while remaining active as an initiator of the ring polymerization.

To initiate the ring opening polymerization, the macromonomer (poly(CPH)) was allowed to stir in the melt at 180°C with tin(II) octoate for fifteen minutes prior to addition of the ring of sebacic acid. This was done to let the tin(II) octoate react with the acid end-groups of the poly(CPH) in order to allow the chain to act as a macromonomer and produce tri-block copolymers by growing the sebacic acid chains at either end (Figure 4.6). Once the ring of SA was added, the reactants were placed under vacuum and allowed to react for an hour and a half before dissolving in chloroform and precipitating in diethyl ether. There were two fractions isolated from the reaction: one soluble in chloroform and a component not soluble in any of the common solvents. An NMR of the soluble fraction revealed poly(CPH)
components along with tin(II) octoate (Figure 4.6). For comparison's sake an NMR of the tin(II) octoate is provided below (Figure 4.7).

Figure 4.6 Soluble fraction of the reaction between P(CHR), tin(II) octoate and sebacic acid anhydride ring at 180°C under vacuum for 1.5 hrs.

Figure 4.7 Tin(II) octoate in CDCl₃
Based on the comparison of the NMR, it appears that the poly(CPH) either just blended with the tin(II) octoate or reacted. Unfortunately, the CPH-Octoate peak that would be present in the NMR if the two species reacted is either disguised or convoluted due to the tin(II) octoate peak at $\delta \sim 2.45$ ppm. Since a considerable amount of the reactants formed an insoluble material, we choose to try this reaction in a solution polymerization to try to reduce whatever insoluble material formed. The reaction was attempted in dry DMF, and the poly(CPH) was allowed to stir with tin(II) octoate for 15 min before addition of the sebacic acid anhydride ring. Upon addition of the tin(II) octoate, the polymer solution turned a hazy white color. The ring of SA was allowed to react with this solution overnight. After the reaction, an insoluble material had formed in the reaction mixture, which was isolated. Before completing any more experiments, a test was conducted to determine if the DMF was reacting with the tin(II) octoate as an unwanted side reaction. After addition of the tin species into DMF a yellow precipitate appeared in the flask. The oil was isolated and the NMR is shown below (Figure 4.8).
Figure 4.8 Tin(II) octoate reaction with DMF at 60°C in CDCl₃

It is difficult to attribute any spectral assignments to specific peaks between the reaction of DMF and tin(II) octoate because the octoate protons drown out any features in the 0-2.7 ppm range. But upon close inspection, one notices the increase in chemical shift of the two methyl peaks of DMF from 2.8, 2.9 ppm to 3.1, 3.2 ppm. This may be an indication that the aldehyde or amine have reacted or interacted with the tin species and the shift has moved down field due to the more electron withdrawing interaction with the tin-carbonyl bond[6]. Kavros et al. reported similar findings when attempting polymerization of ε-caprolactone with dibutyl tin oxide. They noticed a distinct reduction in the rate of reaction when using DMF as a solvent. Furthermore, they attempted the polymerization in another polar solvent, DMSO, and obtained high conversion to polymer. Therefore, they attributed the loss in reactivity to interactions of the dimethyl amine in DMF and the tin catalyst. After determining that DMF was not a good solvent for this reaction, a common solvent of the
anhydride ring, poly(CPH) and tin(II) octoate was sought. The diacid of SA is soluble in THF, methanol, DMSO and DMF, while the ring of SA is soluble in warm benzene, hot THF and DMF. Poly(CPH) however is soluble in chlorinated solvents, DMF and sparingly in THF. With this knowledge, THF was chosen as the solvent to begin conducting our experiments in.

Solution polymerizations were conducted in THF with just the ring of SA to determine the feasibility of the process. The ring of SA was added to THF with tin(II) octoate pre-dissolved at 60°C and allowed to react for three days. After the allotted time, the reaction mixture was a translucent gel within the THF, that upon isolation was insoluble in common solvents. The experiment was repeated and gave the same result. The reaction was repeated with linear sebacic diacid to see what resulted. Indeed, the insoluble material had formed again. To look at the effect of temperature on this gel formation, the reaction was conducted at 0, 25 and 60°C. The rate of formation of insoluble material increased with increasing temperature. At this point a literature search was conducted to find more information on the mechanism of polymerization, with the tin(II) octoate catalysts.

When the initial work was conducted on polymerizing adipic anhydride by Albertsson and coworkers[4], the prevailing mechanism of ring opening polymerization with tin(II) octoate was thought to be through a coordination inserting mechanism. But, further investigations into the mechanism determined that tin(II) octoate is not the initiating species but rather a di-alkoxide of the tin. The di-alkoxide is formed in situ during the reaction, presumably with trace amounts of water or alcohols depending on the solvent the reaction was conducted in[7-11]. Moreover, when MALDI-TOF was conducted on the polymerization products of the ring opening polymerization of ε-caprolactone with tin(II)
octoate as an initiator the data revealed not only polymer that had formed but also di-bridged tin compounds that were cyclized by multiple caprolactone monomers. When MALDI-TOF was conducted on the insoluble material from the solution ring opening polymerization of SA with tin(II) octoate in THF, it was determined that there was macrocyclic formation of a di-bridged tin compound. The tin species are linked together by an oxygen atom (SA-Sn-O-Sn-SA). At this juncture in the project other possibilities were explored to synthesize block copolymers from polyanhydrides. Another possible route was the linking of two previously polymerized homopolymers of polyanhydrides.

4.3 Synthesis of Polyanhydride Block Copolymers via an Ester Amide Linker

A common method of linking polymers together is through an amide bond. Langer et al. [12] formed block copolymers of poly(L-lysine) (poly(Lys)) with PLGA by DCC coupling of the carboxylic acid of the PLGA end-group with the amine end-group of the poly(Lys) in DMF with little to no degradation of the polymers. It was determined that a linking strategy would be a novel method to produce polyanhydride block copolymers. However, polyanhydrides are susceptible to degradation and chain randomization due to the high reactivity of the anhydride bonds throughout the polymer chain. Also, since there are symmetric end-groups on the polyanhydride chain, a tri-block copolymer is the minimum block number one can obtain with a linking strategy, with the possibility of an uncontrolled multi-block copolymer if a symmetric di-functional linker is used. Therefore, it was determined that the easiest way to obtain tri-block copolymers would be to use a di-functional linking agent with multiple reactivities i.e. amine more reactive than alcohol under the right conditions. Presently, dicyclohexylcarbodiimide (DCC) is a popular method of
producing peptides from amines and carboxylic acids. But in the presence of an amino alcohol, DCC tends to give lower yields and even ester products. However, a literature search yielded a paper detailing the synthesis of amides in the presence of alcohols and further reaction of the alcohol to an ester with the same coupling agent. Kunishima et al. have developed an effective coupling agent, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-ethylmorpholiniumchloride (DMT-MM), for the aminolysis of activated carboxylic acids in the presence of water and polar solvents [13-20].

Therefore, this was chosen to be a novel method of functionalizing carboxylic acid containing polymers as well as forming block copolymers. The polyanhydride chosen for the initial amide functionalization was poly(CPH) because of the more robust anhydride bond in comparison to an aliphatic polyanhydride. The pathway to the synthesis of the tri-block copolymer poly(CPH)-b-poly(SA)-b-poly(CPH) is shown in Figure 4.9.

Figure 4.9 Tri-block copolymer synthesis of polyanhydrides by ester-amide linker
The basic functionalization of poly(CPH) was a follows: the polymer ($M_n \sim 10,000$ g/mol) was first dissolved in dimethyl formamide and then added to DMT-MM to form the carboxylate salt of poly(CPH) and dimethoxy triazine. It should be noted that DMF was chosen as the solvent because Kunishima et al. have shown that polar aprotic solvents such as chloroform and methylene chloride cause demethylation of the DMT-MM N-methyl group rendering the agent un-reactive to the coupling reaction [21]. The demethylation reaction occurred within 4 hours with chloroform while taking 2 weeks when the demethylation was tested in methanol. Although not the best solvent for poly(CPH), the demethylation reaction is sufficiently slow enough in DMF for use in the coupling reaction. To complete the reaction, ethanol amine was added and allowed to react for 4 hours at room temperature before precipitating the polymer with petroleum ether. The NMR of the result of the reaction is shown in Figure 4.10.

![Figure 4.10 Reaction product of poly(CPH), ethanol amine and DMTMM end-group modification reaction](image-url)
The reaction did not yield polymer, but rather a dimer of CPH with ethanol amide end- 
groups, it should be noted the diacid is insoluble in chloroform in contrast to the polymer,
which has a high solubility in chloroform. Therefore, a qualitative confirmation of the 
reaction products’ degree of polymerization is made by the products’ solubility in 
chloroform. The reaction was repeated again and produced the same result. It is unclear as 
to whether the DMF or the ethanol amine or the DMT-MM was causing such a marked loss 
in molecular weight of the poly(CPH). Therefore, it was decided to use 2-chloro-4,6-
dimethoxy-1,3,5-triazine(CDMT) with N-methylmorpholine (NMM), a very similar coupling 
method, to produce the amide end-group. The difference between CDMT/NMM and DMT-
MM is that the CDMT and NMM are reacted in a previous step to make DMT-MM and then 
added to the reaction flask, while the CDMT/NMM is just added directly in this case. The 
reaction product was soluble in chloroform, but NMR revealed a CPH tetramer with amide 
end-groups. The reaction was also run without DMT-MM and the NMR revealed monomer 
of poly(CPH).

Since every reaction that was performed in an attempt to make poly(CPH) with amido 
ester end-groups resulted in at best a tetramer, a decision was made to make high molecular 
weight poly(CPH) and try the reaction with the assumption that if degradation of the chain 
occurs it would be possible to obtain polymer with a sufficiently large molecular weight. 
Unfortunately, at the time, the highest molecular weight poly(CPH) that our laboratory could 
make was around $\bar{M}_n \sim 10,000$ g/mol which was already deemed unacceptable for the end-
group modification. Therefore, investigations were conducted to look at what step of the 
synthesis was restricting the ability to obtain high molecular weight poly(CPH). It was 
decided that it had to be lack of monomer purity or lack of sufficient vacuum. Since the
reaction was being conducted at vacuum levels on the order of 0.2 torr, it was determined that the monomer purity was the issue. Moreover, there was always an unassigned peak in the NMR of the CPH diacid, prepolymer and polymer that was never identified (Figure 4.11).

![Figure 4.11 CPH prepolymer with an unknown peak at δ = 4.3 ppm](image)

After making the CPH diacid through a base catalyzed nucleophilic substitution reaction of dibromo hexane and para-hydroxy benzoic acid in water, experiments were conducted with solvents to remove the mystery substance from the diacid. Solvents that worked were hot acetic acid, precipitation from DMSO or DMF and recrystallizing from hot N-methyl pyrrolidinone (NMP).

The most effective method to remove impurities from the diacid was NMP or doing the actual diacid synthesis in DMF. They both resulted in removal of the unknown peak from the diacid, prepolymer and polymer NMR spectra (Figure 4.12, 4.13).

After successful synthesis of pure diacid, prepolymer and later polymer were made. The polymer resulting from the pure diacid had a number average molecular weight of 85,000 g/mol, as determined by GPC. With the high molecular weight poly(CPH) polymer...
synthesized, the next objective was to end functionalize the polymer with ethanol amine using CDMT/NMM because slightly better results were obtained as compared to DMT-MM (in relation to loss of molecular weight). After conducting the reaction with the high molecular weight poly(1CPH) and ethanol amine the product was analyzed by GPC and revealed a number average

![Diagram](image)

**Figure 4.12** Pure CPH diacid recrystallized from NMP
molecular weight of about 1700 g/mol or a pentamer of CPH. The amidation reaction was run for various lengths of time: 1.5 minutes, 4 hours and 12 hours. When the polymer was isolated and analyzed by GPC, the number average molecular weight of all the products from each of the reactions was about 1,700 g/mol. In order determine the cause of this rapid loss of molecular weight, the poly(CPH) was dissolved in DMF (without any CDMT/NMM) and the number average molecular weight determined at times of 5 min, 1 hour and 4 hours. All the number average molecular weights were the same, about 1,700 g/mol. Therefore, it was concluded that the DMF was either reacting with or enhancing a degradation reaction of poly(CPH) which was causing this rapid loss of molecular weight. Because DMF was causing such a drastic loss of molecular weight from the poly(CPH) it was thought that dimethyl acetamide (DMAC) might be a good solvent for the reaction with the assumption
that the loss of molecular weight was being caused by some interaction of the aldehyde of DMF with the anhydride bonds of the polymer. If that was the case, then DMAC should resolve this problem because DMAC contains an acetamide instead of an aldehyde as with DMF. Unfortunately, no further experiments were conducted because of the failure of our GPC; thus there was an inability to determine polydispersity and the average molecular weight of the polymers synthesized. Another problem encountered with this method was determining if block formation had occurred and to what extent. Determining the end-group concentration and extent of reaction is very difficult with NMR due to the small number of end-group protons in comparison to the magnitude of the other peaks resulting from other protons in the chain. Moreover, by producing block copolymers with a linking strategy, the ability to purify the unreacted homopolymers from the block copolymer becomes a serious question.

As a consequence of the inability to construct block copolymers of polyanhydrides the research focused on the second objective of this Thesis: synthesizing short chain polyethylene glycol (PEG) containing diacids for the synthesis of polyanhydrides. The interest in synthesizing PEG containing diacids stems from the desire to improve the control of the hydrophilicity and degradation time of polyanhydrides while being able to stabilize a wide range of drugs and macromolecular drugs with predictable release profiles.
4.4 Synthesis and Characterization of PEG Containing Aromatic Polyanhydrides

4.4.1 Synthesis of PEG Containing Aromatic Polyanhydrides

While PEG diacids have been synthesized and incorporated into polyanhydrides, the segments were on the order of 600 g/mol and were strictly aliphatic glycols modified with succinic anhydride to produce a diacid[22-25]. The result is a polymer that degraded very quickly and is therefore of little use in controlled release technology, with the exception of creating porosity in nanospheres for inhalation devices. Thus, to make PEG anhydrides that had more function in controlled release it was decided that there was a need to increase the hydrophobicity of the PEG monomer. This was accomplished by using shorter PEG chains and end functionalizing them with an aromatic acid such as para-hydroxy benzoic acid (Figure 4.14).

![Synthetic route to PEG containing aromatic diacids](image)

**Figure 4.14** Synthetic route to PEG containing aromatic diacids

To determine the effect of increasing the polymer hydrophilicity on the release of model compounds, tri-ethylene glycol and penta-ethylene glycol (n = 3 and 5) were chosen as the PEG segments to constitute the internal moiety of the aromatic diacids.
One benefit of the choice of tri-ethylene glycol is the commercial availability of the dibromo analog, which is not the case for the penta-ethylene glycol. Therefore, penta-ethylene glycol had to be halogenated in order to obtain a starting material for the diacid synthesis. Two methods were explored in the halogenation of penta-PEG, bromination with phosphorous tribromide (PBr₃) and chlorination with thionyl chloride (SOCl₂) (Figure 4.15).

\[
\begin{align*}
\text{HO} & \quad \text{O} & \quad \text{OH} & \quad \xrightarrow{\text{PBr}_3 \text{ Toluene}} & \quad \text{Br} & \quad \text{O} & \quad \text{OH} \\
\text{HO} & \quad \text{O} & \quad \text{OH} & \quad \xrightarrow{\text{SOCl}_2 \text{ Hexanes}} & \quad \text{Cl} & \quad \text{O} & \quad \text{OH}
\end{align*}
\]

**Figure 4.15** Halogenation of penta-ethylene glycol

Since the synthesis of the PEG containing aromatic diacids are modeled after the synthesis of CPH diacid, it was decided to try to brominate the penta-ethylene glycol first because the bromine would be a better leaving group than the chloride. In the initial studies on the bromination reaction, tetrahydrofuran (THF) was chosen as the solvent but it made purification very difficult because the by-products of this reaction were phosphorous trihydroxide (P(OH)₃) and even phosphorous oxide oligomers, which were soluble in THF. Therefore, the THF solution had to be extracted with water, sodium bicarbonate and sodium chloride, which caused large losses in yield of the final product due to extraction of the brominated product along with the by-products. Therefore, toluene was chosen as a reaction medium, because the PEG and brominated PEG were soluble in it and the by-products were not. By filtration over celite, the by-products may be easily removed. Furthermore, washing with activated charcoal removes any trace amounts of the by-products that were not removed.
by the celite filtrations. Yet, this method still gave poor yields of the brominated penta-ethylene glycol (~ 50 %) and moderately pure product that was tainted with PEG starting material. This was a result of washing the PEG product and not being able to extract the diol completely from the dibromo product. Therefore, the decision was made to explore chlorination of penta-ethylene glycol with thionyl chloride in hexanes. The PEG was not soluble in hexanes but the chlorinated product was. Therefore some purification of the starting material could be done by filtration. The other advantage was that the reaction time was only 2 hours in contrast to the 12 hour bromination reaction. Moreover, the chlorinated product distilled at a lower temperature than the brominated PEG therefore the product may be distilled from the hydroxylated starting material. The yields of the chlorination reactions were also improved since there was no extraction required. Figures 4.16 and 4.17 show the purified products of the two different halogenation reactions; notice the extraneous peaks that show up in the dibromo penta-ethylene glycol spectrum.

Figure 4.16 Purified dibromo penta-ethylene glycol
The ability to vacuum distill the reaction product of the chlorination reaction yields a much purer product that can be used to synthesize the aromatic diacid.

The synthesis of the aromatic diacids of tri-ethylene glycol and penta-ethylene glycol are shown in Figure 4.18

![Diagram of synthetic process]

**Figure 4.18** Synthesis of aromatic PEG diacid (n = 2, 4 for tri and pent-ethylene glycols)
The synthesis of the aromatic PEG containing diacids was initially conducted in water and potassium hydroxide. Unfortunately, the yield of diacid was rather low when the reaction was conducted in water therefore it was concluded that a mixed solvent system would result in higher a yield. Presumably the low yield was caused by the halogenated PEG’s low solubility in water and therefore the reaction has to occur at a water/oil interface instead of in a homogenous mixture of reactants. Although this reaction system works well for dibromo alkanes, the same was not true for halogenated PEGs. Dimethyl acetamide and water was chosen as a solvent system because DMAC is water miscible and should act as a carrier solvent for the halogenated PEG’s. This solvent system worked much better with the exception of the added difficulty in precipitating the product from the DMAC/water solution after completion of the reaction. In order to improve the precipitation yield the mixture was concentrated before addition of the precipitation solvent. The mixed solvent system and concentration resulted in improvement of the reaction yield from about 20% to 60%. Further increase in the reaction yield might be possible by just removing all the solvent before workup but on the scale the reaction was being performed this would take too long due to high boiling point of dimethyl acetamide. Purification of the diacids proved to be the most difficult challenge in the reaction sequence. Initially the crude product was washed with hot water to remove any unreacted hydroxy benzoic acid and then dried overnight before washing with chloroform, to remove any of the unreacted PEG. The results of these washing steps are shown in Figure 4.19 for the tri-ethylene glycol diacid (CPPEG-3). Notice the unreacted hydroxy benzoic acid (δ ~ 7.8 and 6.8 ppm) and PEG (δ ~ 3.4-3.5 ppm). It is important to remove these impurities from the diacid before making prepolymer or
polymerizing because any unreacted starting material will have a deleterious effect on the ability to attain high molecular weight polymer. Therefore further exploration of

Figure 4.19 Impure CPPEG-3 diacid

pure diacid was obtained by washing with warm methanol. The warm methanol removed any unreacted benzoic acid and PEG while not esterifying the acid. The penta-PEG diacid (CPPEG-5) offered the additional challenge of increased solubility in methanol, and ethanol. The impure CPPEG-5 diacid is shown in Figure 4.20, again notice the unreacted benzoic acid and PEG.
Fortunately, isopropyl alcohol was a recrystallization solvent for the CPPEG-5 and also yielded pure CPPEG-5. The NMR of pure CPPEG-3 and CPPEG-5 are shown in Figure 4.21 and 4.22.
After completing the synthesis and purification of the PEG diacids, they were reacted with acetic anhydride and polymerized under vacuum to yield polyanhydrides. The prepolymers
were not isolated before polymerization because they were too hard to work with due to their PEG content and low melting point. An NMR of the crude CPPEG-3 prepolymer is shown in figure 4.23.

![Figure 4.23 Crude CPPEG-3 prepolymer](image)

The crude prepolymer was polymerized under vacuum at 180°C for an hour and a half to produce polymers of about 20,000 g/mol molecular weight. NMR spectra of poly(CPPEG-3) and poly(CPPEG-5) are shown in Figures 4.24 and 4.25. Notice the disappearance of the end-group peaks at δ=2.12 ppm, which indicates an increase in the degree of polymerization.
Figure 4.24 NMR of poly(CPPEG-3) polymerized at 180°C under vacuum for 2 hrs

Figure 4.25 NMR of poly(CPPEG-5) polymerized at 180°C under vacuum for 2 hrs
Table 4.1 contains physical properties collected from the PEG containing diacids and the aromatic PEG polymers. The glycol containing polymers have substantially lower glass transition temperatures \( T_g \) and no melting temperature \( T_m \) peaks due, while the aliphatic poly(sebacic anhydride) (poly(SA)) and poly(1,6-bis-(acetoxycarboxyphenoxy)hexane (poly(CPH)) have glass transition temperatures and melting points of 62°C and 46°C \( T_g \) and 79°C and 136°C \( T_m \) [26].

<table>
<thead>
<tr>
<th>Polymer</th>
<th>( M_n ) (g/mol)</th>
<th>( M_w ) (g/mol)</th>
<th>( T_m ) (°C)</th>
<th>( T_g ) (°C)</th>
</tr>
</thead>
<tbody>
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<td>-</td>
<td>235</td>
<td>-</td>
</tr>
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<td>19</td>
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<td>-</td>
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<tr>
<td>poly(CPH)</td>
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<td>-</td>
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<td>46</td>
</tr>
<tr>
<td>poly(SA)</td>
<td>-</td>
<td>-</td>
<td>79</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 4.1 Summary physical properties of CPPEG-3 and CPPEG-5 polymers as compared to poly(CPH) [26] and poly(SA) [26].

4.4.2 Release and Degradation Studies of PEG Containing Aromatic Polyanhydrides

The degradation rates of poly(CPPEG-3) and poly(CPPEG-5) were determined gravimetrically by degrading 100 mg tablets or spheres in 900 ml of 0.1 M PBS solution at a pH of 7.4 and periodically drying under vacuum for 48 hrs and weighing the sample. The water swelling of the polymer was determined by subtracting the wet weight from the dry weight of the polymer sample. The results of the degradation experiments are shown in Figures 4.26 and 4.27. After 29 days of being subjected to PBS solution at 37°C, the poly(CPPEG-3) eroded about 50 % of the initial 100 mg mass. Under the same conditions, poly(CPPEG-5) almost completely eroded in 7.5 days in PBS solution. It should be noted that extensive bulk erosion was taking place in the poly(CPPEG-5) case as
Figure 4.26 Erosion of 100 mg tablet of poly(CPPEG-3) in 900ml of 0.1 M PBS solution at 37°C, pH=7.4 all error bars are standard deviations of three samples.

Figure 4.27 Erosion of 100 mg sphere of poly(CPPEG-5) in 900ml of 0.1 M PBS solution at 37°C, pH=7.4 all error bars are standard deviations of three samples.
confirmed by the loss of structural integrity of the polymer matrix at about day 4. The
difference in erosion rates was attributed to the extra hydrophilicity added by the addition of
two more ethylene glycol units in the backbone of the monomer. In comparison, poly(SA)
degraded in about 8.5 days while poly(CPH) eroded in 1 year[27]. The incorporation of two
more oxygen’s into the backbone of the CPH monomer (i.e. CPPEG-3) resulted in an
increase in the erosion rate of about 7 times. The lag before erosion occurred was attributed
to the intra-polymer pH decreasing upon initial water uptake resulting in a pH gradient
between the surface and interior of the device. The gradient caused water to continue
swelling within the device until a critical osmotic pressure was attained as a result of a
maximum in the degradation products within the device. At this time, presumably the device
had macro-pores within it, therefore when the degradation products were released from the
device a higher internal surface area was available for the erosion process which in turn lead
to a rapid erosion [28].

The aromatic PEG containing polyanhydrides, poly(CPPEG-3) and poly(CPPEG-5),
were investigated for there release kinetics and degradation profiles. Release studies were
conducted with two model compounds, Rhodamine B Base (Fw = 442.5 g./mol, solubility in
water = 1 mg/ml) and Acid Orange 8 (Fw= 366.4 g/mol, solubility in water = 30 mg/ml),
which are hydrophobic and hydrophilic model compounds respectively. The structures of
the two model compounds are shown in Figures 4.28 and Figure 4.29. Structurally important
features of the dyes are the tertiary diamines of the Rhodamine B base and the sulfonic acid
moiety of the Acid Orange 8. These ionizable groups may have a large impact on the inter-
polymer matrix environmental pH, which will dictate the solubility of the monomer that in
turn plays a role in the polymer degradation.
The release studies were conducted with 5 mg of model drug in 95 mg of polymer as either a tablet or a sphere depending on the polymer used. The dye was loaded by co-dissolving the polymer and dye together in chloroform and vacuum drying the mixture. The poly(CPPEG-5) could not be processed into a tablet by pressing. Therefore spheres of equal diameter (4.75 mm) were made and used in the release study. Poly(CPPEG-3) polymer was pressed into tablets of 10 mm x 1.25 mm (diameter x thickness). All experiments were conducted in a USP 2 dissolution apparatus filled with 900 ml of 0.1 M PBS solution at pH 7.4 with mesh baskets spinning at a rate of 100 rev/min. The release profiles and water uptake of Acid Orange 8 and Rhodamine B Base for poly(CPPEG-3) are shown in Figures 4.30. The release of the hydrophobic dye was complete within seven days of beginning the release study.
Moreover, the Acid Orange 8 release study of poly(CPPEG-3) resulted in a burst release of about 30% in the first day with a more gradual release over the course of seven days. Since there was so little water ingression into the polymer matrix, as was displayed by the water uptake data, the initial 30% burst of the Acid Orange 8 was perhaps caused by dye that had not dissolved in the polymer during processing. After the initial burst, the release was slowed by a lowering of the intra-polymer pH due to the acidity of the Acid Orange 8 and CPPEG-3 monomer. To examine the porosity and loading of model drug within the poly(CPPEG-3), SEM was utilized. The results are shown in Figures 4.31 and 4.32 for an
outer surface and a cross section of each Acid Orange loaded (5 wt%) and Rhodamine B Base loaded poly(CPPEG-3) tablets.

Figure 4.31 SEM micrographs of Acid Orange 8 loaded (5 wt%) poly(CPPEG-3). Outer surface (left) and cross section (right).

Figure 4.32 SEM micrographs of Rhodamine B Base loaded (5 wt%) poly(CPPEG-3). Outer surface (left) and cross section (right).

The Acid Orange 8 loaded sphere has much higher surface roughness and more pools of undissolved dye within the polymer than the Rhodamine B Base filled polymer. The increased roughness may be caused by an increase in the surface hydrophilicity due to the dye, resulting in the rapid onset of erosion beginning shortly after processing the polymer into a sphere. Both the outer surface and cross section seem to be smooth and homogeneous for the
Rhodamine B Base filled polymer. The release profiles of Acid Orange 8 and Rhodamine B Base seem to agree with the aforementioned conclusions from the SEM micrographs. After the initial burst the release profiles change depending on the chosen dye. There was a noticeable concavity difference between the Acid Orange 8 and the Rhodamine B Base release. The Acid Orange 8 release gradually slowed while the release of Rhodamine B Base increased with time. This increase in the rate of release was attributed to the increase in intra-polymer pH. It is hypothesized that the Rhodamine B Base facilitated the dissolution of the CPPEG-3 monomer by increasing the pH within the device. This is borne out by the fact that the Rhodamine B Base release rate is much higher than the rate of water uptake in the polymer. The solubility of the CPPEG-3 was presumably increased, resulting in an increase in the rate of degradation and increase in the dye release rate. Conversely, a decrease in the intra-polymer matrix pH occurred when Acid Orange 8 was subjected to water within the matrix, leading to a slower release of Acid Orange compared to the Rhodamine B Base even though it is much more hydrophilic. This result agrees with the results of Langer and Gopferich [29, 30] for polyanhydrides. They found that the solubility of anhydride monomer is dependent upon the pH inside the pores of the degrading polymer, which in turn was dependent on the pKₐ of the monomer acids. The results showed that SA is five times more soluble than CPP at pH values below 7.4. Furthermore, Mader et. al [31] showed that there was a pH gradient resulting from the degradation of polyanhydrides with the intra-polymer pH < 4 increasing to the buffer solution pH of 7.4 over time. In this system, the lowering of the pH decreased the solubility of the CPPEG-3 monomer and slowed the release and degradation down.
If there was no effect of dye on the release and degradation of the matrices one would expect the Rhodamine B Base to be more soluble in the poly(CPPEG-3) than Acid Orange 8 (due to the more hydrophobic nature of both the poly(CPPEG-3) and Rhodamine B Base). As a result a complete initial burst of Acid Orange 8 and a linear release of Rhodamine B Base dependent on the erosion rate would result. This is the case for most hydrophilic/hydrophobic model dyes released from relatively hydrophobic surface eroding polyanhydride carriers such as poly(CPH) [32].

The results of the poly(CPPEG-5) release study are shown in Figure 4.33 and 4.34. The study was characterized by rapid release of Rhodamine B Base over the course of 5 days. In contrast, the Acid Orange 8 release displayed an initial burst followed by gradual release and then complete discharge of the model drug. Most of the Rhodamine B Base releases within 5 days while the Acid Orange 8 takes about 9 days to release for two of the samples while the other sample steadily released Acid Orange 8 for about 17 days. After the study was complete, the Rhodamine B Base spheres were completely degraded while the Acid Orange 8 containing devices were mostly intact after the two week period. The rapid loss of mass in the Rhodamine B Base release was attributed to the increased pH and accelerated polymer degradation within the polymer matrix as mentioned previously. The lack of degradation in the Acid Orange 8 device was a consequence of the decrease in pH within the matrix as mentioned previously.
Figure 4.33 Release of Rhodamine B Base and mass fraction of water swollen in polymer (without drug) from poly(CPPEG-5) in triplicate error bars are standard deviations.

Figure 4.34 Release of Acid Orange 8 and mass fraction of water swollen in polymer from poly(CPPEG-5), individual runs plotted.
Moreover, the water uptake rate of poly(CPPEG-5) was much greater than that of poly(CPPEG-3) as is noticed by comparing day 5 of the degradation/water uptake study. At day 5 in the study, about 45% of the matrix was water in the poly(CPPEG-5) while only about 15% for poly(CPPEG-3). Also, the release of Rhodamine B and the initial burst of Acid Orange 8 correspond well to the water swelling data for poly(CPPEG-5), but have little relation in the poly(CPPEG-3) case. The burst in the poly(CPPEG-3) release was attributed to un-dissolved dye within the polymer that was released by dissolution.

In summary, it was shown that the degradation and release rates may be tailored by varying the hydrophilicity of the polyanhydride homopolymers by incorporation of PEG units into the monomers. This provides enormous versatility to control the hydrophilicity of the PEG homopolymers and copolymers as well as tailor the inter-polymer environments for macromolecular and small molecular weight drugs.

As part of drug release and macromolecular stabilization, choice of polymer carrier is very important to stabilize the target molecule adequately. Because of the large numbers of possible synthesis conditions for homopolymers and various compositions for copolymers, any technique that can increase the rate of polymer synthesis or ability to screen polymers would be of important use and consequence. The last section of this thesis details the efforts to utilize microwave energy for the high throughput synthesis of polyanhydrides for material library screening.
4.5 High Throughput Microwave Synthesis of Polyanhydrides

The conventional method of polymerizing polyanhydrides is by melt polycondensation of an acetylated dicarboxylic acid prepolymer[33-39]. This method affords high molecular weight polymer in good yields but it takes an hour and a half to three hours to conduct the polymerization. This does not even include the preparation and isolation of the acetylated prepolymer, which can take up to four days including drying time. Therefore, to make a polyanhydride of a diacid through melt polycondensation from beginning to end, one might expect to take about four days at a minimum. In this age of rapid drug discovery and advances in protein and macromolecular therapies, determining the correct polymer compositions and system are of the utmost importance to effectively stabilizing the target drug. Therefore, there is considerable need to be able to synthesize efficiently materials in a high throughput manner for material library screening. Recently, a number of papers in the literature have described the rapid synthesis of many polymers through the use of microwave radiation and dielectric heating, with the exception of polyanhydrides[40-69]. This method appears to be an ideal candidate for the high throughput synthesis of polymers, due to an increased rate of heating as a result of improved internal heating in comparison to conductive oil bath means. Moreover, a number of polycondensation reactions have appeared in the literature with the ability to produce high molecular weight polymer without the need of vacuum to remove the condensation by-product. Therefore, described below are preliminary experiments displaying the utility and speed of the microwave polymerization of polyanhydrides.

The microwave experiments were broken down into two classes: first, reactions of prepolymer to directly produce polyanhydrides and second, in situ formation of prepolymer
from diacid before polymerization in the microwave. The initial studies were conducted on sebacic acid due to the commercial availability of the diacid and it’s general acceptance as a controlled release polymer. The prepolymer was synthesized conventionally in an oil bath by refluxing with acetic anhydride and purification. Before any experiments were conducted, the microwave was calibrated for hot spots by moistening a large piece of filter paper and placing it on the glass plate. The paper was subjected to microwave radiation on high (1100 Watts) for 15 minutes resulting in the calibration shown in Figure 4.35. The microwave used for these experiments had about an 8 in diameter of usable space not subjected to hot spot radiation. All reactions were kept within this ring to keep the field levels consistent.

The pure sebacic anhydride prepolymer (100 mg) was placed in the microwave for anywhere from 2-25 minutes and the degree of polymerization (DP) determined. The degree of polymerization is defined as the number of monomer units divided by the number of polymer chains. The number of monomers was determined from the area under the peak

Figure 4.35 Calibration of microwave hot spots using water moistened filter paper
corresponding to the backbone protons in the NMR of sebacic anhydride and the number of chains, by taking half the number of end-groups, which was also determined from NMR.

The degree of polymerization was determined by proton NMR using the following equation:

\[
DP = \frac{(I_2 + \frac{1}{2} I_1)}{(I_4 + \frac{2}{3} I_3)}
\]

Eq. 4.1

where \(I_1, I_2, I_3\) and \(I_4\) are integrals obtained from NMR at the peaks \(\delta = 1.2\) ppm, 1.5 ppm, 2.2 ppm and 2.4 ppm respectively. The number average molecular weight was obtained by multiplying the DP by the monomer molecular weight, which is 186.2 for sebacic acid.

Figure 4.36 and Figure 4.37 are an NMR of prepolymer of SA and poly(SA) polymerized from the prepolymer in the microwave. Notice the peak at \(\delta = 2.2\) pp in Figure 4.35 and the peak at \(\delta = 2.4\) ppm in Figure 4.36 which correspond to the protons from the acetylated end-group and the protons \(\alpha\) to a carbonyl with acid end-groups.

Figure 4.36 Conventionally prepared prepolymer of sebacic acid
Figure 4.37  NMR of a sebacic acid anhydride prepolymer microwave polymerized for 30 min at 1100 Watts

A decrease in the acetylated end-groups ($\delta = 2.2$ ppm) with no increase in the acid endgroups results in an increase in the DP. Table 4.2 shows all the polymerization experiments attempted using the sebacic anhydride prepolymer in the microwave.

<table>
<thead>
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<tr>
<td>micSA_prepolymer</td>
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Table 4.2  Number average molecular weight of all experiments polymerized in the microwave using sebacic anhydride prepolymer. The sample ID is labeled as type of heating_catalyst_time, mic = microwave, conv = oil bath heating and SA refers to sebacic anhydride prepolymer. MicSA_prepolymer is the unreacted prepolymer.
The microwave polymerizations of sebacic anhydride prepolymer resulted in oligomers forming with a large percentage of acid end-group chains, which caused the polymerization reaction to cease. With this knowledge, it was clear the prepolymer needed something to prevent it from degrading into acid before it polymerized. Therefore it was decided to try adding catalysts such as calcium oxide, calcium carbonate, aluminum oxide and silica, all of which have been used previously in conventional polymerizations to increase the molecular weight of the polymers obtained [35, 38]. The results were similar to what was obtained with neat polymerization, oligomers with acid end-groups. The best results were obtained when the prepolymer was subjected to microwaves on a glass plate or a bath of glass beads. However, results similar to those obtained in microwave polymerization were achieved by just heating the prepolymer in an oil bath without vacuum for a given period of time. The best result of conventional polymerization was obtained when calcium oxide was used as a polymerization catalyst. These results suggested that the prepolymer was too reactive in the microwave and possibly degraded by some thermal mechanism before polymerization could commence.

Therefore it was decide to try forming the prepolymer *in situ* by adding equivalents of acetic anhydride to sebacic acid and reacting the contents of the vial in the microwave with the vial cap on and then removing the acetic anhydride before polymerizing in the microwave. The results of these experiments are summarized in Table 4.3. A given amount of acetic anhydride was allowed to react from 2 minutes before removal and subsequent polymerization.
<table>
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Table 4.3 Number average molecular weight of all experiments polymerized in the microwave with *in situ* sebacic acid prepolymer preparation. The sample ID is labeled as such AA = number of Acetic Anhydride drops (0.0185 ml/drop).

The polymerization times were varied from 2 minutes to 8 minutes. Experiments were performed to determine the effect of adding more acetic anhydride on the degree of polymerization. In general, the number average molecular weight reached a maximum at about 5.5 minutes with 6 equivalents of acetic anhydride producing higher molecular weights than 9 or 12 equivalents of acetic anhydride.

This was explained by the ability to remove the excess acetic anhydride between the acetylation reaction and polymerization. With more acetic anhydride, it took longer to remove the excess between prepolymer formation and polymerization. Furthermore, perhaps less acetic anhydride was removed resulting in a shift in the polymerization equilibrium reaction. Experiments were also performed to determine the effect of the microwaves on the polymerization, results summarized in Table 4.4.
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>$M_n$ (g/mol)</th>
<th>DP</th>
<th>$M_n$ (g/mol)</th>
<th>DP</th>
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<td>Conventional</td>
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<td>13,229</td>
<td>71</td>
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<td>10</td>
<td>3,025 (6 min)</td>
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<tr>
<td>SAc_25AA_8min_1g</td>
<td>1,161</td>
<td>8</td>
<td>3,583 (6 min)</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 4.4 Number average molecular weight of experiments polymerized in the microwave with *in situ* sebacic acid prepolymer preparation compared to conventional oil bath heating without vacuum. The sample ID is labeled as such AA = number of Acetic Anhydride drops (0.0185 ml/drop).

The prepolymer or sebacic acid was subjected to heating in an oil bath with no vacuum under the same conditions as the microwave samples with the exception of the heating method. The results were consistent with the microwave results for small weights of acid (100 mg) but a deviation was shown when a 1 gram sample was polymerized in the microwave as compared to conventional polymerization without vacuum. Figure 4.38 and Figure 4.39 show an example of the product formed from a microwave polymerization and a conventional polymerization with in situ prepolymer formation and polymerization.
Comparing a microwave polymerized sample with a conventionally polymerized sample with no vacuum, the major difference was the lack of acid end-group peak for the microwave longer reaction times. This can be seen directly in Figure 4.38 compared with Figure 4.39 by looking at the lack of peak at $\delta = 2.4$ ppm. These results suggested that microwave polymerization of larger quantities polyanhydrides was possible and produced high molecular weight for reaction times of five to six minutes. Although conventionally
polymerizing sebacic acid with in situ prepolymer formation worked nearly as well as microwave polymerization for 100 mg samples, when larger samples were compared (1 gram at polymerized once), there was a noticeable difference in the resulting number average of molecular weight formed. The number average molecular weights were about a factor of two larger for the microwave polymerized case.

Microwave polymerization was shown to be a novel technique to synthesize rapidly, a large numbers of polyanhydride samples without the need to use vacuum or isolate an acetylated prepolymer before polymerization. This method can produce number average molecular weights comparable to those obtained by conventional polycondensation under vacuum while decreasing the time of reaction from 1.5 hours to 6 minutes. When samples were polymerized on a small scale (100 mg) in the microwave, no significant difference in the number average molecular weight was achieved when compared to conventional oil bath heating without vacuum. However, significant improvements were observed with the microwave technique over the conventional technique without vacuum for larger samples (1 g).
4.6 References


5.1 Introduction to Conclusions

The research conducted within this thesis was broken into three parts all related to the synthesis of polyanhydrides and their copolymers. The results of the research were organized into the synthesis of block copolymers, the synthesis of PEG containing aromatic polyanhydrides and the microwave polymerization of polyanhydrides.

5.2 Attempts at Block Copolymer Synthesis

Two approaches were explored to synthesize block copolymers of polyanhydrides. First, a macromonomer ring opening polymerization of poly(SA) with a tin(II) octoate - poly(CPH) macromonomer was attempted but resulted in a heterocyclic, bridged tin-sebacic acid species that was confirmed by MALDI-TOF. Moreover, ring opening solution polymerization of sebacic anhydride was attempted but resulted in the tin-sebacic acid molecules when conducted in THF and no reaction occurred when attempted in DMF. The lack of reaction in DMF was due to catalyst poisoning by the DMF. The second approach to block copolymer synthesis of polyanhydrides was by an ester amide linking strategy. A short poly(CPH) chain was synthesized and functionalized with a hydroxy amine in DMF in the presence of DMT-MM. The functionalized poly(CPH) could then be reacted with poly(SA) to yield tri-block copolymers. Unfortunately, it was concluded that the DMF was either reacting with or enhancing a degradation reaction of poly(CPH) which was causing a rapid loss of molecular weight. No poly(CPH) chains were functionalized or reacted further with
poly(SA) to make block copolymers due to the inability to sustain molecular weight while functionalizing the poly(CPH).

5.3 Synthesis and Characterization of PEG Containing Aromatic Polyanhydrides

The second aspect of this thesis dealt with the synthesis of PEG containing aromatic polyanhydrides for the controlled release of macromolecular therapies. Aromatic diacids containing triethylene glycol (CPPEG-3) and pentaethylene glycol (CPPEG-5) were synthesized and further polymerized by melt polycondensation into polyanhydrides. The degradation rate, water uptake and release of two model compounds were explored. Poly(CPPEG-5) swelled more water than poly(CPPEG-3) resulting in a faster release and more bulk degrading profile. Intra-polymer pH exerted a large impact on the release of model compounds Rhodamine B Base and Acid Orange 8 from each of the polymer matrices. The Acid Orange 8 slowed the release profile while the Rhodamine B Base enhanced the degradation rate and release rate. In summary, it was shown that the degradation and release rates may be tailored by varying the hydrophilicity of the polyanhydride homopolymers by incorporation of PEG units into their monomers. This provides enormous versatility to control the hydrophilicity of the PEG homopolymers and copolymers as well as tailor the intra-polymer environments for macromolecular and small molecular weight drugs.

5.4 Microwave Polymerization of Polyanhydrides

The last part of this thesis was devoted to the microwave polymerization of polyanhydrides for the high throughput synthesis of material libraries. Microwave polymerization was shown to be a novel technique to synthesize rapidly, a large numbers of
polyanhydride samples without the need to use vacuum or isolate an acetylated prepolymer before polymerization. This method can produce number average molecular weights comparable to those obtained by conventional polycondensation under vacuum while decreasing the time of reaction from 1.5 hours to 6 minutes. When samples were polymerized on a small scale (100 mg) in the microwave, no significant difference in the number average molecular weight was achieved when compared to conventional oil bath heating without vacuum, but significant improvements were seen with the microwave techniques for larger quantities (1 g) as compared to conventional oil bath heating sans vacuum.
CHAPTER 6
FUTURE WORK

6.1 Future Work

The work contained within this thesis was primarily an exploration into novel synthetic routes and characterization of a novel PEG containing aromatic polyanhydrides. The investigations into the synthesis of block copolymers of polyanhydrides were not very successful, unless the ability to perform ring opening polymerization with a carboxylic acid moiety becomes a reality. Some work has been conducted by Dubois and coworkers on the ring opening polymerization of adipic anhydride with aluminum isopropoxide, which produced low molecular weight polymers. Control of degradation is important because if polyanhydride blocks are linked by an anhydride bond, then this bond could break easily resulting in a blend of polyanhydrides. The method of end-group linking investigated in this thesis would overcome that hurdle and the use of DMAC as a solvent for this method looks like a promising alternative to DMF. But the question of purifying the block copolymers without substantial degradation is a major unresolved issue.

As for the poly(CPPEG-3) and poly(CPPEG-5) polymers, these seem like good candidates for water stable protein encapsulation. Experiments should be conducted to determine the protein-polymer interactions and whether any loss of conformation or degradation of the proteins occur. To reduce water inclusion into the PEG polymers, copolymers of CPPEG-3 and CPPEG-5 with SA or CPH would be interesting to look at, and these could also serve to improve the mechanical properties of poly(CPPEG-5). Also, the PEG diacids may be synthesized more efficiently by using a fluoro benzonitrile and the
corresponding PEG diol. Once the dinitrile is made, it may then be hydrolyzed to the carboxylic acid in water and base. This method removes the need to halogenate the PEG chains, which was the slowest step in the synthesis of the diacids.

The microwave polymerization considerably increases the rate of polyanhydride production compared to conventional vacuum assisted polycondensation. As part of the future work of this project, aromatic diacids should be polymerized to determine the polymerization processing parameters in the microwave. Copolymers of diacids should also be attempted to determine if they are feasible in the microwave. Copolymers may not be feasible due to the chance that longer reaction times will be needed for aromatic containing diacids to polymerize, which may result in thermal degradation of the aliphatic diacid prepolymer during the polymerization. This thermal degradation may prevent the attainment of molecular weight due to an end-capping effect. Regardless, microwave induced polymerization is still a very promising route for polymerization of anhydrides that needs to be explored further.
DSC heating and cooling cycle for CPPEG-3 diacid, mp = 235°C

DSC heating and cooling cycle for CPPEG-3 diacid, mp = 165°C
DSC heating and cooling cycle for poly(CPPEG-3), $T_g = 19^\circ C$

DSC heating and cooling cycle for poly(CPPEG-3), $T_g = 3^\circ C$