Engineering bioerodible polymers with tailored micro/nanostructure for vaccine delivery

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Engineering bioerodible polymers with tailored micro/nanostructure for vaccine delivery

by

Matthew J. Kipper

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Chemical Engineering

Program of Study Committee:
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Iowa State University
Ames, Iowa
2004

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This is to certify that the doctoral dissertation of
Matthew J. Kipper
has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor
Signature was redacted for privacy.

For the Major Program
To my family, with love
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This work describes the investigation of bioerodible polyanhydrides as controlled drug delivery vehicles. The polymers studied are based on the 1,6-bis(p-carboxyphenoxy)hexane (CPH) and sebacic acid (SA) monomers. These two materials erode at vastly different rates and can be combined in random copolymers or blends to achieve tailored erosion kinetics. The hydrophobic nature of these materials offers the potential to stabilize proteins, and their mutual incompatibility and semicrystallinity provide an interesting phase behavior, which can be exploited to aid in tailoring the release kinetics. Theoretical and experimental description of the microstructure of polyanhydride copolymers reveals the details of the microstructure, which are essential to understanding the erosion and drug release kinetics. Injectable drug delivery systems based on polyanhydride microspheres are developed and tested in vitro and in vivo to ascertain drug release kinetics and immune responses to a model antigen, tetanus toxoid (TT). Tailored release profiles of small molecular weight drugs are demonstrated by combining microspheres with different erosion kinetics in "cocktails." This concept is extended to vaccine formulations, where it is demonstrated that the in vivo immune response mechanism can be tuned by altering the drug release kinetics. To achieve control of the immune response mechanism, TT-loaded microspheres providing a controlled release are combined with unencapsulated antigen or delivered without the addition of unencapsulated antigen. Hypotheses regarding the phenomena controlling the immune response are discussed. Finally, accurate erosion and drug release kinetics models are developed that incorporate details of the polymer microstructure and offer molecular level descriptions of the complex process of erosion to aid future developments of polyanhydride systems for biomedical applications.
CHAPTER 1
INTRODUCTION

1.1 Bioerodible Polyanhydride Microspheres as Vaccine Delivery Vehicles

History demonstrates that aggressive vaccination campaigns are capable of virtually eliminating many dangerous diseases (e.g. polio, small pox). Public health initiatives aimed at specific diseases, though successful in the United States and other western countries, may fail in less developed countries due to lack of adequate public health services, patient compliance, education, and record keeping. Dropout rates after initial vaccine doses reach as high as 70% in developing regions[1]. Improved vaccine delivery techniques that require only a single dose to confer protective immunity would help make mass immunization programs successful in underdeveloped and developing countries. For instance, tetanus is responsible for over 700,000 neonatal deaths annually, half of which could be prevented by immunization alone[2]. In 2003 The National Institutes of Health listed the development of single dose vaccines as the number one grand challenge in global health[3].

Bioerodible polymers show great potential as vehicles for controlled drug delivery, offering significant improvements over conventional drug delivery methods. For example, devices made from bioerodible polymers have been used to increase the stability of macromolecular drugs by preventing exposure to conditions which could cause denaturing[4], target specific organs and tissues by modifying the surface of the device to confer affinity for specific cell types[5], and achieve sustained and controlled drug release profiles by modulating the release rate[6-12].

There are two primary mechanisms of drug release exhibited by bioerodible, polymeric, controlled-release devices. These two mechanisms of release are illustrated in
Figure 1.1. Diffusion-controlled release occurs when drug release is governed by diffusion of dissolved drug from the device in response to a concentration gradient. This is the typical release mechanism of hydrogel systems that swell in the presence of water, facilitating drug diffusion. Diffusion-controlled drug release occurs over a time scale that is short with respect to the time scale of device erosion. In contrast, erosion-controlled release occurs when diffusion is much slower than device erosion. Erosion-controlled devices typically do not swell. Rather, dissolved drug is released as the device shrinks.

Figure 1.1. Two mechanisms of drug release: Diffusion-controlled release (a.) is governed by swelling of the polymer matrix and diffusion of solute (triangles) within the device (dark gray areas represent unswollen polymer, light gray areas represent swollen polymer). Erosion-controlled release (b.) does not involve appreciable swelling or drug diffusion, but is governed by device shrinkage.

Note that erosion control occurs when water is prevented from penetrating into the bulk of the polymer. Many bioerodible polymers exhibit “bulk” erosion, allowing water to
penetrate into the bulk of the polymer and degrade it from the inside. When swelling and bulk erosion occur over similar time scales, the kinetics of drug release are more complex than simple diffusion-controlled release. A good review of controlled release from polymers is given by Ottenbrite[13].

Langer, Mathiowitz, and co-workers have shown that polyanhydrides are a particularly promising class of polymers for drug delivery, due to their chemistry[6-9, 12, 14-18] and biocompatibility[19-24]. Polyanhydrides of aliphatic and aromatic dicarboxylic acids have hydrophobic regions separated by relatively hydrophilic acid anhydride bonds. The general structures of these polyanhydrides are illustrated in Figure 1.2.

\[
\begin{align*}
\left\{ (CH_2)_n - C - O - C \right\}_x \\
\left\{ \text{aromatic} \right\}_y
\end{align*}
\]

**Figure 1.2.** Generalized chemical structures for aliphatic poly(dicarboxylic alkane anhydride) (top) and aromatic poly[bis-p(carboxyphenoxy) alkane] (bottom).

The anhydride bonds are hydrolyzed under physiological conditions, resulting in polymer degradation and subsequent erosion. However, water does not penetrate into the bulk of the hydrophobic polymer[25]. Thus, degradation and erosion occur at the surface, rather than in the bulk. Since water is prevented from penetrating into the bulk of the device, surface eroding polymers are particularly well suited for sustained release and drug stabilization. Additionally, polyanhydrides of varying hydrophobicity have erosion rates that
span several orders of magnitude[6]. The potential for not only sustaining the release of a
drug, but also achieving desirable release profiles can be realized by combining
polyanhydrides with differing erosion rates in an advantageous way.

Like most polymer blends, binary blends of aliphatic and aromatic polyanhydrides
tend to phase-separate based on thermodynamic compatibility. Polyanhydride copolymers
have also been shown to microphase separate when their composition is rich in one
component[26]. Typically, microphase separation is a property of block copolymers.
However, the polyanhydride monomers discussed here are sufficiently long, and the
segment-segment interaction parameter sufficiently high that microphase separation occurs
even in random copolymers. For extreme compositions, the high relative abundance of one
component results in a “block-like” structure for the copolymer with relatively long
sequences of the more abundant monomer punctuated by short sequences of the less
abundant monomer. This “block-like” characteristic permits microphase separation similar
to that observed for true block copolymers. Copolymers with compositions near 50:50 do
not microphase-separate since relatively long sequences of one monomer are extremely rare.
Microphase separation is an additional property of polyanhydride systems that can be
exploited to aid in the stabilization of macromolecular drugs and tailoring release profiles.

Bioerodible polymer microspheres as drug delivery vehicles offer the advantage of
not requiring surgical implantation, since they can be injected in suspension; they also do not
require surgical removal. Also, since the drug loaded in a microsphere remains separated
from that in other microspheres, a further advantage is the potential to administer multiple
drugs in a single injection, which for compatibility reasons would otherwise need to be
separated.
The design of injectable vaccine delivery vehicles composed of bioerodible polymer microspheres requires a detailed understanding of microstructural characteristics of the polymer, interactions between the polymer matrix and the antigen, and the release characteristics of the system. The microstructural characteristics of interest include the morphology of the microphase separation, the length scale of the microdomains, and the crystallinity of the polymer matrix. All of these characteristics have the potential to affect the erosion and drug release kinetics. Interactions between the antigen and the polymer include desirable interactions that increase the solubility of the antigen in the polymer, conserve its biological activity, and partition it preferentially into phase-separated microdomains. Undesirable interactions may cause the antigen to denature or cause it to become insoluble in the polymer matrix. Also, as the polymer is degraded, the degradation products must not interact unfavorably with the antigen. The release kinetics is determined primarily by the phenomena associated with polymer erosion. For surface eroding polymers, erosion can be described as the sum of three processes: polymer degradation, dissolution of degradation products, and diffusion of monomer and drug from the device. The polymer erosion may also be affected by the presence of the drug itself (e.g. dissolved drug may reduce the crystallinity of the polymer, thereby enhancing the degradation rate).

The overall objective of this work is to develop a controlled-release device based on polyanhydride microspheres for the delivery of tetanus toxoid (TT). This will be accomplished by meeting the following three goals:

1. Describe in detail the microstructure of polyanhydride copolymers and the effects of this microstructure on drug/antigen distribution within and release from microphase-separated polyanhydrides.
2. Design TT-loaded polyanhydride microspheres and perform *in vitro* and *in vivo* studies to discern antigen release profiles and antibody production in order to maximize protective immunity.

3. Formulate and solve a mathematical model to predict and tailor copolymer microstructure effects on drug/antigen release mechanisms.

The polymer system selected for this study is copolymers of sebacic acid (SA) and 1,6-bis(p-carboxyphenoxy)hexane (CPH). The repeat units of this polymer system are shown in Figure 1.3.

![Structures of poly[1,6-bis(p-carboxyphenoxy)hexane], poly(CPH), (left) and poly(sebacic acid), poly(SA), (right).](image)

**Figure 1.3.** Structures of poly[1,6-bis(p-carboxyphenoxy)hexane], poly(CPH), (left) and poly(sebacic acid), poly(SA), (right).

1.2 References


CHAPTER 2

BIODEGRADABLE POLYANHYDRIDES FOR DRUG DELIVERY

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Balaji Narasimhan\textsuperscript{1,2} and Matt Kipper\textsuperscript{1,3}

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2.1 Introduction

Bioerodible polymers offer a unique combination of properties that can be tailored to suit nearly any controlled drug delivery application. By far the most common bioerodible

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\textsuperscript{3} Graduate student, primary author
polymers employed for biomedical applications are polyesters and polyethers (e.g. poly(ethylene glycol), polylactide, polyglycolide and their copolymers). These polymers are biocompatible, have good mechanical properties, and have been used in many controlled release applications. However, their chemistries are limited, thereby restricting structural modifications resulting in tailored properties. Over the past two decades, researchers have begun investigating alternative biodegradable polymers, resulting in a vast body of literature on both the synthesis of, and mechanisms of drug release from, biodegradable polymers.

Drug release may be controlled by several mechanisms including diffusion of the drug through a matrix, dissolution of the polymer matrix, and degradation of the polymer. The chemistry of the polymer matrix may be tailored to facilitate drug stabilization, target delivery to specific tissues, or alter the release kinetics. Bioerodible polymers erode in vivo, thus obviating the need for surgical removal after the useful lifetime of the device has expired. The erosion may actually determine the drug release kinetics, or may occur on a time scale much slower than that of drug release.

It is important to distinguish between erosion and degradation. Erosion is mass loss from a bioerodible polymer and may be a consequence of polymer dissolution or degradation of the polymer backbone, followed by dissolution of the degradation products. Degradation typically occurs by hydrolysis of the polymer backbone, the kinetics of which is a function of the polymer chemistry. Thus, erosion is the sum of several elementary processes, one of which may be polymer degradation.

Some biodegradable chemistries are listed in Table 2.1 (Pierre and Chiellini, 1986; Staubli et al., 1990; Weinberg et al., 1998; Siepmann and Goepferich, 2001). Pierre and Chiellini (1986) have summarized hydrolysis mechanisms for many biomedically relevant systems. Degradation half-lives range from millennia (for amides, carbonates, and
urethanes) to minutes (for the fastest degrading anhydrides) (Pierre and Chiellini, 1986). Though all of these chemistries are hydrolyzable, hydrolysis rates vary depending not only on the functional group (Pierre and Chiellini, 1986; Albertsson, 1995), but also what lies between the functional groups. Polyyanhydrides, for example, are one of the most labile classes, and their hydrolysis is shown in Scheme 2.1.

**Table 2.1. Functional groups found in bioerodible polymers.**

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetal</td>
<td>-O-CH-O-</td>
</tr>
<tr>
<td>Amide</td>
<td>-NH-C-</td>
</tr>
<tr>
<td>Anhydride</td>
<td>-C0-C-</td>
</tr>
<tr>
<td>Carbonate</td>
<td>-O-C-O-</td>
</tr>
<tr>
<td>Cyanoacrylate</td>
<td>C==N</td>
</tr>
<tr>
<td>Ester</td>
<td>-C-O-</td>
</tr>
<tr>
<td>Imide</td>
<td>-O-C-N-</td>
</tr>
<tr>
<td>Ketal</td>
<td>-O-R-</td>
</tr>
<tr>
<td>Ortho ester</td>
<td>-O-C-O-</td>
</tr>
<tr>
<td>Phosphate ester</td>
<td>-O-P-O-</td>
</tr>
<tr>
<td>Phosphazene</td>
<td>-N=O-P=O</td>
</tr>
<tr>
<td>Silyl ester</td>
<td>-C-Si-O-Si-C-</td>
</tr>
<tr>
<td>Urethane</td>
<td>-NH-C-NH-</td>
</tr>
</tbody>
</table>
Erosion is typically characterized by either occurring on the surface or in the bulk. Surface erosion is controlled by the chemical reaction and/or dissolution kinetics, while bulk erosion is controlled by diffusion and transport processes such as polymer swelling, diffusion of water through the polymer matrix, and the diffusion of degradation products from the swollen polymer matrix. The processes of surface and bulk erosion are compared schematically in Figure 2.1. These two processes are idealized descriptions. In real systems, the tendency towards surface versus bulk erosion behavior is a function of the particular chemistry and device geometry (Tamada and Langer, 1993). Surface erosion may permit the stabilization of macromolecular drugs and offers the potential to tailor release profiles by tailoring the composition and drug distribution.

Polyanhydrides are typically characterized as surface eroding because the anhydride bond itself is quite reactive with respect to hydrolysis, but the structure of the dicarboxylic acid monomer can render the polymer very hydrophobic, thereby limiting water ingress. These materials are interesting for controlled drug delivery due to the wide range over which the degradation kinetics can be varied. Thus, polyanhydrides have emerged as an extremely diverse and promising class of polymers for drug delivery and other biomedical applications. This review will discuss the novel chemistries and synthesis, characterization, and applications of polyanhydrides as surface erodible biomaterials for drug delivery.
Figure 2.1. Schematic comparing surface and bulk erosion. In surface erosion (top), water does not penetrate far into the bulk, but hydrolyzes functional groups on the surface. The resulting monomers dissolve and diffuse away from the device. In bulk erosion (bottom), water penetrates into the bulk, polymer may dissolve, and is ultimately hydrolyzed into monomer.

Several synthesis routes have been investigated to design polyanhydrides, and these are discussed in Section 2.2. Section 2.3 reviews the microstructural characterization of homopolymers, blends, and copolymers of polyanhydrides. Section 2.4 discusses the important features that affect erosion and drug release kinetics and reviews some of the modeling efforts that have been undertaken to predict erosion and drug release. Section 2.5 discusses the design of polyanhydride drug carriers with respect to delivery routes, mechanisms of release, and factors affecting release profiles. Finally, Section 2.6 presents some of the future directions for polyanhydride research. Polyanhydrides have a variety of microstructural characteristics that affect the release profiles of encapsulated drugs. It is important to accurately describe the microstructure to predict and tailor drug release profiles. If the effects of these microstructural characteristics can be accurately understood, they can
be exploited to control drug release profiles and effectively design controlled release formulations.

2.2 Chemistry and Synthesis

2.2.1 Early synthesis of polyanhydrides

Synthesis of polyanhydrides from the aromatic dicarboxylic acids (isophthalic and terephthalic acids) by melt polycondensation was first reported by Bucher and Slade in 1909. In the early 1930’s Hill and Carothers explored the synthesis of aliphatic polyanhydrides for use as fibers for the textile industry. Hill (1930) reported the polymerization of the aliphatic adipic acid, and later, Hill and Carothers (1932) reported the polymerization of sebacic acid, both by melt polycondensation and dehydrochlorination. The melting points of these polymers were too low and hydrolysis was too fast for them to be of use as fibers, and the study of anhydrides was abandoned.

In the late 1950’s through the mid 1960’s Conix (1957; 1958; 1966) reported the synthesis of the poly[a,co-bis(p-carboxyphenoxy)alkanes], improving the fiber and film forming properties of polyanhydrides. From 1959-1962, Yoda (1959; Yoda and Akihisa, 1959), being encouraged by the work of Conix, synthesized random copolymers by melt polycondensation and alternating copolymers by dehydrochlorination, from a variety of aliphatic and aromatic monomers in attempts to improve the fiber and film properties of polyanhydrides. Windholz (1965) later patented a similar process for producing polyanhydrides as intermediates in the production of polyesters. Polyanhydride homopolymers and copolymers containing heterocyclic rings (Yoda, 1962a; Yoda, 1962b), and aliphatic and aromatic thioethers (Yoda, 1962c) were also synthesized by Yoda. Despite
these efforts, polyanhydrides remained inferior to polyesters and other classes of polymers, never gaining prominence in the textile industry.

2.2.2 Synthesis of polyanhydrides for drug delivery

Interest in polyanhydrides waned until the 1980's when Langer and coworkers (Rosen et al., 1983) suggested that their biodegradability would make them suitable for controlled drug delivery applications. Their initial study was conducted with poly[bis(p-carboxyphenoxy)methane] (PCPM) made by melt polycondensation and they showed near zero order release kinetics of a model drug (cholic acid) from compression-molded PCPM slabs (Rosen et al., 1983). These first results on drug release from polyanhydrides initiated what has now been two decades of extensive research. The same group studied additional chemistries (Leong et al., 1985) as well as alternate synthetic routes (Leong et al., 1987). The melt polycondensation and dehydrochlorination syntheses discussed in Section 2.2.1 were explored, along with a third route, dehydrative coupling (Leong et al., 1987; Chasin et al., 1988). An alternative solution technique for the polymerization of poly(terephthalic acid) (PTA) is offered by Subramanyam and Pinkus (1985). Domb et al. (1993) reviewed several polymerization methods including melt polycondensation, ring opening polymerization, solution polymerization (dehydrohalogenation and dehydrative coupling), and interfacial polymerization (dehydrohalogenation). A review of the important polyanhydride synthesis routes follows.

2.2.2.1 Melt polycondensation. Melt polycondensation is performed by first acetylating the dicarboxylic acids by refluxing in excess acetic anhydride in a dry atmosphere, and then melting under vacuum to remove the condensation byproduct. This procedure is represented in Scheme 2.2. Domb and Langer (1987) improved upon the melt
polycondensation technique (Scheme 2.2) to obtain higher molecular weight homopolymers and copolymers of aliphatic and aromatic dicarboxylic acids. They obtained weight average molecular weights of up to 137,300, for poly(sebacic acid) (PSA). In the same study (Domb and Langer, 1987), the synthesis of poly[1,3-bis(p-carboxyphenoxy)propane] (PCPP), poly[1,6-bis(p-carboxyphenoxy)hexane] (PCPH), poly(1,4-phenylenedipropionic acid) (PPDP), and poly(dodecanedioic acid) (PDDA), as well as the copolymers P(CPP-SA), P(CPP-DDA), and the copolymer of sebacic acid with isophthalic acid P(IPA-SA) was reported. A method for copolymer synthesis was patented by the same authors (Domb and Langer, 1988b).

\[
\text{HO-U—R—J—OH + HO-M—CH}_2—\text{OH} \xrightarrow{\text{reflux \ Ar, N}_2} \text{H}_3\text{C—}\text{[O—O—R—O]_n—O—CH}_3
\]

\[
\text{H}_3\text{C—[O—O—R—]}_n—\text{O—CH}_3 \xrightarrow{\text{heat \ vacuum}} \text{H}_3\text{C—[O—O—R—]}_{m>n—\text{O—CH}}_3
\]

**Scheme 2.2.** Polyanhydride synthesis via melt polycondensation involves first the formation of oligomeric acetylated prepolymers, followed by condensation under vacuum. Acetic acid is formed as a byproduct of the second reaction.

Methods employing a variety of coordination catalysts were also reported (Domb and Langer, 1987). The anhydride interchange reaction mechanism for the melt polycondensation (Scheme 2.3) has been proposed by Albertsson and Lundmark (1990a). This mechanism may also result in the formation of lower molecular weight cyclic macromers and contribute to the high polydispersity characteristic of the resulting polymers (Domb and Langer, 1987). Gupta (1989) patented a melt polycondensation procedure from a bis(trimethylsilyl)ester of a dicarboxylic acid and a diacid chloride that produces alternating
copolymers. The majority of recent work with polyanhydrides has been conducted using the polycondensation synthesis originated by Conix (1966) and later improved upon by Domb and Langer (1987).

![Chemical structure](image)

**Scheme 2.3.** Anhydride interchange mechanism proposed for polymerization. The same mechanism may be responsible for cyclization.

### 2.2.2.2 Dehydrochlorination.

In the dehydrochlorination synthesis developed by Yoda (1959; Yoda and Akihisa, 1959) diacid chlorides are first formed by either reacting dicarboxylic acids with phosphorous pentachloride or refluxing dicarboxylic acids in thionyl chloride. Reaction is then carried out in the presence of pyridine. Dehydrochlorination, (Schotten-Baumann condensation) offers two main advantages over melt polycondensation. First, it can be performed at much milder temperatures. Second, the copolymer sequence can be precisely controlled to form alternating copolymers. Leong *et al.* (1987) studied this route both as a solution technique, and at aqueous and non-aqueous interfaces. In general, somewhat lower molecular weights are obtained by this method than by the melt polycondensation (Leong *et al.*, 1987).

### 2.2.2.3 Dehydrative coupling.

The third synthesis mechanism studied by Leong *et al.* (1987) is an extension of a technique used by previous researchers (Cabré-Castellví *et al.*, 1981; Mestres, 1981) to form monomeric anhydrides, employing strong dehydration agents (e.g. organophosphorous compounds) such as those employed in peptide synthesis. A variety
of dehydration agents were studied. Of the three synthesis methods studied by Leong et al. (1987), this one yielded the lowest molecular weight, and presented the most difficulties with respect to product purification.

To address purification, Domb and Langer (1988a) developed two techniques involving phosgene or diphosgene as coupling agents, both of which are single step polymerizations yielding pure product, by selective dissolution of either the polymer or the byproducts. A variety of polymers were synthesized including PSA, PCPP, PTA, PAA, PDDA, though only with PSA was a weight-average molecular weight above 15,000 (16,300) obtained. Most of the polymers had weight-average molecular weights less than 10,000. The advantages of this method are that relatively pure polymers are obtained without the exposure to extreme temperatures (Domb and Langer, 1988a).

2.2.2.4 Ring opening polymerization. Dicarboxylic acid monomers that form monomeric anhydride rings, such as adipic anhydride (oxepane-2,7-dione), can be polymerized by ring-opening polymerization (Albertsson and Lundmark, 1988). A catalyst such as tin 2-ethylhexanoate, tin octanoate, aluminum isopropoxide, or n-butyl lithium is added and the reaction proceeds via an insertion mechanism (Albertsson and Lundmark, 1990b; Edlund and Albertsson, 1999). Ring opening can be performed both in solution and in the melt (Albertsson and Lundmark, 1988; Albertsson and Lundmark, 1990b). Ropson et al. (1997) reported a mechanism for insertion in living polymerizations of adipic anhydride using aluminum alkoxides as initiators. Ring opening polymerizations are limited to chemistries capable of forming rings, but offer the capability of easily forming block copolymers via living polymerizations. Block copolymers of adipic anhydride with ε-caprolactone (Ropson et al., 1997) and trimethylene carbonate (Edlund and Albertsson, 1999) have been formed by this synthetic route. Deng et al. (2003) have cleverly
surmounted the chemistry limitation by using potassium poly(ethylene glycol)ate as a macro-initiator, thereby synthesizing a poly(adipic acid-\textit{block}-ethylene glycol) copolymer. The same group has also recently studied the use of dibutylmagnesium as an alternative initiator (Li \textit{et al.}, 2003).

2.2.2.5 Polymerization with ketene. In an attempt to avoid the polymerization/depolymerization equilibrium that occurs during melt polycondensation, Albertsson and Lundmark (Albertsson and Lundmark, 1988) also studied the irreversible reaction of adipic anhydride with ketene. However, they reported very little difference in molecular weights when two ketene syntheses were compared to melt polycondensation and ring-opening polymerization using a zinc catalyst (Albertsson and Lundmark, 1988).

2.2.3 Chemistries of polyanhydrides used in drug delivery

We have already mentioned a few of the polyanhydride chemistries that have been studied in drug delivery applications. Tables 2.2 through 2.7 present some of the polyanhydrides that have been explored for drug delivery applications and we briefly discuss the literature on each one. Copolymers are discussed separately.

2.2.3.1 Aliphatic polyanhydrides. Aliphatic polyanhydrides (Table 2.2) together with the $\alpha,\omega$-bis($p$-carboxyphenoxy)alkanes are the most commonly studied polyanhydrides for drug delivery applications. Poly(sebacic acid) (PSA) was first suggested as a polymer for drug delivery by Langer and coworkers in 1987 and was among the monomers on which they studied alternative synthesis methods (Domb and Langer, 1987; Leong \textit{et al.}, 1987). Poly(dodecanedioic acid) (PDDA) is also synthesized by melt polycondensation and yields similar molecular weights (Domb and Langer, 1987). The synthesis of poly(adipic acid) (PAA) by multiple methods was discussed in Section 2.2.2. Poly(1,4-cyclohexyldicarboxylic
acid) (PCDA) was first synthesized via melt polycondensation by Zhang et al. (2000; 2001). Domb and Nudelman (1995) reported the synthesis of the series of aliphatic polyanhydrides from PAA to poly(dodecanedicarboxylic acid) (PDX).

**Table 2.2. Aliphatic polyanhydrides.**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>x = 4 Poly(adipic acid)</td>
<td>PAA</td>
</tr>
<tr>
<td>x = 5 Poly(pimelic acid)</td>
<td>PPA</td>
</tr>
<tr>
<td>x = 6 Poly(suberic acid)</td>
<td>PSA</td>
</tr>
<tr>
<td>x = 7 Poly(azalaic acid)</td>
<td>PAZ</td>
</tr>
<tr>
<td>x = 8 Poly(sebacic acid)</td>
<td>PSA</td>
</tr>
<tr>
<td>x = 10 Poly(dodecanedioic acid)</td>
<td>PDDA</td>
</tr>
<tr>
<td>x = 12 Poly(dodecanedicarboxylic acid)</td>
<td>PDX</td>
</tr>
<tr>
<td>O-C-(CH₂)_x-C-O</td>
<td>Poly(1,4-cyclohexane dicarboxylic acid)</td>
</tr>
</tbody>
</table>

2.2.3.2 Polyanhydrides from unsaturated and fatty acid-derived monomers. Polyanhydrides based on unsaturated and fatty acid-derived monomers are shown in Table 2.3. Poly(fumaric acid) (PFA) was first synthesized by Domb et al. (1991) by both melt polycondensation and solution polymerization. The copolymer of fumaric acid and sebacic acid (P(FA-SA)) has been synthesized and characterized (Mathiowitz et al., 1990b; Domb et al., 1991). The mucoadhesive properties of this polymer have been shown to aid in increasing the bioavailability of encapsulated model drugs in oral delivery experiments (Chickering et al., 1995; Chickering et al., 1996).
Table 2.3. Polyanhydrides from unsaturated and fatty acid derived monomers.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Poly(fumaric acid) structure" /></td>
<td>Poly(fumaric acid) (PFA)</td>
</tr>
<tr>
<td><img src="image" alt="Poly(Fatty acid dimer) (erucic acid) structure" /></td>
<td>Poly(Fatty acid dimer) (PFAD)</td>
</tr>
<tr>
<td><img src="image" alt="Poly(Dimer acid) structure" /></td>
<td>Poly(Dimer acid) (PDA)</td>
</tr>
</tbody>
</table>

Fatty acids have also been converted to difunctional monomers for polyanhydride synthesis by dimerizing the unsaturated erucic or oleic acid to form branched monomers. These monomers are collectively referred to as fatty acid dimers and the polymers are referred to as poly(fatty acid dimer) (PFAD). PFAD (erucic acid dimer) was synthesized by Domb and Maniar (1993) via melt polycondensation and was a liquid at room temperature. Desiring to increase the hydrophobicity of aliphatic polyanhydrides such as PSA without adding aromaticity to the monomers (and thereby increasing the melting point), Teomim and Domb (1999) and Krasko et al. (2002) have synthesized fatty acid terminated PSA.
Octanoic, lauric, myristic, stearic, ricinoleic, oleic, linoleic, and lithocholic acid acetate anhydrides were added to the melt polycondensation reactions to obtain the desired terminations. As desired, a dramatic reduction in the erosion rate was obtained (Teomim and Domb, 1999; Krasko et al., 2002).

Teomim and Domb (Teomim and Domb, 2001) report the termination of PSA with monoesters of ricinoleic acid (i.e. cis-12-hydroxyoctadeca-9-enoic acid) and fatty acids. The fatty acids used in this study range in length from C10 to C18. The combination of PSA with FAD is not limited to terminal modification. P(FAD-SA) and P(fatty acid trimer-SA) (P(FAT-SA)) copolymers have been synthesized (Domb and Maniar, 1993) and their release properties have been studied (Tabata et al., 1993; Tabata and Langer, 1993; Shieh et al., 1994; Tabata et al., 1994).

Xu et al. (2001) synthesized the copolymers of a dimer fatty acid (dimer of oleic and linoleic acids) and sebacic acid (P(DA-SA)) by melt polycondensation of acetylated prepolymer. Degradation and drug release kinetics showed that increasing dimer acid content decreased the release rate (Xu et al., 2001).

Another class of PSA-fatty acid-based copolymers has been synthesized from the ricinoleic acid and ricinoleic half-esters with maleic and succinic anhydride, poly(sebacic-co-ricinoleic acid maleate), poly(sebacic-co-ricinoleic acid succinate), and poly(sebacic-co-12-hydroxystearic acid succinate) (P(SA-RAM), P(SA-RAS), and P(SA-HSAS)) (Teomim et al., 1999; Krasko et al., 2003). These syntheses result in poly(anhydride-co-esters).

2.2.3.3 Aromatic polyanhydrides. Aromatic polyanhydrides (Table 2.4) are typically characterized by slow degradation rates, high melting temperatures, brittle mechanical properties, and low solubility in organic solvents compared to the aliphatic polyanhydrides. PCPM was the first aromatic polyanhydride to be synthesized as a candidate for controlled
Table 2.4. Aromatic polyanhydrides.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure" /></td>
<td>x = 1 Poly[bis(p-carboxyphenoxy) methane]</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure" /></td>
<td>x = 3 Poly[1,3-bis(p-carboxyphenoxy) propane]</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure" /></td>
<td>x = 6 Poly[1,6-bis(p-carboxyphenoxy) hexane]</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure" /></td>
<td>Poly(Terephthalic acid)</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure" /></td>
<td>Poly(isophthalic acid)</td>
</tr>
<tr>
<td><img src="image6.png" alt="Structure" /></td>
<td>Poly(phenylene dipropionic acid)</td>
</tr>
<tr>
<td><img src="image7.png" alt="Structure" /></td>
<td>Poly[2,2'-(p-xylenedithio)diacetic acid]</td>
</tr>
<tr>
<td><img src="image8.png" alt="Structure" /></td>
<td>x = 1 Poly[2-(p-carboxyphenoxy) acetic acid]</td>
</tr>
<tr>
<td><img src="image9.png" alt="Structure" /></td>
<td>x = 4 Poly[5-(p-carboxyphenoxy) valeric acid]</td>
</tr>
<tr>
<td><img src="image10.png" alt="Structure" /></td>
<td>x = 7 Poly[8-(p-carboxyphenoxy) octanoic acid]</td>
</tr>
<tr>
<td><img src="image11.png" alt="Structure" /></td>
<td>x = 3 Poly[1,3-bis(o-carboxyphenoxy)propane]</td>
</tr>
<tr>
<td><img src="image12.png" alt="Structure" /></td>
<td>x = 6 Poly[1,6-bis(o-carboxyphenoxy)hexane]</td>
</tr>
</tbody>
</table>
Table 2.4. (continued)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure 1" /></td>
<td>Poly[ø-bis(p-carboxyphenoxy)xylene] Po-p-CPX</td>
</tr>
<tr>
<td><img src="image2" alt="Structure 2" /></td>
<td>Poly[m-bis(p-carboxyphenoxy)xylene] Pm-p-CPX</td>
</tr>
<tr>
<td><img src="image3" alt="Structure 3" /></td>
<td>Poly[o-bis(o-carboxyphenoxy)xylene] Po-o-CPX</td>
</tr>
<tr>
<td><img src="image4" alt="Structure 4" /></td>
<td>Poly[m-bis(o-carboxyphenoxy)xylene] Pm-o-CPX</td>
</tr>
<tr>
<td><img src="image5" alt="Structure 5" /></td>
<td>Poly[p-bis(o-carboxyphenoxy)xylene] Pp-o-CPX</td>
</tr>
<tr>
<td><img src="image6" alt="Structure 6" /></td>
<td>Poly[4,4’-(hexafluoroisopropylidine)bis-benzoic acid] PHFB</td>
</tr>
</tbody>
</table>

Drug delivery (Rosen et al., 1983). Other polymers in family of poly[α,ω-(p-carboxyphenoxy)alkanes that had originally been synthesized by Conix (1957; 1958; 1966) soon followed including PCPP, poly(terephthalic acid) (PTA) (Leong et al., 1985), and PCPH (Leong et al., 1987). Also included in the later study were poly(terephthalic-alt-sebacic acid) (P(SA-alt-TA)), poly(1,4-phenylene dipropionic acid) (PPDP) and poly[2,2’-(p-xylylenedithio)diacetic acid] (PXDA) (Leong et al., 1987). Domb et al. (1989) synthesized several polyanhydrides based on ω-carboxyphenoxyalkenoic acids including poly(carboxyphenoxy acetic acid) (PCPA), poly[5-(p-carboxyphenoxy)valeric acid] (PCPV), and poly[8-(p-carboxyphenoxy)octanoic acid] (PCPO) by melt polycondensation and studied the release of model drugs from them. Domb (1992) also synthesized poly(isophthalic acid) (PIPA) and poly(terephthalic acid) (PTPA) by melt polycondensation.
Campo et al. (1999) synthesized the ortho- isomers of PCPP and PCPH, poly[1,3-bis(o-carboxyphenoxy)propane] (Po-CPP) and poly[1,6-bis(o-carboxyphenoxy)hexane] (Po-CPH), in an attempt to improve the solubility and processability of these two polymers. Solubility was improved and crystallinity reduced, but $T_g$s were also lowered to below physiological temperature, which may limit their applicability as biomaterials.

In an attempt to increase $T_g$ of the poly[bis(o-carboxyphenoxy)alkanes], Anastasiou and Uhrich (2000a) replaced the alkane moiety by ortho-, meta-, and para-xylenes producing poly[o-/m-bis(p-carboxyphenoxy)xylene]s (Po-p-CPX, and Pm-p-CPX) and poly[o-/m-/p-bis(o-carboxyphenoxy)xylene]s (Po-o-CPX, Pm-o-CPX, and Pp-o-CPX). They found Po-p-CPX to be relatively insoluble and were unable to synthesize poly[p-bis(p-carboxyphenoxy)xylene] because of the insolubility of the dicarboxylic acid (Anastasiou and Uhrich, 2000a). Po-o-CPX and Pm-o-CPX demonstrated the most favorable solubility and neither exhibited a melting temperature. All of the polymers synthesized had $T_g$s between 71 and 101 °C (Anastasiou and Uhrich, 2000a).

2.2.3.4 Copolymers of aliphatic and aromatic polyanhydrides. Researchers interested in polyanhydrides as candidates for drug delivery realized the value of co polymerizing aliphatic and aromatic residues. In this way, a large number of polymers could be made from only a handful of chemistries and chemical and physical properties could be tailored by combination. Initially, the goal was to obtain a variety of release times by making simple changes to the copolymer composition. The first such copolymer was P(CPP-SA) synthesized via melt polycondensation by Leong et al. (1985). The alternating copolymers of adipic acid, sebacic acid, and dodecanedioic acid with terephthaloyl chloride (P(AA-alt-TA), P(SA-alt-TA), and P(DDA-alt-TA)) and sebacic acid with isophthaloyl chloride and P(IPA-alt-SA)) were produced by dehydrochlorination and the random copolymers P(CPM-
SA) and P(CPH-SA) were produced by melt polycondensation for the first time in the extensive study by Leong et al. (1987). The copolymers P(IPA-SA) and P(CPP-DDA) via melt polycondensation were added to the repertoire of copolymers by Domb and Langer (1987). Domb (1992) later synthesized the copolymers P(CPP-IPA), P(IPA-TA), P(IPA-FA), P(CPP-FA), P(FA-TA), P(SA-TA), and P(IPA-SA).

Sanders et al. (1999) attempted to lower the melting points of aromatic polyanhydrides by substituting branched alkyl groups in place of the linear alkyls of P(CPP-SA). They synthesized poly[1,2-bis(\(p\)-carboxyphenoxy)propane-co-sebacic acid] (P(1,2-CPP-SA)), poly[1,3-bis(\(p\)-carboxyphenoxy)-2-methyl propane-co-sebacic anhydride] (P(CPMP-SA)), and poly[1,3-bis(\(p\)-carboxyphenoxy)-2,2-dimethyl propane-co-sebacic anhydride] (P(CPDP-SA)), all of which had melting points below 165 °C.

2.2.3.5 Poly(anhydride-co-imide)s. Another important class of polyanhydrides is the poly(anhydride-co-imide)s (Table 2.5). This class of polymers was first synthesized by Fontán and co-workers (De Abajo et al., 1971; González et al., 1976) as potential candidates for fiber forming polymers. Staubli et al. (1990) developed a technique for incorporating amino acids into polyanhydrides by first reacting them with N-trimellitic acid. Uhrich et al. (1995) synthesized copolymers of trimellitylimido glycine, pyromellitylimido alanine and the monomers of PSA and PCPH by melt polycondensation and proposed the use of (P(TMAgly-SA), P(TMAgly-CPH), P(PMAala-SA), and P(PMAala-CPH)) as potential candidates to improve the mechanical properties of polyanhydrides. Hanes et al. (1996) later synthesized the copolymer of trimellitylimido \(L\)-tyrosine with PSA and PCPP (P(TMAtyr-CPP-SA)) as a candidate polymer for vaccine delivery.
Table 2.5. Poly(anhydride-co-imide)s.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Poly(trimellitylimido glycine)" /></td>
<td>PTMAgly</td>
</tr>
<tr>
<td><img src="image" alt="Poly(pyromellitylimido alanine)" /></td>
<td>PMAala</td>
</tr>
<tr>
<td><img src="image" alt="Poly(trimellitylimido tyrosine)" /></td>
<td>PTMAtyr</td>
</tr>
</tbody>
</table>

2.2.3.6 Poly(anhydride-co-ester)s and poly(anhydride-co-ether)s. Poly(anhydride-co-ester)s (Table 2.6) were suggested as potential polymers for drug delivery and synthesized by Pinther and Hartmann (1990), and Kricheldorf and Jürgens (1994). Other poly(anhydride-co-ester)s already mentioned in Section 2.2.2.4 include poly(adipic acid-block-ε-caprolactone) (P(AA-block-ε-CL)), poly(adipic acid-block-trimethylene carbonate)
<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>Poly(Riconleic acid maleate) RAM</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>Poly(Riconoleic acid succinate) RAS</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>Poly(12-hydroxystearic acid succinate) HSAS</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure 4" /></td>
<td>Poly[bis(o-carboxyphenoxy) sebacate] PCPS</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure 5" /></td>
<td>x = 2 poly(p-carboxyphenoxy succinic monoester anhydride) PCPSM</td>
</tr>
<tr>
<td><img src="image6.png" alt="Structure 6" /></td>
<td>x = 4 poly(p-carboxyphenoxy adipic monoester anhydride) CPAM</td>
</tr>
</tbody>
</table>
(P(AA-block-TMC)), and poly(adipic acid-block-ethylene glycol) (P(PAA-block-EG)). Others have synthesized poly(anhydride-block-ethylene glycol) copolymers. Jiang and Zhu (1999) synthesized and characterized poly(sebacic acid-block-ethylene glycol) (P(SA-block-EG)) and poly[(sebacic acid-co-trimellitylimidoglycine)-block-ethylene glycol] (P[(SA-co-TMA)-block-EG]) by melt polycondensation. The ethylene glycol segments were added by first acetylating polyoxyethylene dicarboxylic acid and then adding it to the PSA polymerization (Jiang and Zhu, 1999). Qiu and Zhu (2001) proposed the use of this material in laminated devices for pulsed release. P(SA-co-TMA-block-EG) and PSA were also used by Qiu and Zhu to make blends of poly[bis(glycine ethyl ester)phosphazene] in order to regulate the degradation rate of the phosphazene as well as to decrease its cost (Qiu and Zhu, 2000). The in vitro and in vivo erosion kinetics of the P[(SA-co-TMA)-block-EG] containing blend was later studied in detail by Qiu (Qiu, 2002).

Wu et al. (2000) showed the formation of self-assembled nanoparticles of P(SA-block-EG) in an aqueous environment and studied their degradation as a function of pH and temperature. Fu et al. (2002) recently repeated the synthesis of (P(SA-block-EG) and studied the morphology and erosion kinetics of microspheres which they propose as vehicles for mucosal drug delivery.

Poly(lactic acid) (PLA) has also been added to poly(SA) via melt polycondensation to produce the triblock copolymers poly(lactic acid-block-sebacic acid-block-lactic acid) (P(LA-block-SA-block-LA)) by Slivaniak and Domb (2002). The PLA (D-, L-, and DL-) was incorporated by acetylation and addition to the PSA synthesis. The showed the formation of stable stereocomplexed particles with increased melting points and reduced solubility, and studied the degradation and drug release characteristics of the same (Slivniak and Domb,
29

2002). The stereocomplexes self-assemble as a consequence of the chirality in the PLA portions of the chains (Slivniak and Domb, 2002).

Erdmann et al. (2000; Erdmann and Uhrich, 2000) recently synthesized novel poly(anhydride-co-ester)s containing salicylic acid in the backbone, by melt polycondensation of the disalicylic acid ester of sebacic acid, poly[bis(o-carboxyphenoxy)sebacate] (PCPS) and the copolymer P(CPH-CPS). The release of salicylic acid (the active form of aspirin) from the former was studied in vitro and from the latter was studied in vivo (Erdmann et al., 2000; Erdmann and Uhrich, 2000). Similar polymers that release 5-amino salicylic acid, and p-nitro salicylic acid have been prepared by the same group for the treatment of Crohn’s disease and tuberculosis, respectively (Anastasiou and Uhrich, 2000b; Krogh-Jespersen et al., 2000).

Jiang and Zhu (2001) recently reported on the synthesis of poly(p-carboxyphenoxy succinic monoester anhydride) and poly(p-carboxyphenoxy adipic monoester anhydride) (PCPSM and PCPAM), and the copolymer P(CPAM-CPSM) as polymeric antimicrobial prodrugs for diseases such as malaria and hepatitis B. They also reported that PCPSM exhibits strong fluorescence, the intensity of which increases linearly with its molecular weight (Jiang et al., 2001a; Jiang et al., 2001b). They showed that when co-polymerized the fluorescence is maintained, though diminished approximately in proportion to the copolymer composition.

2.2.3.7 Poly(anhydride-co-amide)s. The synthesis of poly(anhydride-co-amide)s (Table 2.7) of various chemistries was pursued by Hartmann and Schulz (1989) as a means of improving biocompatibility and extending the degradation times of polyanhydrides. This work also contains calorimetry data on the thermal transitions and spectroscopic characterization.
Table 2.7. Poly(anhydride-co-amide)s.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /></td>
<td>$x = 2 \text{ poly}{p-\text{carboxyphenoxy (ethyl)formamido} \text{benzoic acid}}$</td>
</tr>
<tr>
<td><img src="image2" alt="Structure" /></td>
<td>$x = 3 \text{ poly}{p-\text{carboxyphenoxy (propyl)formamido} \text{benzoic acid}}$</td>
</tr>
<tr>
<td><img src="image3" alt="Structure" /></td>
<td>$x = 4 \text{ poly}{p-\text{carboxyphenoxy (butyl)formamido} \text{benzoic acid}}$</td>
</tr>
</tbody>
</table>

Jiang and Zhu (2001) became interested in synthesizing additional polyanhydrides with fluorescence after their discovery of the fluorescent properties of PCPS. They synthesized the series of poly(anhydride-co-amide)s poly\{$p$-\{carboxyphenoxy (ethyl/propyl/butyl)formamido\}benzoic anhydride\} (PCEFB, PCPFB, and PCBFB) (Jiang et al., 2001c). Only the ethyl polymer emitted strong fluorescence, which was consistent with their previous study of the poly(anhydride-co-ester)s of similar chemistry (Jiang and Zhu, 2001). PCEFB can be modified with an acetyl ortho to the anhydride bond to form poly\{$o$-acetyl-$p$(carboxyethylformamido)benzoic acid\} (PACEFB), which also fluoresces and may have potential as a polymeric prodrug for the treatment of tuberculosis (Jiang et al., 2001b). The copolymers of P(CEFB-SA) and P(CACEFB-SA) were also synthesized and shown to exhibit decreased fluorescence in proportion to the decrease in mole fraction of the fluorescent monomer (Jiang et al., 2001b). These polymers may prove to be very valuable
for combining *in vivo* controlled release and drug targeting studies with non-invasive imaging techniques. The dependence of fluorescence on molecular weight may offer a powerful mechanism to conduct *in situ* analysis of *in vivo* degradation profiles (Jiang and Zhu, 2002).

2.2.3.8 Other novel anhydride chemistries. The chemistry of polyanhydrides is by no means limited to the categories discussed in the preceding sections. A brief review of some of the additional chemistries that have recently been synthesized follows with a mention of their potential for application in drug delivery.

2.2.3.8.1 Branched polyanhydrides. Branched PSA was synthesized by Maniar *et al.* (1990) by reacting sebacic acid in the presence of 1,3,5-benzenetricarboxylic acid and polyacrylic acid to improve the processability and mechanical properties of PSA. Weight average molecular weights above 200,000 were obtained in four of the eight compositions tested and all of the branched polymers had weight average molecular weights above 140,000, though very little difference in the polymer properties from the properties of PSA other than molecular weight were observed (Maniar *et al.*, 1990). Degradation profiles of the branched polymers were also similar to that for PSA (Maniar *et al.*, 1990). Drug release profiles for these polymers are discussed in Section 2.4.1.

2.2.3.8.2 Poly(anhydride-co-alkylene carbonate)s. Xiao and Zhu (2000) suggested accelerating the degradation of polycarbonates by incorporating anhydrides into the polymer backbone. This was accomplished by melt polycondensation of acetylated bis-\(\alpha,\omega\)-(hydroxy)alkalene carbonate oligomers. The polymers synthesized were poly(tetramethylene carbonate succinic half-ester anhydride) (PTMCSA) and poly(hexamethylene carbonate succinic half-ester anhydride) (PHMCSA). They observed an initially fast loss of molecular weight followed by much slower degradation in *in vitro*
degradation studies and attributed this to initial hydrolysis of the more labile anhydride bond, followed by slower hydrolysis of the carbonate bonds.

2.2.3.8.3 Fluorinated polyanhydrides. Kaur et al. (2002) synthesized poly[4,4'-(hexafluoroisopropylidene)bis benzoic acid] (PHFB) as an alternative to aromatic polyanhydrides with relatively low solubilities. Acetylated prepolymer did not polymerize readily by melt polycondensation, so trifluoroacetylated prepolymer was used instead and weight average molecular weight of up to 14,000 was obtained with some un-reacted monomer (Kaur et al., 2002). The authors suspected cyclization in the case of the acetylated prepolymer. The stability and degradation kinetics of PHFB were reported in the same study (Kaur et al., 2002).

2.2.3.8.4 Poly(lithocholic acid). Gouin et al. (2000) recently reported the synthesis of poly(lithocholic acid) (PLCA) and its copolymer with sebacic acid (P(LCA-co-SA)) via both melt polycondensation and dehydrative coupling. The material was characterized thermally, and drug release kinetics and biocompatibility studies were also reported. Modulation of the release kinetics was shown via changes in the copolymer composition (Gouin et al., 2000).

2.2.3.8.5 Poly(anhydride-co-urethane)s. In their investigation of polyanhydrides with novel chemistries, Hartmann et al. (1993) synthesized several poly(anhydride-co-urethane)s and compared their degradation kinetics to the poly(anhydride-co-ester)s and poly(anhydride-co-amide)s with similar structures. Poly(anhydride-co-amide)s, and poly(anhydride-co-urethane)s degraded by hydrolysis of the anhydride bond only, but poly(anhydride-co-ester)s degraded at both the ester and the anhydride bond.
2.3 Polyanhydride Characterization

2.3.1 Chemical characterization of polyanhydrides

2.3.1.1 Chemistry of polyanhydrides assessed by FTIR and \(^1\)H NMR. Fourier transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance spectroscopy (\(^1\)H NMR) have become standards for verifying the chemistry of polyanhydrides. The reader is referred to the synthesis literature in the previous section for spectra of specific polymers. The FTIR spectrum for PSA is shown in Figure 2.2. In FTIR the absorption characteristic of the anhydride doublets are typically found around 1740 and 1810 cm\(^{-1}\) for the aliphatic residues and 1720 and 1780 cm\(^{-1}\) for the aromatic residues (Domb et al., 1993). Excitation of the anhydride bond also absorbs at 1050 cm\(^{-1}\) (Leong et al., 1985). The acidic O-H bond absorbs between 3300 and 2500 cm\(^{-1}\) (Rosen et al., 1983). The combination of these absorbances can be used to assess hydrolytic degradation, and the relative intensities of the anhydride bonds can be used to verify copolymer composition.

The analysis of \(^1\)H NMR spectra of aliphatic and aromatic polyanhydrides has been reported by Ron et al. (1991) and McCann et al. (1999), and Shen et al (2002), and \(^{13}\)C NMR has been reported by Heatly et al. (1998). In \(^1\)H NMR, the aliphatic protons have chemical shifts between 1 and 2 ppm, unless they are adjacent to electron withdrawing groups. Aliphatic protons appear at about 2.45 ppm when \(\alpha\) to an anhydride bond and can be shifted even further when adjacent to ether oxygens. Aromatic protons typically appear with chemical shifts between 6.5 and 8.5 ppm and are also shifted up by association with anhydride bonds.
Figure 2.2. FTIR spectra for PSA showing characteristic anhydride peaks between 1750 and 1900 cm$^{-1}$.

The sequence distribution of copolymers can be assessed, for example in P(CPH-SA), by discerning the difference between protons adjacent to CPH-CPH bonds, CPH-SA bonds, and SA-SA bonds (Shen et al., 2002). FTIR and $^1$H NMR spectra for many of the polymers mentioned in Section 2.2 can be found in their respective references. Spectroscopy can also be used to assess drug-loading in these systems. Figure 2.3 is a $^1$H NMR spectrum for $p$-nitroaniline-loaded P(CPH-SA) (50:50). The combination of these two techniques provides a standard for verifying the chemistry of polyanhydrides. UV spectroscopy has also been reported for determining the chemistry of copolymers (Leong et al., 1985).
2.3.1.2 Solubility of polyanhydrides. When Bucher and Slade first synthesized PTA and PIPA, they reported insolubility in low pH, aqueous media, and solubility of PTA in alkaline solutions. Most polyanhydrides synthesized in the century that has passed since then show similar behavior. Many polyanhydrides also exhibit extremely limited solubility in organic solvents. This can cause problems in both characterization and processing as many characterization techniques are conducted in solution, and co-dissolution is a common method of fabricating both polymer/polymer blends and polymer/drug systems. A careful survey of the literature reveals that chlorinated solvents (chloroform and dichloromethane (DCM)) are almost universal solvents (and in some cases the only solvents) for polyanhydrides. Leong et al. (1985) reported that PCPP and PCPH were soluble in tetrahydrofuran (THF) and N,N’-dimethylformamide (DMF) only immediately following polymerization, making characterization by GPC on these polymers rather inconvenient.
Domb et al. (Domb and Langer, 1987; Domb and Langer, 1988a; Domb et al., 1989) report the use of chloroform as a solvent for P(CPP-SA), PSA, PCPH, PPDP, PDDA, PCPV, and PCPO, but that PCPP, and PCPA are both insoluble in chloroform (Domb and Langer, 1988a; Domb et al., 1989). PDDA and PAA are also reported to be soluble in chloroform (Albertsson and Lundmark, 1990b). Domb (1992) also studied the solubilities of PTA, PCPP, PIPA, and PFA in DCM, chloroform, and carbon tetrachloride and reported that all of them had less than 0.1% solubility (w/v). However, altering copolymer composition proved to be an effective method of improving the solubility of aliphatic polyanhydrides. Domb (1992) indicated slightly increased solubilities of the 70:30 copolymers P(TA-SA), P(CPP-SA), P(IPA-SA), and P(FA-SA), and increasing solubility as the PSA fraction was increased. More surprisingly, the copolymers made exclusively of the aromatic moieties (the homopolymers of which were insoluble) showed solubilities of greater than 1% (w/v) for some compositions (Domb, 1992).

Several of the synthetic efforts outlined in Section 2.2 were motivated partially by the necessity of increasing the processability of polyanhydrides. Solubilities of the 20:80 copolymers of P(CPP-SA) and P(FAD-SA) are compared by Domb and Maniar (1993). They reported improved solubility of the later over former in several organic solvents including (in order of decreasing solubility) THF, 2-butanone, 4-methyl-2-pentanone, acetone, and ethyl acetate.

Altering the linearity of aromatic polyanhydrides has proven to be a successful strategy for increasing solubility. Campo et al. (1999) reported the solubility of Po-CPP and Po-CPH in THF to be 124 mg/ml and 130 mg/ml respectively. Anastasiou and Uhrich (2000a) reported that the ortho-isomers Po-o-CPX, Pm-o-CPX, Pp-o-CPX and Po-p-CPX also had improved solubilities in DMF, and all but the Pp-o-CPX had improved solubility in
THF, whereas the Pp-p-CPX could not be synthesized because its corresponding methyl ester monomer wasn’t even soluble due to the rigidity of the three para- aromatic moieties in sequence. Other chemistries also demonstrated improved solubilities. The poly(anhydride-co-ester)s and poly(anhydride-co-imide)s synthesized by Jiang et al. (Jiang and Zhu, 2001; Jiang et al., 2001c) demonstrated solubility in THF and the esters were also soluble in DMSO in addition to DCM.

2.3.2 Characterization of thermal properties, crystallinity, phase behavior of polyanhydrides

2.3.2.1 Thermal transitions. It is important to characterize the thermal properties of polyanhydrides that are proposed for drug delivery applications, as changes in crystallinity can affect degradation profiles and drug release kinetics. The anticipated dependences of chain structure on glass transition temperature (T_g) are evident in most of the polyanhydrides studied. The most rigid polymer, PTA, has a glass transition temperature of 245 °C and a melting point reported alternatively at 372 °C (Leong et al., 1985) and 400 °C (Yoda, 1963). As methylene groups are added to the para-aromatic polyanhydrides, the T_g and T_m generally exhibit systematic reductions. PCPM has a T_g reported at 86 and 92 °C and a T_m reported at 196 °C. PCPP has a T_g that has been reported to be between 92 and 96 °C and T_m of between 230 and 266 °C, while PCPH has a T_g that is difficult to detect, but found at 123-147 °C (Rosen et al., 1983; Leong et al., 1985; Leong et al., 1987; Domb and Langer, 1988a; Mathiowitz et al., 1990b; Domb, 1992; Campo et al., 1999).

The branched aromatic polyanhydrides synthesized by Sanders et al. (Mathiowitz et al., 1990b; Sanders et al., 1999) demonstrated lower T_g's than the corresponding P(PCPP-SA) copolymers. The para- xylyl polymers synthesized by Anastasiou and Uhrich (2000a) (Pp-
CPX and \( \text{Pp-}m-\text{CPX} \) had systematically higher \( T_g \)s than the ortho- isomers (\( \text{Po-}o-\text{CPX}, \text{Pm-}o-\text{CPX}, \text{Pp-}o-\text{CPX} \)).

For the aliphatic polyanhydrides, Albertsson and Lundmark (1990a) report that the melting point increases as the number of methylenes between the anhydride bonds increases. For the series PAA, PSA, and PDDA, the melting points are 73, 80 and 107 °C, respectively (Albertsson and Lundmark, 1990a). Also, altering PSA by addition of fatty acid terminals lowers the melting point by as much as 12 °C from 82 °C to as low as 70 °C, depending on the specific fatty acid used (Teomim and Domb, 1999; Teomim and Domb, 2001). And PFAD is completely amorphous (Tabata and Langer, 1993).

Staubli et al. (1991) offer an in depth analysis of the effects of sequence distribution on the \( T_g \) of poly(anhydride-co-imide)s and discuss the experimental results with respect to several applicable theoretical models of \( T_g \).

The change in melting point and glass transition of the copolymers as a function of copolymer composition are also of particular interest because this reveals information about the copolymer microstructure. This is discussed along with the crystallinity characterization in the following section.

2.3.2.2 Crystalline Morphology of Polyanhydrides. Most of the commonly used polyanhydrides, including the copolymers, are semicrystalline. Crystallinity is characterized by a variety of techniques including differential scanning calorimetry (DSC) small-angle X-ray scattering (SAXS) and X-ray diffraction (XRD). Optical microscopy of films can also be used to investigate the crystallinity of polyanhydrides. Because most polyanhydrides have \( T_m \)s near or above room temperature, the crystallinity is a strong function of the thermal history. Therefore, the weight percents of crystallinity (\( W_c \% \)) reported here are primarily for neat polymer purified and precipitated from the synthesis reaction and dried under vacuum.
Most of the polyanhydride homopolymers discussed here have $W_c\%$ in the range of 50-60. For the aromatic polyanhydrides PTA and PCPP $W_c\%$ is around 60 (Mathiowitz et al., 1990b; Domb, 1992). As chain flexibility is increased, a corresponding decrease in the crystallinity is observed. PIPA and PCPH have $W_c\%$ of 50 and 20 respectively (Mathiowitz et al., 1990b; Domb, 1992). PFA, PSA, and PDDA all have a $W_c\%$ between 55 and 66 (Mathiowitz et al., 1988; Mathiowitz et al., 1990b; Domb, 1992). Mathiowitz et al. (1990b) provide an excellent summary of the crystallinity of homopolymers and copolymers of PSA, PCPP, PCPH, PFA, P(SA-FA), P(SA-CPP) and P(SA-CPH) (Figure 2.4). Of the copolymers studied, only the copolymers P(FA-SA) in the composition range from 20:80 to 70:30 exhibited two melting temperatures, indicating two separate types of crystals (Mathiowitz et al., 1990b). Data on thermal transitions, $W_c\%$, and heats of fusion ($\Delta H_f$) are presented for an extensive range of copolymer ratios. Plots of the copolymer crystallinities as a function of the composition are reproduced in Figure 2.4. Crystallinity and heat of fusion data are summarized in Table 2.8. X-ray diffraction spectra can be found in the work by Subramanyam and Pinkus (1985), Leong et al. (1985), Mathiowitz et al. (1990b), and Jiang et al. (2001c).
Table 2.8. Crystallinity and thermal properties for a variety of polyanhydrides.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>T&lt;sub&gt;g&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;m&lt;/sub&gt; (°C)</th>
<th>ΔH&lt;sub&gt;f&lt;/sub&gt; (J/g)</th>
<th>W&lt;sub&gt;c&lt;/sub&gt; %</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aliphatic polyanhydrides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAA</td>
<td>70-79</td>
<td>37-78</td>
<td>(Albertsson and Lundmark, 1988; Albertsson and Lundmark, 1990b; Domb and Nudelman, 1995)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPA</td>
<td>71.5</td>
<td></td>
<td>(Domb and Nudelman, 1995)</td>
<td></td>
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<tr>
<td>PSU</td>
<td>77.9</td>
<td></td>
<td>(Domb and Nudelman, 1995)</td>
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<tr>
<td>PAZ</td>
<td>71.8</td>
<td></td>
<td>(Domb and Nudelman, 1995)</td>
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<tr>
<td>PSA</td>
<td>60</td>
<td>80-89</td>
<td>126-153</td>
<td>57-66</td>
<td>(Domb and Langer, 1987; Mathiowitz et al., 1988; Albertsson and Lundmark, 1990b; Mathiowitz et al., 1990b; Domb and Nudelman, 1995)</td>
</tr>
<tr>
<td>PDDA</td>
<td>88-95</td>
<td>107-123</td>
<td>56</td>
<td>(Domb and Langer, 1987; Mathiowitz et al., 1988; Albertsson and Lundmark, 1990b; Domb and Nudelman, 1995)</td>
<td></td>
</tr>
<tr>
<td>PDX</td>
<td>94.4</td>
<td></td>
<td>(Domb and Nudelman, 1995)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aromatic polyanhydrides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTA</td>
<td>245</td>
<td>372-400</td>
<td>60</td>
<td>(Yoda, 1963; Leong et al., 1985)</td>
<td></td>
</tr>
<tr>
<td>PIA</td>
<td>259</td>
<td></td>
<td>50</td>
<td>(Domb, 1992)</td>
<td></td>
</tr>
<tr>
<td>PDP</td>
<td>a</td>
<td>100-113</td>
<td></td>
<td>(Domb and Langer, 1987; Leong et al., 1987)</td>
<td></td>
</tr>
<tr>
<td>PCPM</td>
<td>86-92</td>
<td>196</td>
<td></td>
<td>(Rosen et al., 1983; Leong et al., 1987)</td>
<td></td>
</tr>
<tr>
<td>PCPP</td>
<td>92-96</td>
<td>230-266</td>
<td>96.3-111</td>
<td>53-61.4</td>
<td>(Leong et al., 1985; Domb and Langer, 1988a; Mathiowitz et al., 1988; Mathiowitz et al., 1990b; Domb, 1992; Campo et al., 1999)</td>
</tr>
</tbody>
</table>

a\textit{Leong et al.} (Leong et al., 1987) reported that no T<sub>g</sub> was observed above room temperature.
b\textit{Leong et al.} (Leong et al., 1987) reported that no T<sub>g</sub> was observed above -20 °C.
cMathiowitz et al. (Mathiowitz et al., 1992) reported to be amorphous.
dNo melting point observed (Anastasiou and Uhrich, 2000a).
Table 2.8. (continued)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$T_e$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_f$ (J/g)</th>
<th>$W_c$%</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCPH</td>
<td>47-48$^b$</td>
<td>123-143</td>
<td>7.1</td>
<td>20</td>
<td>(Leong et al., 1985; Domb and Langer, 1987; Leong et al., 1987; Mathiowitz et al., 1990b; Campo et al., 1999)</td>
</tr>
<tr>
<td>PCPA</td>
<td></td>
<td>185-205</td>
<td></td>
<td></td>
<td>(Domb et al., 1989)</td>
</tr>
<tr>
<td>PCPV</td>
<td>12</td>
<td>50-74$^c$</td>
<td></td>
<td></td>
<td>(Domb et al., 1989; Mathiowitz et al., 1992)</td>
</tr>
<tr>
<td>CPO</td>
<td></td>
<td>48-54</td>
<td></td>
<td></td>
<td>(Domb et al., 1989)</td>
</tr>
<tr>
<td>Po-CPP</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td>(Campo et al., 1999)</td>
</tr>
<tr>
<td>Po-CPH</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td>(Campo et al., 1999)</td>
</tr>
<tr>
<td>Po-o-CPX</td>
<td>82</td>
<td>d</td>
<td></td>
<td></td>
<td>(Anastasiou and Uhrich, 2000a)</td>
</tr>
<tr>
<td>Pm-o-CPX</td>
<td>71</td>
<td>d</td>
<td></td>
<td></td>
<td>(Anastasiou and Uhrich, 2000a)</td>
</tr>
<tr>
<td>Pp-o-CPX</td>
<td>84</td>
<td>114</td>
<td></td>
<td></td>
<td>(Anastasiou and Uhrich, 2000a)</td>
</tr>
<tr>
<td>Po-p-CPX</td>
<td>101</td>
<td>d</td>
<td></td>
<td></td>
<td>(Anastasiou and Uhrich, 2000a)</td>
</tr>
<tr>
<td>Pm-p-CPX</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
<td>(Anastasiou and Uhrich, 2000a)</td>
</tr>
</tbody>
</table>

Other polyanhydrides

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$T_e$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_f$ (J/g)</th>
<th>$W_c$%</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFA</td>
<td>41</td>
<td>246</td>
<td>67</td>
<td>60</td>
<td>(Mathiowitz et al., 1990b; Domb, 1992)</td>
</tr>
<tr>
<td>PFAD</td>
<td></td>
<td>25-30</td>
<td>0</td>
<td></td>
<td>(Domb and Maniar, 1993)</td>
</tr>
<tr>
<td>PCPAM</td>
<td>35.8</td>
<td></td>
<td></td>
<td></td>
<td>(Jiang and Zhu, 2001)</td>
</tr>
<tr>
<td>PCPSM</td>
<td>36.4</td>
<td></td>
<td></td>
<td></td>
<td>(Jiang and Zhu, 2001)</td>
</tr>
<tr>
<td>CEBF</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
<td>(Jiang et al., 2001c)</td>
</tr>
<tr>
<td>CPF B</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
<td>(Jiang et al., 2001c)</td>
</tr>
<tr>
<td>CBFB</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td>(Jiang et al., 2001c)</td>
</tr>
</tbody>
</table>
When drugs are incorporated into semicrystalline polymers, the crystallinity may be altered, depending on the interactions between the polymer and the drug (Shen et al., 2001a). The effects of drug loading on polymer crystallinity may offer some insights into release kinetics as will be discussed in Section 2.4.1. Mathiowitz et al. (1990a) reported the changes in melting point and degree of crystallinity for PSA and P(CPP-SA) 50:50 loaded with various model drugs at different loading levels. The effects on polymer crystallinity and melting point for different drugs provides information on the solubility of the drugs in the polymer matrix which, may be used to predict how drug loading will modify the polymer erosion kinetics and thus the drug release kinetics. Shen et al. (2001b) used wide-angle X-ray diffraction (WAXD) and DSC to characterize the changes in crystallinity of PSA as a function of the loading of a compatible drug, p-nitroaniline (PNA), and an incompatible drug,
brilliant blue (BB) (Shen et al., 2001b). The compatible drug reduces the crystallinity, while the incompatible drug has no effect on the polymer crystallinity (Figure 2.5).

**Figure 2.5.** WAXD spectra for: A) BB-loaded PSA, and B) PNA-loaded PSA. A) BB loading is (a) 0, (b) 15, (c) 30, and (d) 45. Note that as loading increases, the spectrum shows no change for the PSA crystallinity, but crystals of BB appear, indicating that the solute and polymer are immiscible. B) PNA loading is (a) 0, (b) 5, (c) 10, and (d) 15. Note that there are no peaks corresponding to PNA as the loading increases, however, the polymer crystallinity decreases with increased loading, indicating polymer/solute compatibility. From (Shen et al., 2001a) Reprinted with permission.

2.3.2.3 Amorphous phase behavior and microstructure of polyanhydrides. Blending of polymers is a strategy commonly used to design materials with desirable properties for many applications. Few studies have investigated the amorphous phase behavior of polyanhydrides. Domb developed two techniques for qualitatively assessing polymer miscibility and reported the results for a variety of binary polyanhydride blends as well as blends of polyanhydrides with other biodegradable polymers (1993). Shakesheff et al.
(1995) studied the phase behavior of PSA blends with poly(DL-lactic acid) (PLA) and the effects of the phase behavior on erosion kinetics by novel techniques allowing in situ atomic force microscopy (AFM) and surface plasmon resonance (SPR). Surface enrichment in PSA/PLA blends has also been assessed by AFM (Chen et al., 1998). Chan and Chu (2002) used calorimetry and IR to characterize the phase behavior of PSA/poly(ethylene glycol) blends. Rigorous analysis of the phase behavior of polyanhydrides based on theoretical predictions is not found in the published literature.

When describing erosion of and drug release from surface erodible polymers, it is often implicitly assumed that the matrix erodes uniformly, resulting in a uniform release profile for a homogenously dispersed drug. While this may be a valid assumption for some homopolymer systems, neglecting the effects of crystallinity, some multicomponent polymer matrices may exhibit microphase separation, even when the copolymers are random (Shen et al., 2001a). In such phase-separated systems, a drug will thermodynamically partition.

The erosion of copolymers requires the hydrolytic cleavage of three bond types: the A-A bond, the A-B bond, and the B-B bond. If the degradation rates of these three bonds are unequal, as is likely the case, then the erosion will be inhomogeneous. And, if drugs are inhomogeneously distributed in the polymer matrix, the drug release profile will not follow overall device erosion (Shen et al., 2002). Therefore, it is necessary to accurately describe the microstructure of microphase-separated systems.

The length scale on which this microphase separation occurs can be obtained by considering the sequence distribution of monomers in the copolymers. For instance, number-average sequence lengths can be determined from $^1$H NMR (Mathiowitz et al., 1990b; Ron et al., 1991; Tamada and Langer, 1992; Shen et al., 2002). One may estimate that the length scale of the phase-separated domains is likely to be less than <10 nm. The characterization
proves to be challenging as there are few microscopy or spectroscopy techniques that can resolve such small length scales. However, the effects on drug release kinetics are apparent (see Section 2.4.1).

2.3.3 Biocompatibility of polyanhydrides

Biocompatibility is an essential property of new biomaterials for drug delivery. Biocompatibility is always assessed with respect to specific applications and may be assessed with respect to cytotoxicity, allergic responses, irritation, inflammation, mutagenicity, teratogenicity, and carcinogenicity (Katti et al., 2002). The reviews by Katti et al. and Domb et al. (1997) provide good discussions on the biocompatibility studies that have been conducted with polyanhydrides over the past two decades.

Leong et al. (1986) conducted experiments with the degradation products of P(CPP-SA) to determine mutagenicity and teratogenicity. In the same study, PCPP and PTA were implanted in rat corneas and PCPP was implanted subcutaneously in rat abdomens for histology. Endothelial and smooth muscle cell cultures on P(CPP-SA), P(SA-TA), and PTA were also conducted to assess cytotoxicity. Mutagenicity and teratogenicity tests were both negative, and the in vivo experiments revealed no inflammation. Cell cultures exhibited normal proliferation and no abnormal morphologies (Leong et al., 1986).

The biocompatibility of P(CPP-SA) implants in the brain was assessed by Brem et al. (1989) and Tamargo et al. (1989). In the former study the 50:50 copolymer was implanted in rabbit brains and compared to a gelatin based implant used in neurological surgery (Gelfoam) and induced similar mild reactions (Brem et al., 1989). In the latter study the 20:80 copolymer was implanted in rat brains and was compared to Gelfoam® and a cellulose-derived product (Surgicel®). Inflammatory response was similar to that induced by the
Surgicel®, but more severe than the Gelfoam®. No local or systemic toxicity was observed (Tamargo et al., 1989). The brain biocompatibility of P(FAD-SA) was investigated by Brem et al. (1992) and found to be comparable to that of P(CPP-SA).

Laurencin et al. (1990) conducted extensive local and systemic toxicity studies with P(CPP-SA), which also showed excellent biocompatibility and toxicology. Domb (1992) studied the biocompatibility of P(CPP-IPA), P(CPP-IPA-SA), and P(CPP-SA) by subcutaneous and intramuscular implants in rabbits. Inflammation occurred at week one and was more pronounced for the intramuscular implants, but subsided in all cases by week 4 (Domb, 1992). Domb and Nudelman (1995) conducted subcutaneous biocompatibility studies in rats with poly(pimelic acid) (PPA), poly(azelaic acid) (PAZ), PSA, and PDDA resulting in mild inflammation but no encapsulation or other pathologies. The systemic and local biocompatibility of the ricinoleic acid-based polymers was investigated and confirmed by Teomim et al. (1999) by subcutaneous implantation in rats. Jiang et al. (2001a) assessed the biocompatibility of the poly(anhydride-co-ester)s PCPA, PCPS, and P(CPA-co-CPS) by subcutaneous implants in rats. Mutagenicity and toxicity were not observed, though mild inflammatory responses were observed.

2.4 Degradation, Erosion, and Drug Release Kinetics

2.4.1 Experiments

2.4.1.1 Polymer stability. The degradation kinetics of several polyanhydrides have been assessed under different storage conditions, to determine the useful shelf life. Rate constants and activation energies for degradation of a variety of polyanhydrides in solution have been reported (Domb and Langer, 1989). In solution, degradation rate is an increasing function of temperature. Aromatic polymers such as PCPM, PCPP, and PCPH, and PDP all
maintain their molecular weights both in the solid state and in organic solution for up to a year, but aliphatic polymers show a first order decrease in molecular weight with time (Domb and Langer, 1989; Chasin et al., 1990). Domb et al. (1989) reported that the PCPV and PCPO were stable for six months when stored in vacuo at room temperature. However, when stored in concentrated chloroform solution, the molecular weights of both polymers were reduced by 50% in only about 3 hours. The degradation products could be repolymerized, proving that the degradation occurred primarily via the anhydride interchange and could be reversed (Domb et al., 1989). Chang and Chu (2003) showed that in humid environments, depolymerization results primarily in the formation of diacid products, and therefore occurs by hydrolysis. Domb (1992) also demonstrated the stability of aromatic copolymers stored both under dry argon and in DCM solution, and under exposure to γ-irradiation. The ortho-substituted aromatic polyanhydrides, salicylic acid-based poly(anhydride-co-ester)s, and ricinoleic acid based poly(anhydride-co-ester)s also demonstrate stability to γ-irradiation (Erdmann et al., 2000; Bedell et al., 2001a; Krasko et al., 2003). From a study of these results and the studies of other polyanhydrides, storage in a dry atmosphere below −20 °C is recommended if polymers are not going to be used within a few days of synthesis (Tamada and Langer, 1992).

2.4.1.2 *In vitro* degradation, erosion, and drug release kinetics. *In vitro* kinetics experiments are usually conducted on compression molded monolithic polymer tablets, slabs, or cylinders with well-defined surface areas. Compression molding is done above the glass transition and near the melting point. Drugs are incorporated by co-dissolution with the polymer or mechanical mixing in the melt. If erosion profiles are desired, the polymer samples can be removed from the dissolution media at the specified times, dried, and massed. Degradation and drug release requires an assay for the monomer or drug content of the
dissolution media. UV Spectrophotometry or HPLC are common techniques. Monitoring
the appearance of a single component in the dissolution media is not a reliable method for
characterizing the overall erosion rate of a multicomponent system, even when that system is
surface-erodible. Such generalizations should be carefully avoided, particularly when the
system contains hydrophilic and hydrophobic moieties. For example, Shieh et al. (1994)
demonstrate that different drugs release from the same matrix with different kinetics. In this
study, the model hydrophilic drug acid orange (AO) released faster than the PSA monomer
from P(FAD-SA) (50:50) systems, diffusing out of the polymer matrix, while rhodamine b
table (RhoB) released more slowly than the PSA monomer from the same system (Shieh et
al., 1994). For other compositions, the AO release profile more closely matched the PSA
degradation profile (Shieh et al., 1994).

The in vitro degradation and drug release of polyanhydride formulations is not
necessarily equivalent to the in vivo kinetics. For information on the in vivo kinetics, the
interested reader is referred to the recent review by Katti et al. (2002) and the review by
Domb et al. (1997).

2.4.1.2.1 Modulating erosion rates and drug release rates. The erosion rate
constants reported in the literature or estimated from degradation or erosion data for many of
the polyanhydrides discussed in this review are summarized in Table 2.9. Many of the
homopolymers exhibit zero-order degradation over the majority of the release time. As
polymer hydrophobicity is increased, the erosion rates generally decrease, presumably due to
the decrease in reactivity of the anhydride bond. However, increase in polymer
hydrophobicity corresponds to increase in monomer hydrophobicity as well. The
corresponding decrease in erosion may therefore be due to both degradation kinetics and/or
monomer dissolution kinetics (Hanes et al., 1998). Evidence has also been presented (see for
Table 2.9. Erosion rate constants for many common polyanhydrides.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Erosion rate constant (mol cm$^{-2}$ day$^{-1}$)</th>
<th>Weight-average molecular weight</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>$2.7 \times 10^{-5}$</td>
<td>23,900</td>
<td>(Leong et al., 1987)</td>
</tr>
<tr>
<td>PDDA</td>
<td>$5.4 \times 10^{-5}$</td>
<td>32,700</td>
<td>(Albertsson and Lundmark, 1990a)</td>
</tr>
<tr>
<td>PCDA</td>
<td>$9.3 \times 10^{-5}$</td>
<td></td>
<td>(Zhang et al., 2001)</td>
</tr>
<tr>
<td>PCPA</td>
<td>$3.1 \times 10^{-5}$</td>
<td></td>
<td>(Domb et al., 1989)</td>
</tr>
<tr>
<td>PCPV</td>
<td>$1.3 \times 10^{-5}$</td>
<td>44,600</td>
<td>(Domb et al., 1989)</td>
</tr>
<tr>
<td>PCPO</td>
<td>$2.5 \times 10^{-6}$</td>
<td>33,300</td>
<td>(Domb et al., 1989)</td>
</tr>
<tr>
<td>PTA</td>
<td>$3.2 \times 10^{-5}$</td>
<td></td>
<td>(Leong et al., 1985)</td>
</tr>
<tr>
<td>PCPM</td>
<td>$3.4 \times 10^{-5}$</td>
<td>11,800</td>
<td>(Rosen et al., 1983)</td>
</tr>
<tr>
<td>PCPP</td>
<td>$1.1 \times 10^{-5}$</td>
<td>15,000</td>
<td>(Leong et al., 1985)</td>
</tr>
<tr>
<td>Po-CPP</td>
<td>$6.3 \times 10^{-7}$</td>
<td></td>
<td>(Bedell et al., 2001a)</td>
</tr>
<tr>
<td>PCPH</td>
<td>$1.4 \times 10^{-8}$</td>
<td>9530</td>
<td>(Leong et al., 1985)</td>
</tr>
<tr>
<td>Po-CPH</td>
<td>$1.2 \times 10^{-5}$</td>
<td></td>
<td>(Bedell et al., 2001a)</td>
</tr>
<tr>
<td>PCPS</td>
<td>$1.2 \times 10^{-5}$</td>
<td></td>
<td>(Jiang et al., 2001a)</td>
</tr>
<tr>
<td>PCPA</td>
<td>$5.3 \times 10^{-5}$</td>
<td>21,000</td>
<td>(Jiang et al., 2001a)</td>
</tr>
<tr>
<td>PXDA</td>
<td>$3.1 \times 10^{-5}$</td>
<td>7920</td>
<td>(Leong et al., 1987)</td>
</tr>
<tr>
<td>PFAD</td>
<td></td>
<td></td>
<td>(Tabata and Langer, 1993)</td>
</tr>
<tr>
<td>PHFB</td>
<td>$1.6 \times 10^{-6}$</td>
<td>15,700</td>
<td>(Kaur et al., 2002)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Copolymer P(CPP-SA)</th>
<th>Erosion rate constant (µg cm$^{-2}$ day$^{-1}$)</th>
<th>Weight-average molecular weight</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0</td>
<td>1.4</td>
<td>15,000</td>
<td>(Leong et al., 1985)</td>
</tr>
<tr>
<td>85:15</td>
<td>6.0</td>
<td>9,840</td>
<td>(Leong et al., 1985)</td>
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<tr>
<td>45:55</td>
<td>80.0</td>
<td>6,140</td>
<td>(Leong et al., 1985)</td>
</tr>
<tr>
<td>21:79</td>
<td>160.0</td>
<td>12,030</td>
<td>(Leong et al., 1985)</td>
</tr>
<tr>
<td>0:100</td>
<td>210</td>
<td>23,900</td>
<td>(Leong et al., 1987)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Polyanhydrides with other functional groups</th>
<th>Erosion rate constant (μg cm$^{-2}$ day$^{-1}$)</th>
<th>Number-average molecular weight</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(A-co-U)</td>
<td>8400</td>
<td>5900</td>
<td>(Hartmann et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>3600</td>
<td>9100</td>
<td>(Hartmann et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>2160</td>
<td>13,700</td>
<td>(Hartmann et al., 1993)</td>
</tr>
<tr>
<td>P(A-co-A)</td>
<td>9120</td>
<td>6800</td>
<td>(Hartmann et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>5280</td>
<td>10,800</td>
<td>(Hartmann et al., 1993)</td>
</tr>
<tr>
<td>P(A-co-E)</td>
<td>2880</td>
<td>6,390</td>
<td>(Hartmann et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>480</td>
<td>10,900</td>
<td>(Hartmann et al., 1993)</td>
</tr>
</tbody>
</table>

*Erosion experiment conducted at pH 7.2

1Estimated from linear portion of sigmoidal profile
example Shakesheff et al., 1994) that crystalline domains erode much more slowly than amorphous domains. Thus, careful control of crystallinity may be necessary to accurately modulate erosion and drug release kinetics.

Erosion rates of copolymers can also be modulated by changing the copolymer composition. As an example, erosion rates for three compositions of P(CPP-SA) are reported in Table 2.9. Similar results were reported by Domb and Maniar (1993) for the copolymers of P(FAD-SA). This study also showed that the copolymers degrade in a heterogeneous fashion, that is, at later times, the composition is richer in the more slowly degrading monomer. Note that erosion rates are varied over two orders of magnitude (Table 2.9). The same phenomenon was demonstrated by Shakesheff et al. (1995) for PSA/PLA blends by a novel technique allowing in situ AFM and SPR measurements. Further characterization of these blends revealed surface segregation of the PLA phase, which slowed erosion for high PLA content blends (Davies et al., 1996).

Whether in copolymers or blends, inhomogeneous erosion has a non-trivial effect on drug release kinetics as will be shown later. Leong et al. (1985) demonstrated that the pH of the degradation media also has a dramatic effect on the erosion rate, which increases with increasing pH. The acceleration of degradation of polyanhydrides with increase in pH is widely reported and has been used to speed up experiments (Shakesheff et al., 1994).

Molecular weight may also affect the erosion rate. Table 2.9 shows the degradation rate of a representative poly(anhydride-co-urethane), a poly(anhydride-co-amide), and a poly(anhydride-co-ester) of different molecular weights (Hartmann et al., 1993). For all of these polymers, the reported erosion rate decreases as the molecular weight increases.

In their study of branched PSA (1990), Maniar et al. found that the molecular architecture of branched polymers affects the release kinetics in a variety of ways. They
found that the branched polymers degraded faster than linear PSA of comparable molecular weight (Maniar et al., 1990). They also noted that drug (morphine) release profiles were more characteristic of bulk erosion than surface erosion: An initial lag time during which very little drug was released was associated with the time required for water to swell the polymer. This was followed by a period of relatively fast release, which tapered off as the device disintegrated. The polymer matrix lost its mechanical integrity before the release experiment was complete (Maniar et al., 1990). Despite the increase in degradation rate, release rates from the polymer randomly branched with 1,3,5-benzene tricarboxylic acid were much lower than release rates from PSA (Maniar et al., 1990). The release from the graft type polymer branched with poly(acrylic acid) approached that of PSA (Maniar et al., 1990).

Evidence that drug loading modifies the erosion rate can be found in many drug release studies. Particularly at higher loadings, hydrophilic drugs tend to increase the overall erosion rate of the polymer (Park et al., 1996; Shen et al., 2002). This phenomenon is attributed to the contribution that the drug makes to the overall chemistry of the system, as well as porosity and voids that may form as hydrophilic drug crystals rapidly dissolve from the exposed surface (Sandor et al., 2002). One study of drug release from bioerodible polyanhydrides found a change in the drug release kinetics from zero-order to first order by simply changing the pH of the media or by changing the hydrophobicity of the drug (Park et al., 1997).

Finally, drug release profiles can be altered by altering the distribution of the drug in the polymer matrix. For purely surface eroding systems, it is theoretically possible to obtain any desired drug release profile by fabricating a device with the corresponding drug
distribution profile. Design and fabrication of devices with non-uniform drug distribution is discussed in Section 2.5.

2.4.1.2.2 Surface changes during erosion. Albertsson and Lundmark (1990a) reported that during the degradation of PDDA, the surface showed a lower C/O ratio (from electron spectroscopy for chemical analysis (ESCA) studies) than in the neat polymer, indicating partial oxidation. Mathiowitz et al. (1993) discussed the effects of crystallinity and liquid crystallinity on the degradation kinetics in P(CPH-SA) and P(CPP-SA) copolymers. Evidence that crystalline domains degrade more slowly than amorphous domains is also reported in several studies (Shakesheff et al., 1994).

The monomer solubility has a crucial effect on the surface characteristics of eroding polymer systems. Undissolved monomer deposited on the surface complicates erosion and release kinetics by presenting a diffusional barrier for drug release as well as water ingress. The compounding effect slows not only the release of monomer and drug, but also the prerequisite hydrolysis of the polymer backbone that results in release (Goepferich et al., 1996). The solubilities of the class of aliphatic polyanhydride monomers from adipic acid (six carbons) to dodecandicarboxylic acid (14 carbons) vary from 50 mg/ml to <0.01 mg/ml, generally decreasing as the length of the methylene chain increases (Domb and Nudelman, 1995).

2.4.1.2.3 Chemical changes during erosion. Because the degradation products of polyanhydrides are acidic (see pKa's reported in Goepferich and Langer, 1993a), and the degradation is a strong function of pH, it has been hypothesized that during erosion the pH of the microenvironment very near the surface of a device may not be the same as that of the dissolution media. Dissolved drugs may also affect the local pH. This local pH is difficult to measure or estimate (Goepferich and Langer, 1993a), but may have profound effects on the
erosion and drug release profiles. Mädter et al. (1997) employed spectral spatial paramagnetic resonance imaging and measured pH values inside eroding samples of P(CPP-SA) as low as 4.5, though the dissolution media was buffered at 7.4.

The composition of copolymers usually changes during erosion due to the disparity between the degradation kinetics of the two corresponding homopolymers. Actually, in binary copolymers there are three types of bonds that may all have different degradation kinetics: the A-A bond, the A-B bond, and the B-B bond. Spectroscopic techniques such as IR and NMR can be used to follow the kinetics of specific bond cleavage in copolymers (Heatley et al., 1998; Uhrich et al., 1998; McCann et al., 1999). Changes in molecular weight during degradation are frequently reported. The formation of relatively stable oligomers in copolymer erosion studies has been shown (Santos et al., 1999). The changes in molecular weight may also result in drastic shifts in thermal transitions during erosion (Bedell et al., 2001b).

Figure 2.6 summarizes some of the important effects contributing to drug release kinetics discussed in this section. Drug release from a simple, homogeneous surface eroding system is shown schematically in Figure 2.6a and graphically in Figure 2.6c. Zero order release is obtained when the drug (represented by the circles) is uniformly distributed and the system erodes uniformly from the surface. Drug release from a phase-separated surface eroding system is shown schematically in Figure 2.6b. In this system, two polymer phases are present, one which erodes quickly (light gray), and one which erodes slowly (dark gray). Drug release accelerates initially because the inhomogeneous erosion and bursting of drug from the slow eroding phase lead to increase in surface area. At later times, the fast eroding phase is completely gone, and the degradation products from the slow eroding phase (triangles) form an insoluble barrier to transport, retarding the release. A sigmoidal release
profile results, as shown in Figure 2.6d. Additional effects, such as partitioning of the drug are not represented.

Figure 2.6. Mechanism of drug release from (a) homogenous, surface-eroding system, and (b) phase-separated surface-eroding system demonstrating some of the key factors affecting release as discussed in Section 2.4.1. Length scale of phase separation is enlarged for emphasis.
2.4.2 **Modeling degradation, erosion and drug release kinetics**

Modeling the behavior of bioerodible polyanhydrides is complicated by the many phenomena contributing to release profiles described in the previous section. The degradation kinetics may be coupled to other processes, such as diffusion and dissolution, and the overall erosion kinetics represent the sum of all of these multiple processes (Goepferich, 1996a). The phenomena that contribute to erosion kinetics may be difficult or impossible to study independently (Goepferich, 1996b). Therefore, great care must be taken when formulating or applying a model to ensure that the phenomena described by the model are the dominant phenomena controlling the kinetics. A variety of models have been developed that account for different aspects of polymer microstructure, degradation kinetics, and drug loading. The recent review by Goepferich and Tessmar (2002) discusses degradation and erosion of polyanhydrides with an eye to developing more accurate models. And the review by Seipman and Goepferich (2001) discusses many of the recent models and could be used to aid in selection of the appropriate model for a given system.

Burkersroda et al. (2002) provide a model that can be used to estimate whether a polymer is more accurately characterized as surface eroding or bulk eroding. In this model, the ratio of a characteristic time scale for diffusion to a characteristic time scale for degradation (comparable to a Deborah number) determines the 'erosion number,' \( \varepsilon \) (Burkersroda et al., 2002). For \( \varepsilon \gg 1 \), a device is surface eroding, whereas for \( \varepsilon \ll 1 \), a device is bulk eroding (Burkersroda et al., 2002). A key component of this model is the device dimensions. Theoretically, even very hydrophobic polymers can be bulk eroding, provided the device is sufficiently small. If the polymer matrix itself is hydrophobic, the polymer degradation rate can be decreased by several orders of magnitude.
The simplest model for pure erosion control with kinetics dominated by a single rate constant and uniformly distributed drugs was described by Hopfenberg (1976). This model says nothing about the various physical phenomena that contribute to erosion, and therefore fails to describe drug release profiles from many polyanhydride systems. Below we classify some of the models that can be found in the literature.

2.4.2.1 Phenomenological models. The broadest class of models, phenomenological models, account explicitly for individual phenomena such as swelling, diffusion, and degradation by incorporation of the requisite transport, continuity, and reaction equations. This class of models is useful only if it can be accurately parameterized. As phenomena are added to the model, the number of parameters increases, hopefully improving the model’s accuracy, but also requiring additional experiments to determine the additional parameters. These models are also typically characterized by implicit mean-field approximations in most cases, and model equations are usually formulated such that explicit solutions may be obtained. Examples from the literature are briefly outlined below.

The transport and continuity equations for surface eroding polymers with two moving boundaries (defining a diffusion zone for drugs inside the polymer matrix) were solved by Thombre and Himmelstein (1984). No account was made for inhomogeneities either in polymer matrix or the drug distribution, but the model was extended to account for the presence of a membraneous diffusive barrier at the surface. A later extension of the model accounted for an external mass transfer coefficient and changes in degradation rate and drug diffusivity with pH and the progress of degradation (Thombre and Himmelstein, 1985). This model was solved numerically.

Batycky et al. (1997) developed a model applicable for bulk eroding systems. An interesting component of this model is the explicit accounting of the changes in the molecular
weight distribution with time via both end chain scission and random chain scission. Larobina et al. (2002) developed a model for release from copolymers that accounts for microphase separation in copolymers and partitioning of drugs into the phase separated microdomains. Two moving erosion fronts are assumed, leading to three regimes of release. Analytical solutions are obtained.

2.4.2.2 Discretized models. Zygourakis and coworkers (1990; Zygourakis and Markenscoff, 1996) developed a discretized model in which cells are assigned a degradation time, upon exposure to solvent, based on their identity as either drug, polymer, solvent, or void. The initial distribution of cells can be modeled after the microstructure of the polymer matrix and multiple phases are explicitly accounted for. The solution is found numerically.

Goepferich and Langer (1993b) developed a similar model, except that finite probabilities are assigned for the erosion of each cell type rather than predetermined erosion times. No account of drug release was made in this model, but the model was applied to materials with two types of polymer cells, designed to signify crystalline and amorphous phases. In a second publication, Goepferich and Langer also accounted for monomer diffusion through the eroding zone (Goepferich and Langer, 1995). The solution to this model is also obtained numerically.

2.5 Design of Polyanhydride Carriers for Controlled Release

Many model formulations of polyanhydrides have been tested both in vitro, and in vivo. The delivery schemes that polyanhydrides have been used for can be broadly grouped into three classifications – implantable systems for localized drug release, injectable systems, and aerosols for mucosal delivery. Each of these delivery routes presents a unique set of challenges and these are discussed below.
2.5.1 **Implantable systems**

2.5.1.1 BCNU-loaded polyanhydride discs for treatment of glioblastoma multiforma. The encapsulation and release of 1,3-bis(2-chloroethyl)nitrosourea (BCNU) in P(CPP-SA) 20:80 wafers was the first implantable controlled release device based on polyanhydrides that was FDA-approved and marketed (Gliadel®) (Chasin *et al.*, 1988). BCNU was encapsulated by two techniques, trituration and co-dissolution, resulting in different release profiles (Chasin *et al.*, 1990; Chasin *et al.*, 1991). The triturated samples released faster than those prepared by co-dissolution, presumably due to more homogeneous loading in the samples prepared by co-dissolution.

2.5.1.2 Laminated devices for pulsatile release. Jiang and Zhu (2000) and Qiu and Zhu (2001) have reported the fabrication of multilayered devices composed of stacks of compression-molded disks of alternating compositions. One type of disk is either P(SA-EG) or P[SA-co-TMAgly)-b-EG] and the other is a pH-sensitive, protein-loaded blend of, for example, poly(methacrylic acid) and polyethoxazoline. The release of model proteins, myoglobin, bovine serum albumin, and FITC-dextran, and compounds such as brilliant blue have been studied and pulsatile release profiles have been demonstrated (Jiang and Zhu, 2000; Qiu and Zhu, 2001).

2.5.1.3 Other devices. Erdmann *et al.* (2000) report the fabrication of devices for the localized delivery of salicylic acid from the poly(anhydride-co-ester)s mentioned in Section 2.2.3. A unique feature of this drug delivery system is that the drug compound is part of the polymer backbone. Devices were implanted intraorally and histopathology was reported (Erdmann *et al.*, 2000). Chasin *et al.* (1990) review fabrication and testing of implantable
formulations for other drugs including angiogenesis inhibitors for treatment of carcinomas and bethanecol for the treatment of Alzheimer's disease.

2.5.2 Injectable systems

Injectable polyanhydride systems for drug delivery usually consist of polymer microspheres suspended in an injection media. Langer and co-workers reported on three techniques for the fabrication of drug loaded polyanhydride microspheres: hot-melt, solvent removal, and spray drying (Mathiowitz and Langer, 1987; Bindschaedler et al., 1988; Mathiowitz et al., 1988; Mathiowitz et al., 1990a; Mathiowitz et al., 1992). The hot-melt technique used by Mathiowitz and Langer (1987) is performed by heating the polymer and drug in a non-solvent to a temperature above the melting point of the polymer and stirring to disperse the molten droplets. Subsequent cooling freezes polymer microspheres loaded with dissolved drug. This technique is only useful for polymers with melting points sufficiently low that the activity of the drugs is not affected by the heating. In the spray drying technique, polymer and drug are dissolved in a suitable solvent and a spray dryer is used to disperse small droplets into air where precipitation occurs (Mathiowitz et al., 1992). The third and most common technique found in the literature is solvent removal. In this technique, a polymer solution (containing drug) is dispersed in a non-solvent (Mathiowitz et al., 1988). An emulsion is formed. The solvent is extracted out of the droplets by the non-solvent, precipitating the microspheres. Variations on the solvent removal technique have been optimized for several polymer/drug systems. Double emulsion or phase inversion techniques are used when the drug and polymer are not soluble in the same solvent (Chiba et al., 1997; Chickering et al., 1997; Thomas et al., 1997). Double walled microspheres have been produced by precipitating a second polymer solution onto previously fabricated
microspheres (Goepferich et al., 1994), and by allowing thermodynamic partitioning of two polymer solutions during the solvent removal process (Pekarek et al., 1994; Leach et al., 1999). *In vitro* and *in vivo* degradation studies showed that the inner layer of P(CPP-SA) degraded before an outer layer of PLA in double walled systems (Leach and Mathiowitz, 1998; Leach et al., 1998).

Microspheres with precisely controlled sizes have been produced by a novel apparatus that uses acoustic excitation and a non-solvent carrier stream to form each droplet in the emulsion separately (Berkland et al., 2004). The morphology and hence the drug release kinetics of the microspheres are affected by the fabrication technique. The size of the microspheres can be modulated by changing the stirring rate in the hot melt and solvent removal techniques, however both of these techniques produce highly polydisperse size distributions (Mathiowitz and Langer, 1987; Mathiowitz et al., 1988). The surface and internal morphology of microspheres produced by various techniques have also been characterized, as these will affect the drug release kinetics. The hot-melt technique produces non-porous microspheres with crenellated surfaces (Mathiowitz and Langer, 1987). Solvent removal techniques can produce smooth microspheres, though porosity is difficult to control and crystalline polymers tend to have greater surface roughness (Mathiowitz et al., 1988; Mathiowitz et al., 1990a). Spray dried microspheres also have polydisperse size distributions and can have very porous structures. Mathiowitz et al. (1992) had difficulty preventing the microspheres made from some polymers from fusing into aggregates with this technique. Drug loading efficiencies and uniformity can vary depending on the compatibility of the drug with the polymer matrix and other characteristics of the fabrication technique. These morphological variations will also have a significant impact on the drug release kinetics, which are discussed in the next subsection.
An advantage of this type of delivery system is that microspheres displaying different release profiles (e.g. being composed of different polymers or different sizes) can be combined in cocktails to obtain release profiles that are the sum of the various release profiles from the individual formulations (Kipper et al., 2002). Multiple drugs could also be delivered this way in a single injection.

Berkland et al. (Berkland et al., 2004) showed that for systems made by solvent removal, the precipitation kinetics play a crucial role in determining drug distribution within the microspheres, and thus the release profiles. For drugs that are incompatible with the polymer matrix, slow precipitation may result in surface segregation of the drug (Berkland et al., 2004). One way of controlling the precipitation kinetics is to carefully control the microsphere size. Smaller microspheres precipitate more quickly and therefore exhibit the most extended release profiles when the polymer/drug compatibility is low (Berkland et al., 2004). In a study comparing release profiles from tablets and injectable granules (Tabata et al., 1994), it was shown that inhomogeneously distributed drug has little or no detectable effect on release profiles from tablets, while the release profiles from granules exhibit drug bursts at the beginning of the experiment.

Proteins may be stabilized by encapsulation in polyanhydride microspheres. Stability of proteins with respect to water-induced aggregation has been demonstrated to be a function of polymer hydrophobicity for insulin and bovine somatotropin as model proteins (Ron et al., 1993). Encapsulation and enzymatic activity of a variety of other proteins encapsulated in P(SA-FAD) was studied by Tabata et al. (Tabata et al., 1993).
2.5.3 Aerosols and systems designed for mucosal delivery

Many therapeutic proteins must be delivered by injection as alternative delivery routes (e.g. oral) result in low bioavailability. This can be difficult, inconvenient, and painful, particularly for long-term treatments, for example, in the case of insulin administration for diabetes patients. Mucosal delivery offers an attractive alternative to injection, but poses some unique challenges. The formulation must be capable of stabilizing the drug, targeting delivery to the mucosa, remaining at the delivery site for extended periods, and facilitating trans-mucosal transport of the drug (Harris and Robinson, 1990). The characteristics of the various mucosa (buccal, nasal, gastrointestinal, and ocular) that can be quantified for design of controlled release devices are summarized by Harris and Robinson (1990). Theories of bioadhesion are briefly outlined by Chickering et al. (1995).

Chickering and Mathiowitz (1995) developed a technique for investigating the bioadhesive properties of polymers and showed that p(FA-SA) demonstrated good bioadhesion. Two mechanisms of bioadhesion were proposed: surface free energy effects and hydrogen bonds between carboxylic acid residues in degradation products and mucin or epithelia (Chickering and Mathiowitz, 1995). The same authors showed that encapsulation of a model drug (ducimerol) in P(FA-SA) improved bioavailability in oral delivery experiments (Chickering et al., 1996).

Fu et al. (2002) report the optimization of a fabrication procedure for microspheres based on the poly(anhydride-co-ether) P(SA-EG). The microspheres are fabricated by solvent removal process that produces a porous structure with densities in the range of 0.344 and 0.077 g cm\(^{-3}\) and sizes that are optimized for delivery to the deep lung by inhalation (Fu
et al., 2002). An appropriate in vitro cell culture model for characterization of the particle-epithelia system was also developed (Fiegel et al., 2003).

2.6 Conclusions and Future Opportunities

The past two decades have produced a revival of interest in the synthesis of polyanhydrides for biomedical applications. These materials offer a unique combination of properties that includes hydrolytically labile backbone, hydrophobic bulk, and very flexible chemistry that can be combined with other functional groups to develop polymers with novel physical and chemical properties. This combination of properties leads to erosion kinetics that is primarily surface eroding and offers the potential to stabilize macromolecular drugs and extend release profiles from days to years. The microstructural characteristics and inhomogeneities of multicomponent systems offer an additional dimension of drug release kinetics that can be exploited to tailor drug release profiles.

The development of new polyanhydrides has sparked researchers to developed new device fabrication and characterization techniques, instrumentation, and experimental and mathematical models that can be extended to the study of other systems. The growing interest in developing new chemistries and drug release systems based on polyanhydrides promises a rich harvest of new applications and drug release technologies, as well as new characterization techniques that can be extended to other materials. Future endeavors will likely focus on multicomponent polyanhydride systems, combining new chemical functionalities to tailor polyanhydrides for specific applications.

The release characteristics of polyanhydride systems could be used not only to develop clinical treatments, but also to induce chronic disease states as models for studying
immune function. Many current models of chronic diseases are based on induction of acute
effects, which do not exhibit the same long-term behavior as the disease being modeled.

2.7 References


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CHAPTER 3
RESEARCH OBJECTIVES AND DISSERTATION ORGANIZATION

3.1 Specific Goals

In Chapter 1, compelling justification was provided for the development of controlled release vaccine formulations based on bioerodible polymer microspheres. A great deal of research has already been done to characterize the release properties of systems composed of several bioerodible polyanhydrides. The examination of this body of work, presented in Chapter 2, reveals that the release kinetics are significantly affected by a variety of system characteristics, from composition and processing to drug incorporation and the thermodynamics of the system. However, very little work has been done to connect the combined effects of the several important microstructural characteristics on the release kinetics. For example, no study to date has attempted to characterize the phase behavior of any binary polyanhydride blend system, let alone discern the effects of phase separation on release kinetics. Such fundamental characterization is essential for the continued study of polyanhydrides in controlled release devices. Though several mathematical models developed to describe the drug release kinetics from such systems have increased our understanding of the mechanisms of degradation, erosion, and release, no model developed to date has effectively solved the problem of predicting the release from polyanhydride microspheres by accounting for the several processes and system characteristics that have significant impact on release.

In light of this review, the overall objective of our research is the design of an injectable system based on bioerodible polyanhydride microspheres for the controlled
delivery of vaccines. The design will include a predictive mathematical model that can be used to design systems with tailored release profiles. Such a model is necessary for tailoring the release from polyanhydrides to specific applications. The many processes affecting the release kinetics, occurring on both microscopic and macroscopic length scales, have their roots in the molecular architecture of the polymer/drug system. Thus, a comprehensive predictive model describing the release characteristics must link molecular descriptions of the system and their microscopic effects to macroscopic phenomena. As stated in Chapter 1, the specific goals (SGs) that must be met in order to attain the overall objective are:

SG1. To describe in detail the microstructure of polyanhydride copolymers and the effects of this microstructure on drug/antigen distribution and release from microphase-separated polyanhydrides.

SG2. To design TT-loaded polyanhydride microspheres and perform in vitro and in vivo studies to discern antigen release profiles and antibody production in order to maximize protective immunity.

SG3. To formulate and solve a mathematical model to predict and tailor copolymer microstructure effects on drug/antigen release mechanisms.

SG1 requires accurate characterization of the polymer system from both kinetic and thermodynamic standpoints. This includes a prediction of the phase behavior of binary homopolymer blends, crystallinity of the homopolymers and copolymers, and a detailed description of the amorphous microphase separation in "block-like" random copolymers.
Data obtained from *in vitro* release kinetics of both small molecular weight compounds and proteins will provide valuable information about the erosion and release kinetics of different compositions of the polymer system and different polymer/drug systems.

SG2 requires the development of efficient microsphere fabrication techniques. Characterization of the microspheres will include drug loading, loading efficiency and size distributions in addition to *in vitro* release kinetics and *in vivo* responses to encapsulated drugs. Tetanus toxoid (TT) will be used as the model antigen. Antibody production will be monitored and the system will be optimized to maximize protective immunity in an animal model.

The data obtained from these studies will provide the necessary foundation for the development of a mathematical model to predict and tailor drug and antigen release profiles (SG3). The model will be developed in two stages. The first will be a phenomenological model with several simplifying assumptions of the microscopic structure of the polymer system. The model solution and fits to experimental data will help identify aspects of the system to which the release kinetics are sensitive and hence, which microscopic aspects of the system require more detailed description. In the second stage, further experiments will be designed to address these specific questions.

### 3.2 Dissertation Organization

The following seven chapters document the progress toward completion of each of these three goals. SG1 is met by the work described in Chapters 4, 5, and 6. Chapter 4 details the phase behavior of the poly(CPH)/poly(SA) blend system as investigated by small-angle X-ray scattering (SAXS), atomic force microscopy (AFM), optical microscopy, and molecular simulation. The phase behavior of the blend system is interpreted via the segment-
segment interaction parameter. This parameter can be used to predict the phase behavior and microstructure of the copolymers. Chapter 5 presents the crystallization kinetics of the copolymers determined by SAXS. The details of the copolymer crystallinity will be incorporated into the release kinetics models presented in subsequent chapters. A detailed understanding of the crystallization kinetics also provides insights on the effect of processing conditions on copolymer microstructure. In Chapter 6 the microphase separation of the copolymers is studied by solid-state NMR and SAXS. These results are briefly compared to theoretical descriptions of the polymer chain conformation based on parameters determined from the molecular simulations reported in Chapter 4. The details of this microstructure will be incorporated into the release kinetics models.

Chapters 7 and 8 describe the work done in pursuit of SG2. Chapter 7 details the fabrication and characterization of drug loaded polyanhydride microspheres. *In vitro* release kinetics and tailored release profiles are discussed. Chapter 8 describes the fabrication and characterization of TT-loaded microspheres and their *in vitro* release kinetics. The results of *in vivo* antibody production studies are also reported.

Chapters 9 and 10 contain the two erosion and drug release kinetics models developed for SG3. Chapter 9 presents the first generation of release kinetics models based on the experimental work presented in the previous chapters. This model makes several simplifying assumptions, strategically chosen to reveal the sensitivity of the model to the parameters of the system. In this way, several key characteristics of the system are identified which require further experimental work in order to improve the model. The second generation of this model is presented in Chapter 10. Improvements to the model include a detailed description of the copolymer microstructure and the explicit consideration of several additional phenomena that were previously neglected.
Chapter 11 offers some conclusions based on this research and discusses the future application of polyanhydrides to controlled release formulations.

Figure 3.1 shows how all of the research endeavors discussed in the following seven chapters contribute to the completion of the specific goals of this research.

**Figure 3.1.** Relationship of research activities discussed in Chapters 4 – 10 to specific goals.
CHAPTER 4
UNDERSTANDING POLYANHYDRIDE BLEND PHASE BEHAVIOR USING SCATTERING, MICROSCOPY, AND MOLECULAR SIMULATIONS

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4.1 Abstract

The phase behavior of a biocompatible binary polyanhydride blend system composed of poly[1,6-bis(p-carboxyphenoxy)hexane] (poly(CPH)) and poly(sebacic acid) (poly(SA)) is described. The phase behavior is determined from the CPH-SA segmental interaction parameter, χ, obtained from in situ small angle X-ray scattering (SAXS) experiments. The predicted phase diagram has an upper critical solution temperature (UCST) with a critical point of 114 °C. The phase diagram is validated by optical microscopy (cloud point determination) of blend films. However, the full range of blend compositions is not accessible via cloud point measurements, because the melting point of poly(CPH) is above the critical point. Additionally, the poly(CPH) crystallinity interferes with cloud point determination because the length scale of the amorphous phase separation and that of the crystallinity are both near the limit of resolution of the optical microscope. The poly(CPH)-

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rich region of the phase diagram was investigated by \textit{ex situ} atomic force microscopy on thin blend films. Finally, in order to validate the use of molecular simulations to study energetic and structural properties of this system, $\chi$ is also computed from molecular dynamics both above and below the critical point. Excellent agreement is obtained for all three experimental methods and the computational technique. The results are compared to a simple group contribution method for computing the solubility parameters of the polymers. This technique fails to accurately predict the phase diagram.

**Keywords:** Polyanhydrides, polymer blend phase behavior, SAXS, molecular dynamics

### 4.2 Introduction

The use of multicomponent polymeric materials for drug delivery offers the potential to provide tailored drug release profiles \cite{1-4}, drug stabilization \cite{5}, and drug targeting \cite{6}. However, multicomponent polymer systems often exhibit phase separation, and many of the properties of interest are governed by the phase behavior of the material. Though phase separation may be exploited to achieve desired characteristics in a particular system, if poorly understood, it may also have effects on the ability of a device to control the drug release profile \cite{7, 8}. Thus, design of drug delivery devices based on multicomponent polymer systems requires a detailed understanding of the phase behavior of the system of interest.

Biodegradable polymers have found widespread acceptance as carriers for therapeutic compounds. In particular, many researchers have focused on poly(ester)s such as poly(lactide-co-glycolide) and poly(ether)s such as poly(ethylene oxide) as biomaterials for drug delivery, due to their biocompatibility. These systems are hydrophilic and release encapsulated drugs via bulk erosion. We are interested in drug release from hydrophobic systems. Hydrophobic biodegradable systems exhibit surface-erosion, which may stabilize
macromolecular drugs [9, 10]. They also have release profiles governed by the erosion kinetics, rather than diffusion and swelling, as is the case for hydrophilic, bulk-eroding systems. Polyanhydrides are ideal because the chemistry of the monomer units can be made hydrophobic, while the polyanhydride bond in the backbone remains hydrolytically labile. Polyanhydrides have been shown to have good biocompatibility. (See the recent reviews by Katti et al. [11] and Narasimhan and Kipper [12].)

We are interested in polyanhydrides composed of the monomers 1,6-bis(p-carboxyphenoxy)hexane (CPH) and sebacic acid (SA) (Figure 4.1). These two materials erode at vastly different rates offering the opportunity to tailor release profiles by altering the composition [4, 8, 13].

![Chemical structures of poly(CPH) (top) and poly(SA) (bottom).](image)

**Figure 4.1.** Chemical structures of poly(CPH) (top) and poly(SA) (bottom).

We have previously characterized the crystallinity of poly(CPH:SA) copolymers [13, 14], the effects of drug loading on crystallinity [13], and the drug release kinetics of both microspheres [4, 15] and tablet [8] devices. Others have studied the release kinetics of similar polyanhydride systems, without providing details of the phase behavior [1, 16]. Our
previous work [8, 17] has revealed that release from the individual phases determines drug release profiles from surface-erodible, two-component systems when the two phases have different release kinetics. In surface-erodible multicomponent systems, drug release may either lead or lag the overall polymer erosion rate. The drug release kinetics is only explained by considering the release kinetics from the individual phases. In phase-separated systems, the drug release kinetics is governed by the phase behavior of the polymer system, the relative erosion rates of the constituent phases, the partition coefficient of the drug in the two phases, and the composition (relative amounts of the two phases) [17]. Thus, a comprehensive description of the release mechanisms requires intimate knowledge of the phase behavior.

The miscibility of several polyanhydride systems has been qualitatively assessed experimentally by Domb [18], who investigated a variety of polyanhydride blends both in solution and melt. The miscibility of polyanhydrides with polyesters and polyethers has been studied by Shakesheff et al. [19], Chen et al. [20], and Chan and Chu [21]. However, the phase behavior of binary polyanhydride systems has not yet been comprehensively investigated. Our previous work has shown preliminary evidence that microphase separation exists in random copolymers of CPH and SA, and that this microphase separation affects the release profiles of dissolved drugs when the drugs partition in the two-phase system [8, 17].

In the present study, we determine the phase diagram of the poly(CPH)/poly(SA) blend system. The phase diagram is predicted from the CPH-SA segmental interaction parameter, $\chi$, determined from small-angle X-ray scattering (SAXS). The phase diagram is verified by optical microscopy and atomic force microscopy (AFM). The experimental work is complemented by the prediction of the poly(CPH)/poly(SA) blend phase behavior from molecular dynamics. Molecular dynamics also allows the prediction of the interaction
parameter in the phase-separated region, which SAXS cannot do. This prediction is compared to the SAXS data, in order to validate the molecular dynamics technique. Knowledge of the phase behavior of the blend system will be extended to copolymer systems (both random and block) in future work as we design new polyanhydride copolymers with precisely tailored architectures for drug delivery.

4.3 Theoretical Background

4.3.1 Polymer miscibility

The Flory-Huggins theory for predicting the miscibility of polymers A and B calculates the Gibbs' free energy of mixing, $\Delta G_{\text{mix}}$, as [22]

$$\frac{\Delta G_{\text{mix}}}{RT} = \frac{\phi_A}{N_A} \ln \phi_A + \frac{\phi_B}{N_B} \ln \phi_B + \chi_{\text{FH}} \phi_A \phi_B$$  \hspace{1cm} (4.1)

In equation 4.1 the $\phi$'s are the volume fractions of polymer A and B and the N's are their degrees of polymerization. The Flory-Huggins interaction parameter, $\chi_{\text{FH}}$, represents the enthalpic component of the free energy of mixing. Note that volume change on mixing is neglected and the entropic terms only include combinatorial entropy. Because the entropic components are small and always favor mixing, $\chi_{\text{FH}}$ is a very important parameter.

In the original Flory-Huggins theory, $\chi_{\text{FH}}$ is predicted by [22]

$$\chi_{\text{FH}} = Z V_{\text{seg}} \frac{\Delta E_{\text{mix}}}{RT}$$ \hspace{1cm} (4.2)

In equation 4.2, $\Delta E_{\text{mix}}$ is the energy of mixing per pair of monomers, scaled by the unit volume, $Z$ represents the lattice coordination number and $V_{\text{seg}}$ is the volume of a mole of
lattice sites. For off-lattice fluids, the coordination number loses some physical significance and becomes an adjustable parameter [23]. Methods to predict $Z$ have been based on Monte Carlo packing algorithms that assume $Z$ as the number of nearest-neighbor segments for each segment [24, 25]. Use of equation 4.2 requires a prediction of the energy of mixing and assumes that the interaction parameter scales with reciprocal temperature.

Alternative temperature functionality can be introduced by modifying the equation for $\chi_{FH}$ resulting in a generalized interaction parameter, $\chi$, such as

$$\chi = A + \frac{B}{T} \quad (4.3)$$

$A$ and $B$ are empirical constants for a particular system but can be related to non-combinatorial entropic effects ($A$) and enthalpic interactions ($B$). Computational techniques for predicting $\chi$ rely on predictions of the energy of mixing.

The solubility parameter approach predicts miscibility based on the relative values of the Hildebrand solubility parameter, $\delta$ [26]. The solubility parameter is the square root of the cohesive energy density

$$\delta = \left( \frac{E_{coh}}{V} \right)^{1/2} \quad (4.4)$$

$E_{coh}$ is the cohesive energy, which is defined as the increase in the potential energy of a system when all intermolecular interactions are turned off. $\delta$ can either be obtained experimentally or it can be predicted. Predictions usually rely on group contribution techniques (such as those reviewed in [27]) and experimental techniques involve comparing the solubility of the polymer of interest in solvents with known solubility parameters. The
former technique may require a database of known compounds and does not account for the particular interactions between specific moieties in the materials of interest. The latter follows the line of reasoning that if A dissolves B and A dissolves C then B dissolves C. An alternative computational technique for obtaining $E_{\text{coh}}$ is described below in the section on the Amorphous Cell algorithm.

Hildebrand and Scott [26] predict $\chi_{FH}$ as

$$\chi_{FH} = V \frac{(\delta_A - \delta_B)^2}{RT}$$

(4.5)

The solubility parameter method can be used as a fast and simple screening method, but may provide misleading results when specific interactions between moieties in the two polymers (such as hydrogen bonds) that are not present in either of the homopolymers affect the solubility [28]. Another limitation is that since the interaction parameter computed from equation 4.5 is always positive, decreasing with temperature, and independent of composition, only UCST behavior can be predicted by this technique.

4.3.2 Small-angle X-ray scattering (SAXS)

SAXS experiments designed to probe the microstructure of amorphous polymers can be used to determine the interaction parameter. The intensity of scattered radiation, $I$, as a function of the scattering vector, $q$ ($q = 4 \pi \sin (\theta/2)/\lambda$, $\theta$ = scattering angle and $\lambda$ is the wavelength of the incident radiation), is related to the structure factor, $S(q)$, which represents the root-mean-square electron concentration fluctuation by

$$\frac{1}{S(q)} = (\Delta \eta)^2 \sigma_s V \frac{1}{I(q)}$$

(4.6)
Here $\Delta \eta$ is the difference in electron density between the two components, $V$ is the volume of a monomer (chosen as an SA monomer in our study), and $\sigma_e$ is the scattering cross section of an electron ($6.653 \times 10^{-9}$ Å$^2$). The electron densities are computed from the known mass densities of the polymers. (For the poly(CPH)/poly(SA) pair, $\Delta \eta = 0.0315, 0.0322, 0.0331$ and $0.0341$ e$^-$/Å$^3$ at 140, 150, 160, and 170 °C respectively.) The structure factor is then related to the interaction parameter by de Gennes’ random phase approximation [29].

$$S(q)^{-1} = \chi_s f_D^{-1}(q^2, R^2_\phi) - 2\chi$$ (4.7)

Here $f_D$ is the Debye structure factor and $R^2_\phi$ is the mean square radius of gyration. $\chi_s$ is the value of the interaction parameter at spinodal conditions. The parameters can be computed as [30, 31]

$$\lim_{q \to 0} f_D(q^2, R^2_\phi) \approx N \left( 1 - \frac{1}{3} q^2 R^2_\phi \right)$$ (4.8)

$$R^2_\phi = (1 - \phi) R^2_A + \phi R^2_B$$ (4.9)

$$\chi_s = 0.5 \left( \frac{1}{\sqrt{N_A}} + \frac{1}{\sqrt{N_B}} \right)^2$$ (4.10)

Here $N_A$ is the degree of polymerization of component A based on the monomer volume, $V$, in equation 4.7. (Because the volume of a CPH monomer is about twice that of the SA monomer, the monomer volume is taken as the volume of an SA monomer. Since the CPH monomer unit is symmetric, the degree of polymerization is computed assuming that
monomer is half the CPH monomer shown in Figure 4.1 – essentially resulting in a head-to-head polymer). In equation 4.9, \( \phi \) is the volume fraction of component A, and \( R_A^2 \) is the mean square radius of gyration of molecules in component A. Substituting equation 4.8 into equation 4.7, one obtains (for \( \phi = 0.5 \))

\[
\lim_{q \to 0} S(q)^{-1} = 2\left[\chi_s - \chi(T)\right] + \frac{2\chi_s R_A^2}{3}q^2
\]

(4.11)

Linear extrapolation of the Zimm plot (\( S^{-1}(q \to 0) \) vs. \( q^2 \)) gives the interaction parameter (from the intercept) and the radius of gyration (from the slope in a \( q \) region where \( qR_\phi < 1.3 \)). The approximation in equation 4.8 is valid in Guinier range (\( I(q) \sim \exp(-Cq^2) \)) that can be identified by a linear region in a Guinier plot (\( \ln[I(q)] \) vs. \( q^2 \)) where \( qR_\phi < 1.3 \) [32].

4.3.3 Molecular dynamics (MD)

Case and Honeycutt review several computational techniques for studying the phase behavior of polymer systems [23]. Here we use the Amorphous Cell algorithm available in the software package Materials Studio® from Accelrys Inc., which employs MD calculations, to predict the phase diagram of the poly(CPH)/poly(SA) blend system. This algorithm has been shown to be reliable for predicting bulk properties of polymeric systems [23, 33-35]. We compare the MD predictions to a simple solubility parameter prediction technique. Our overall goal is to lay the foundation for studies aimed at characterizing blends of CPH-SA copolymers and drug solubilities and release kinetics from this polymer system.

The Amorphous Cell algorithm computes cohesive energy densities from MD simulations on models of bulk amorphous polymer systems [36]. Periodic boundary
conditions are used to eliminate edge effects. As with any molecular simulation, it is imperative to begin with a reasonable starting configuration, since the CPU time required to simulate even very small times (e.g. nanoseconds) becomes prohibitively large for polymers [23]. Simulations are typically conducted over times on the order of picoseconds to a few nanoseconds on structures that are already assumed to be near equilibrium. Thus, the phenomena of mixing and de-mixing cannot be directly observed [36]. Rather, the energies of mixed and de-mixed configurations are compared to discern which is the more favorable.

Theodorou and Suter [36] described an algorithm for the construction of amorphous cells based on a modified form of Flory’s rotational isomeric states (RIS) theory [37]. Since then, some of their assumptions have been relaxed and the present model is fully atomistic, with no ‘united-atom’ groups. Bond stretching, angle bending, and out-of-plane bending are all allowed in addition to dihedral angle rotation. The temperature in the current model is explicit, rather than simply being implied by the density. And, the newer condensed-phase optimized molecular potentials for atomistic simulation studies (COMPASS) force field [38] is used, rather than the consistent valence force field (CVFF). The amorphous cells are equilibrated and evolved using molecular dynamics. The energy of mixing per unit volume is computed from the cohesive energy densities of the two homopolymers and the blend via

\[
\Delta \tilde{E}_{\text{mix}} = \phi_A \left( \frac{E_{\text{coh}}}{V} \right)_A + (1 - \phi_A) \left( \frac{E_{\text{coh}}}{V} \right)_B - \left( \frac{E_{\text{coh}}}{V} \right)_{\text{blend}}
\]  

(4.12)

\(\chi\) can then be computed from

\[
\chi_{FH} = V_{\text{seg}} \frac{\Delta \tilde{E}_{\text{mix}}}{RT}
\]  

(4.13)
The coordination number $Z$ has been dropped (c.f. equation 4.2) because in this algorithm $\Delta \bar{E}_{\text{mix}}$ is a bulk (rather than pairwise) energy of mixing per unit volume.

The advantage of this technique is that direct simulation of the bulk state can be obtained, with careful system construction. Therefore, any non-combinatorial entropic effects can be implicitly included in the enthalpic calculation. For example, if one is interested in volume changes on mixing, MD can be performed in the NPT ensemble, allowing the density to change. Additionally, enthalpic effects that may be unique to the blend system (i.e. interactions between pairs of atoms or groups that do not occur in the homopolymer systems) are accounted for. These are neglected in the solubility parameter approach.

4.4 Experimental

4.4.1 Materials

Sebacic acid (99%), N-methyl-2-pyrolidinone, and $p$-carboxy benzoic acid (99+%) were purchased from Aldrich (Milwaukee, WI). 1,6-dibromohexane (98%) was purchased from Acros (Fairlawn, NJ). Acetic anhydride, chloroform, and methylene chloride were purchased from Fisher (Fairlawn, NJ) and deuterated chloroform was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Petroleum ether (hexanes, 55% n-hexane) was purchased from Fisher and dried and distilled over sodium and benzophenone (Fisher) before use.

4.4.2 Polymer synthesis

The homopolymers, poly(SA) and poly(CPH), were synthesized as previously reported [4]. Briefly CPH diacid was synthesized by a method similar to that described by
Conix [39] for 1,3-bis(p-carboxyphenoxy) propane and purified by recrystallization from N-methyl-2-pyrolidinone three times. CPH and SA diacids were acetylated to form the prepolymers by refluxing in excess acetic anhydride for 30 minutes (SA) or 60 minutes (CPH) under dry nitrogen sweep. Purification of the crude prepolymers was done using the methods previously reported [4].

Melt polycondensation of the prepolymers was performed at 180 °C under vacuum (<0.5 mmHg) for 90 minutes. About 2 ml of acetic anhydride was added to 4 g of prepolymer prior to polymerization to ensure complete acetylation. The polymer was isolated by dissolution in methylene chloride and precipitation in dry hexane, followed by filtration and drying under vacuum. The polymers were desiccated under dry argon to prevent degradation. Blends were formed by co-dissolution in chloroform or methylene chloride, and evaporation at room temperature with gentle agitation when dry samples were required.

4.4.3 Polymer characterization

Polymers were characterized by $^1$H NMR in deuterated chloroform on a Varian VXR 300 MHz spectrometer (Varian Inc. Palo Alto, CA). Molecular weight was assessed via gel permeation chromatography (GPC). GPC samples were dissolved in HPLC-grade chloroform and separation was done using PL Gel columns from Polymer Laboratories (Amherst, MA) on a Waters GPC system (Milford, MA). 50 μl samples were eluted at 1 ml/min. Elution times were compared to poly(methyl methacrylate) standards from Fluka (Milwaukee, WI). Differential scanning calorimetry on a DSC-7 (Perkin Elmer, Shelton, CT) was used to characterize the thermal transitions of the polymers. Samples were heated at 5 °C/min and the data were taken on the second heating cycle. The molecular properties are
listed in Table 4.1. The polycondensation synthesis typically results in high polydispersity index as noted in Table 4.1.

Table 4.1. Molecular properties of polymers used in this study.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$</th>
<th>PDI</th>
<th>$T_g$ (°C)</th>
<th>$T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CPH)</td>
<td>14000</td>
<td>2.5</td>
<td>47</td>
<td>143</td>
</tr>
<tr>
<td>Poly(SA)</td>
<td>10000</td>
<td>3.5</td>
<td>60</td>
<td>82</td>
</tr>
</tbody>
</table>

4.4.4 SAXS Experiments

Samples for SAXS were prepared by melting the polymer into custom aluminum sample holders with a thickness of 35 μm. These make ideal sample holders because the aluminum is fairly transparent to 12 keV X-rays used in these experiments, and the rigid pans maintain the sample thickness when the polymer is melted in the vertically mounted SAXS sample chamber. The sample thickness was 2.2 mm. SAXS measurements were carried out on the instrument at 12-ID beam line at the Advanced Photon Source [40]. A 15 cm × 15 cm CCD detector was used to measure the intensity of scattering and the direct beam intensity was measured using a photodiode. The sample chamber was equipped with a custom furnace for heating the samples without interfering with the beam, permitting in situ data collection. A 50:50 (v/v) blend sample was used and data were collected at 140, 150, 160 and 170 °C. We know from prior experiments that the blend would be phase separated at room temperature [14]. And, because we perform copolymerization under vacuum at 180 °C, yielding random copolymers [8], we anticipate that the blend would become miscible as the temperature approaches 180 °C.
Five data sets were collected at each temperature with exposure times of 0.5 seconds at an incident beam energy of 12 keV (\(\lambda = 1.035 \, \text{Å}\)). The distance between the detector and the sample was 4 m. The scattering data were appropriately corrected and azimuthally averaged to obtain \(I'(q)\).

The one-dimensional data were averaged for the five runs to obtain a single one-dimensional data set for each condition. These data were then corrected by subtracting the scattering due to a blank aluminum holder and normalized to an absolute scale with a polyethylene standard according to

\[
I(q) = I'(q) \times \frac{25.4 \, \text{cm}^{-1}}{I'_{\text{PE}}(q = 0.0245)} \times \frac{d_{\text{PE}}}{d_{\text{sample}}} \tag{4.14}
\]

Here \(I(q)\) represents the normalized intensity on an absolute scale, \(I'_{\text{PE}}\) is the intensity of the polyethylene standard measured at identical configuration, and \(d_{\text{PE}}\) and \(d_{\text{sample}}\) are respectively, the thicknesses of the PE standard (0.078 cm) and the sample, measured with a micrometer. The PE standard produces a peak at \(q = 0.0245 \, \text{Å}^{-1}\) whose absolute intensity is 25.4 cm\(^{-1}\).

4.4.5 AFM experiments

Silicon wafers (approximately 1.5 cm x 1.5 cm) were cleaned by a modified version of the RCA clean procedure [41]. Briefly, wafers were soaked for ten minutes in an organic clean solution (70:15:15 deionized water: ammonium hydroxide: hydrogen peroxide), rinsed, and then soaked for ten minutes in an ionic clean solution (70:15:15 deionized water: hydrochloric acid: hydrogen peroxide). Both solutions were at 70 – 80 °C. No etch solution was used. Wafers were then cleaned using a CO\(_2\) Snow Jet (Applied Surface Technologies,
New Providence, NJ). A 20 nm layer of gold was deposited on the wafers by sputter coating. Films were spun cast at 1500 rpm for 30 sec using a spin coater (Headway Research Inc., Garland, TX) from a 1% (w/v) solution of polymer in HPLC-grade chloroform, filtered through 0.2 μm PVDF membrane syringe filters (Pall Gelman, Portsmouth, UK) onto the gold-coated silicon wafers. This procedure results in films of uniform thickness of 200 nm. Films were dried at room temperature and atmospheric pressure for one hour. Films were annealed at temperatures of interest for up to 12 hours under vacuum (< $10^{-4}$ torr) in a custom built annealing oven equipped with a hot stage, turbo molecular pump, roughing pump, and vacuum gauge (MKS Instruments, Boulder, CO). Annealed films were quenched on dry ice under dry argon to below the glass transition temperature, in order to “freeze” the phase morphology prior to AFM experiments.

AFM images were obtained on a Dimension 3000 Scanning Probe Microscope (Digital Instruments, Santa Barbara, CA). The AFM was operated in contact mode using an Ultrasharp silicon cantilever (Mikromasch, Tallinn, Estonia) with a force constant of 0.30 N/m. All the films were about 200 nm thick, as measured by scratching the surface of the film and performing a line profile measurement with the AFM. Root-mean-square roughnesses were computed from AFM measurements as well.

4.4.6 Optical microscopy

Round glass coverslips from Fryer Company Inc. (Bloomington, MN) were cleaned by treatment in acetone, methanol, and chloroform at room temperature. Samples for optical microscopy were spun cast (500 rpm, 30 sec) onto the coverslips from 10% (w/v) solutions of polymer blends in HPLC-grade chloroform filtered with 0.2 μm PVDF membrane syringe filters. The resulting films were approximately 50 to 100 μm thick. Samples were dried in
air and observed under a Nikon Eclipse ME600L microscope (Fryer) in reflected light mode using a 100x long-working-distance objective. The microscope was equipped with a CCD camera (Hitachi Kokusai Electric Inc., Tokyo, Japan) and an A-200 heating stage (Fryer). The long working distance objective is necessary to protect the optics from the heating stage.

4.4.7 Molecular dynamics simulations

The MD simulations were conducted on a Dell Optiplex™ PC with an Intel® Pentium® 4 (3.06 GHz) processor and 1024 GB of RAM. The model systems were constructed using the Amorphous Cell module of the Materials Studio® 2.1 software package (Accelrys Inc., San Diego, CA).

4.5 Results and Discussion

4.5.1 Phase diagram from SAXS

The SAXS data for the melts had significant contribution from voids especially in the low q region. The fast decay of the scattering from the voids becomes insignificant at q > 0.01 Å⁻¹. This parasitic scattering from the voids, Iₚ, behaves as

\[ I_p \sim \frac{C}{q^4} \]  \hspace{1cm} (4.15)

and must be subtracted. Provided that the voids have radii larger than the radii of gyration of the polymer chains, the constant, C, can be determined from the intercept of a plot of Iq⁴ vs q⁴ [42]. The region over which this correction is fit was chosen as the region below the Debye region from the Kratky-Porod plot [30]. The corrected scattering data are shown in Figure 4.2, and the Zimm plots of the same data at four temperatures are shown in Figure 4.3.
Interaction parameters were obtained from the intercepts of the Zimm plots following equation 4.11. The values of the interaction parameter are plotted in Figure 4.4, from which the temperature dependence of $\chi$ can be extracted as:

$$\chi = -2.04 + \frac{802}{T} \quad (4.16)$$

![Figure 4.2. Corrected SAXS data for poly(CPH)/poly(SA) blend at $T = 140 \, ^\circ C (\times), 150 \, ^\circ C (\bigcirc), 160 \, ^\circ C (\triangle), \text{and} 170 \, ^\circ C (\square)$.]

Because $\chi$ decreases with temperature, the system becomes less miscible at lower temperatures, and an upper critical solution temperature (UCST) is predicted. This is consistent with the observations noted earlier. The critical temperature ($\chi = \chi_s$) for the particular molecular weights studied here is 114 °C (from equations 4.10 and 4.16). The mean square radius of gyration, $R_g^2$, is determined from the slope in the Zimm plot by equation 4.11. The average value of $R_g$ is 107 Å. The relatively high value of the $R_g$ results from the high polydispersity of these polymers (see Table 4.1) as the $R_g$ from scattering corresponds to the z-average radii of gyration of the polymers [43].
Equation 4.16 can now be used in conjunction with the Flory-Huggins formulation of \( \Delta G_{\text{mix}} \) (equation 4.1) to predict the phase diagram. The spinodal curve (boundary between metastable and unstable regions) is defined by the locus of inflections in \( \Delta G_{\text{mix}}/RT \) vs. \( \phi \)
plotted on the T-φ plane [31]. And, the binodal curve (boundary between metastable and stable regions) is the locus of points of common tangency (equal chemical potential) in ΔG_{mix}/RT vs. φ plotted on the T-φ plane [31]. The phase diagram obtained is shown in Figure 4.5.

![Figure 4.5](image.png)

**Figure 4.5.** Phase diagram for poly(CPH)/poly(SA) blend system obtained from SAXS, and cloud point data from optical microscopy (□). The spinodal curve is indicated by the gray line, and the binodal curve is indicated by the black line.

### 4.5.2 Cloud point curve from optical microscopy

In order to verify the phase diagram obtained from the SAXS experiments, we performed *in situ* optical microscopy of blend films on a heating stage and recorded the cloud points. Below the critical point of the blend, phase separation is apparent, as concentration fluctuations in the film make the film appear cloudy. Upon heating through the first-order phase transition, the film homogenizes and becomes transparent. The cloud points for the poly(CPH)/poly(SA) blend system are plotted in Figure 4.5 along with a prediction of the binodal and spinodal curves predicted for the blends used to make the microscopy samples.
The cloud point curve closely matches the predicted binodal curve. The error bars on the cloud point data indicate the 5 °C confidence with which the temperature of the film is controlled and the cloud points can be accurately observed.

Cloud points can only be obtained in regions of the phase diagram where inhomogeneities due to crystallinity are not observed. (Both poly(SA) and poly(CPH) are semicrystalline, as stated before.) For compositions rich in poly(SA) crystallinity does not interfere, as poly(SA) melts at 82 °C. Unfortunately, poly(CPH) has a melting point above the critical point of the blend and a glass transition temperature of only 47 °C. (See Table 4.1.) So attempts to anneal blend films to rid them of poly(CPH) crystals are thwarted by crystallization during the cloud point observation. A further complication is that the length scale of the phase separation is very near the limits of the resolution of the microscope, so inhomogeneities due to amorphous phase separation cannot be discerned from those due to crystallinity. As the poly(CPH) content was increased, the apparent cloud point approached the melting temperature of poly(CPH) (143 °C), indicating that what was observed was actually the transition in the crystalline phase. Therefore, we were unable to accurately discern the cloud point for blend samples rich in poly(CPH) from in situ optical microscopy.

4.5.3 AFM for validating the CPH-rich region of the phase diagram

Observation of cloud points in the poly(CPH)-rich region of the phase diagram requires microscopic methods with higher resolution than that provided by optical microscopy, so that crystallinity can be discerned from amorphous phase separation. To accomplish this, ex situ atomic force microscopy (AFM) experiments were performed. It was not our goal with the AFM experiments to actually find the cloud point, as this would be very inefficient without the ability to perform AFM in situ. Rather we performed the AFM
experiments in order to: A) determine an approximate length scale for the phase separation to verify that the cloud points observed in optical microscopy are real; and B) determine whether the predicted phase diagram accurately described the phase behavior in the poly(CPH)-rich region. Table 4.2 shows the compositions and temperatures studied. A temperature of 180 °C was chosen because this is the temperature at which we perform melt polycondensation to make copolymers. The 47:53 and 78:22 compositions were chosen because these are compositions from which we make copolymers.

The AFM scans obtained are shown in Figures 4.6, 4.7, and 4.8. We observe that for thin films, the length scale of the phase separation is on the order of 1 μm (Figures 4.6 and Figure 4.7), which is about the limit of resolution of the optical microscope. Figure 4.6 shows clear evidence of phase separation in the 47:53 and 78:22 blends at room temperature. The phase separation persists in the films annealed at 90 °C (Figure 4.7). Figure 4.8 shows the 47:53 and 78:22 blend films annealed at 180 °C. The root-mean-square roughnesses obtained from AFM are summarized in Table 4.2. It is instructive to note that the surface roughnesses for the phase-separated films are an order of magnitude higher than those for the homogenous films. The phase behavior observed in the AFM scans is consistent with the phase diagram shown in Figure 4.5.

Table 4.2. RMS roughness of blend films obtained from AFM experiments. * indicates phase-separated systems.

<table>
<thead>
<tr>
<th>Blend Composition</th>
<th>RMS Roughness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room Temperature</td>
</tr>
<tr>
<td>47:53 CPH:SA</td>
<td>39.2*</td>
</tr>
<tr>
<td>78:22 CPH:SA</td>
<td>73.5*</td>
</tr>
</tbody>
</table>
Figure 4.6. AFM scans of as-cast poly(CPH)/poly(SA) blend films. (A) 5 μm x 5 μm scan of 47:53; (B) 10 μm x 10 μm scan of 78:22. RMS roughness is shown in Table 4.2.
Figure 4.7. AFM scans of poly(CPH)/poly(SA) blend films after annealing at 90°C and < 10^{-4} torr. (A) 5 μm x 5 μm scan of 47:53; and (B) 20 μm x 20 μm scan of 78:22. RMS roughness is shown in Table 4.2.
Figure 4.8. AFM scans of poly(CPH)/poly(SA) blend films after annealing at 180 °C and <10^4 torr. (A) 5 µm x 5 µm scan of 47:53 and (B) 5 µm x 5 µm scan of 78:22. RMS roughness is shown in Table 4.2.
4.5.4 MD simulations to predict energetic and structural parameters

We conducted molecular dynamics simulations using the Amorphous Cell® algorithm described earlier to predict $E_{\text{coh}}$ for each of the homopolymers and the 51:49 poly(CPH)/poly(SA) blend. Systems were constructed in cubic simulation boxes with periodic boundary conditions, using a modification of Flory’s rotational isomeric states (RIS) theory, employing the Meirovich scanning method [44]. This method looks ahead six bonds, while considering a maximum of 128 configurations when constructing the amorphous cells. The boxes were constructed according to the parameters in Table 4.3, containing CPH tetramers (80 backbone bonds) and/or SA heptamers (81 backbone bonds). The acid end groups of each chain were acetylated to eliminate the otherwise unrealistic concentration of acidic protons. For each set of conditions, five simulation boxes were constructed at a density of 0.6 g/cm$^3$. The raw structures were equilibrated to the target density via subsequent NPT molecular dynamics. Special care was taken to ensure that speared and catenated phenyl rings were eliminated. Where necessary, configurations were manually repaired followed by short NVT molecular mechanics runs (1000 steps) to equilibrate the structures.

Experimental densities for polyanhydrides are not reported in the literature, though Thomas et al. [3] estimate a density of 1.1 g/cm$^3$. We predicted the densities listed in Table 4.3 via NPT molecular dynamics simulations using the Andersen thermostat and Andersen barostat [45]. Van der Waals and Coulombic interactions were both summed by the Ewald method. (See description in [46] and references therein.) Runs of 100 ps (100,000 time steps of 1 fs) were required to equilibrate the density.
Table 4.3. Parameters used to define simulation boxes for NVT MD simulations.

<table>
<thead>
<tr>
<th>Composition</th>
<th># of atoms</th>
<th>Temperature (K)</th>
<th>Density (g/cm$^3$)</th>
<th>Box length (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CPH)</td>
<td>1544</td>
<td>363</td>
<td>1.1192</td>
<td>25.90</td>
</tr>
<tr>
<td>(8 CPH tetramers)</td>
<td></td>
<td>453</td>
<td>1.0756</td>
<td>26.24</td>
</tr>
<tr>
<td>51:49 Blend</td>
<td>1636</td>
<td>363</td>
<td>1.0656</td>
<td>26.11</td>
</tr>
<tr>
<td>(4 CPH tetramers, 4</td>
<td></td>
<td>453</td>
<td>1.0185</td>
<td>26.50</td>
</tr>
<tr>
<td>SA heptamers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(SA)</td>
<td>1728</td>
<td>363</td>
<td>1.0017</td>
<td>25.20</td>
</tr>
<tr>
<td>(8 SA heptamers)</td>
<td></td>
<td>453</td>
<td>0.9397</td>
<td>26.99</td>
</tr>
</tbody>
</table>

NVT MD simulations were then used to predict cohesive energy densities using the Andersen thermostat [45]. Simulations were run for 400 ps (400,000 time steps of 1 fs). The cell multipole method [47] was used for computing Coulombic interactions. At each temperature, the cohesive energy densities were sampled at 4 ps intervals. Temperature and potential energy were monitored at 1-ps intervals. The cohesive energy density was averaged over each of several starting configurations for each set of conditions. The energies of mixing were computed according to equation 4.12 and the interaction parameter was computed according to equation 4.13. The values for $\chi$ are plotted in Figure 4.9 along with the values obtained from SAXS. These results are consistent, validating extrapolation of SAXS $\chi$ into the immiscible region of the phase diagram, and demonstrating the value of the MD approach to predict energetics of the poly(CPH)/poly(SA) blend system.
Figure 4.9. Comparison of $\chi$ values obtained from SAXS (●) and those computed from molecular dynamics (○).

The molecular simulations permit the calculation of a variety of interesting structural parameters. In particular, characteristic ratios can be estimated ($C_{\infty,\text{CPH}} = 6.8$ and $C_{\infty,\text{SA}} = 4.8$). These parameters will be used in future mesoscale studies, along with the predicted interaction parameters to investigate the phase behavior of both random and block copolymers.

We compare the results from the molecular simulations to solubility parameter predictions based on a group contribution method. The method of Fedors (reproduced in [27]) was used to compute $E_{\text{coh}}$, which was scaled with our predictions of the densities to get $E_{\text{coh}}/V$. The results are reported in Table 4.4 and compared to the solubility parameters of known solvents. The interaction parameter for the poly(CPH)/poly(SA) blend computed from equation 4.5 is 0.125 at 298 K. However, from equation 4.16, the value of $\chi$ is 0.65. The solubility parameters obtained from the group contribution method have no temperature dependence, so the critical temperature can be computed by rearranging equation 4.5 and
Computing the spinodal interaction parameter from equation 4.10. The critical temperature obtained in this way from the solubility parameters is 812 °C. Clearly, the solubility parameter approach is insufficient for accurately predicting the phase behavior of this system.

Table 4.4. Solubility parameter predictions from the group contribution method of Fedors reproduced in [27]. Solubility parameters are reported at 298 K.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_{coh}/V$ ($J/cm^3$)</th>
<th>$\delta$ ($J/cm^3)^{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CPH)</td>
<td>443.6</td>
<td>21.1</td>
</tr>
<tr>
<td>Poly(SA)</td>
<td>390.1</td>
<td>19.8</td>
</tr>
<tr>
<td>Chloroform</td>
<td></td>
<td>19.0</td>
</tr>
<tr>
<td>Methylene Chloride</td>
<td></td>
<td>19.8</td>
</tr>
</tbody>
</table>

aData from [26].

4.6 Conclusions

The phase behavior of the poly(CPH)/poly(SA) blend system was studied by measuring the CPH-SA segmental interaction parameter, $\chi$, using in situ SAXS experiments. The predicted phase diagram is in good agreement with the cloud point measurement of poly(SA)-rich blends using optical microscopy. However, it was impossible to obtain cloud points for poly(CPH)-rich blends by optical microscopy, due to crystallinity, so this region of the predicted phase diagram was validated by AFM. The information from these three complementary techniques provides a complete description of the phase diagram of this system. The use of molecular dynamics to study this system was validated by the prediction
of the cohesive energy density and comparison to the experimental results. Both the experimental methods and the computational techniques were shown to be superior to the solubility parameter approach.

More importantly, the accurate temperature dependence of $\chi$ can now be used to predict the phase behavior of copolymer systems that is of interest for drug delivery applications. Knowledge of the phase behavior will enable the development of accurate drug release models and the rational design of controlled release devices.

4.7 Acknowledgements

We wish to thank the Whitaker Foundation for financial support. We are grateful to Luke Brubaker, an undergraduate research assistant in the Chemical Engineering Department at Iowa State University for help with the polymer synthesis and characterization, and Dr. Hajime Takano for his expertise on AFM. This work benefited from the use of BESSRC-CAT at APS and IPNS, funded by the U.S. DOE, BES under contract W-31-109-ENG-38 to the University of Chicago.

4.7 References


CHAPTER 5
MORPHOLOGY OF POLYANHYDRIDE COPOLYMERS:
TIME-RESOLVED SAXS STUDIES OF CRYSTALLIZATION

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5.1 Abstract

Synchrotron Small Angle X-ray Scattering (SAXS) is used to study the isothermal crystallization kinetics of a family of polyanhydride copolymers, consisting of 1,6-bis(p-carboxyphenoxy)hexane and sebacic acid monomers. *In-situ* SAXS experiments permit the direct observation of crystallization kinetics. Structural parameters (long period, lamellar thickness, and degree of crystallinity) are obtained from Lorentz-corrected intensity profiles, one-dimensional correlation functions, and interface distribution functions in order to obtain a comprehensive picture of the crystal morphology. The combination of these three analyses provides information not only on the lamellar dimensions, but also on the polydispersity (non-uniformity) of these dimensions. Where possible, the crystallization kinetics are interpreted using a modified version of the Avrami equation. The results can be used to

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⁴ Intense Pulsed Neutron Source, Argonne National Laboratory. Assisted with interpretation of SAXS data.
⁵ Major professor, corresponding author.
perform rational design of controlled drug release formulations as crystallinity affects drug release kinetics.

**Keywords:** SAXS, crystallization, biomaterials, biodegradable

### 5.2 Introduction

Polyanhydrides have been extensively studied as potential vehicles for controlled drug delivery\textsuperscript{1-14}. Their hydrophobicity, combined with the very high reactivity of the backbone with respect to hydrolytic degradation leads to surface erosion in most cases\textsuperscript{15,16}. In this process, the overall hydrophobicity of the polymer matrix prevents water from penetrating into the bulk of the polymer. This may be advantageous for the stabilization of macromolecular drugs. Additionally, because diffusion is limited in the bulk polymer, drug release is determined by the erosion profile\textsuperscript{14,17}. However, ample evidence exists to conclude that multiple phases exist in most bulk polyanhydride devices designed for drug delivery\textsuperscript{11,12,18-22}. The semicrystalline nature of many polyanhydride homopolymers has also been reported. (See references in\textsuperscript{23}). Because the erosion rate is strongly affected by the molecular interactions between water and bulk polymer, it is hypothesized that crystalline regions do not erode at the same rate as amorphous regions. The resulting non-homogenous surface erosion has been demonstrated experimentally\textsuperscript{19,20,24}. Additionally, it has been shown that blends and copolymers of polyanhydrides exhibit phase separation\textsuperscript{25} and microphase separation\textsuperscript{11} respectively, which can have profound effects on drug release kinetics, particularly when drugs partition and when the two phases have different erosion rates\textsuperscript{12,17}.

Our research has focused on polymers based on the monomers 1,6-bis(\textit{p}-carboxyphenoxy)hexane (CPH) and sebacic acid (SA) (Figure 5.1) because random
copolymers can be synthesized and these two materials erode at vastly different rates. Further, we have proposed that by exploiting the inherent microstructure of this system we can further tailor release profiles\textsuperscript{17}. Thus we have pursued a detailed description of the microstructure of this system\textsuperscript{11,12,14,17,25,26}. Previously we have characterized the microstructure by wide-angle X-ray diffraction, differential scanning calorimetry, and atomic force microscopy\textsuperscript{11,26}. From these experiments, degrees of crystallinity and lamellar thickness in addition to thermal properties were determined.

\textbf{Figure 5.1.} Poly\text{(CPH)} (top) and Poly\text{(SA)} (bottom).

In the present work we investigate the kinetics of crystallization of the series of copolymers by synchrotron small-angle X-ray scattering (SAXS). This has proven to be a powerful technique for assessing the microstructure of semicrystalline polymers by permitting the acquisition of detailed data on time scales on the order of the crystallization kinetics\textsuperscript{27-31}. 


5.3 Experimental

5.3.1 Polymer synthesis and characterization

Polymers were synthesized as previously reported\textsuperscript{13}, and characterized by differential scanning calorimetry (DSC 7, Perkin Elmer, Shelton, CT), gel permeation chromatography (GPC) (Waters Corp. Milford, MA), and proton nuclear magnetic resonance spectroscopy (\textsuperscript{1}H NMR) in deuterated chloroform (VXR 300, Varian Inc., Palo Alto, CA). The results of the characterization are reported in Table 5.1. DSC samples were heated at 5 °C per minute and transitions were identified on the second heating cycle to confirm the previously obtained values\textsuperscript{23}. GPC samples were dissolved in HPLC-grade chloroform and separation was done using PL Gel columns from Polymer Laboratories (Amherst, MA) on a Waters GPC system. 50 µl samples were eluted at 1 ml/min. Elution times were compared to poly(methyl methacrylate) standards from Fluka (Milwaukee, WI). The \textsuperscript{1}H NMR spectra were interpreted as follows\textsuperscript{32,33}: Poly(SA): aliphatic protons $\delta = 1.32$ (multiplet, H$^8$), $\delta = 1.65$ (multiplet, H$^4$), $\delta = 2.35$ (triplet, H$^2$ to carboxylic acid end group), and $\delta = 2.45$ (triplet, H$^2$ to anhydride bond); acetyl protons $\delta = 2.22$ (singlet, H$^3$ acetylated end). Poly(CPH): aromatic protons $\delta = 6.95$ (multiplet, H$^4$), $\delta = 7.99$ (multiplet, H$^4$ β to acetylated and carboxylic acid ends), and $\delta = 8.08$ (doublet, H$^4$ α to internal anhydride bond); aliphatic protons $\delta = 4.06$ (triplet H$^2$ α to ether oxygen); acetyl protons $\delta = 2.22$ (singlet, H$^3$ acetylated end). Copolymers: CPH aromatic protons $\delta = 7.9 - 8.15$ (multiplet H$^4$) and $\delta = 6.85 - 7.05$ (multiplet H$^4$); CPH aliphatic protons $\delta = 4.06$ (triplet H$^3$ α to ether oxygen); SA aliphatic protons $\delta = 2.35$ (triplet, H$^2$ α to carboxylic acid end group), and $\delta = 2.45$ (triplet, H$^2$ α to anhydride bond); acetyl protons $\delta = 2.22$ (singlet, H$^3$ acetylated end).
### Table 5.1. Characterization of polymers used in this study.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>T&lt;sub&gt;g&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;m&lt;/sub&gt; (°C)</th>
<th>Actual Composition (CPH:SA)</th>
<th>M&lt;sub&gt;n&lt;/sub&gt;</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(SA)</td>
<td>62</td>
<td>81</td>
<td>0:100</td>
<td>10,300</td>
<td>3.5</td>
</tr>
<tr>
<td>20:80 (CPH:SA)</td>
<td>50</td>
<td>67</td>
<td>17:83</td>
<td>9600</td>
<td>2.2</td>
</tr>
<tr>
<td>50:50 (CPH:SA)</td>
<td>-&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50</td>
<td>48:52</td>
<td>6700</td>
<td>2.0</td>
</tr>
<tr>
<td>80:20 (CPH:SA)</td>
<td>33</td>
<td>114</td>
<td>78:22</td>
<td>9700</td>
<td>2.1</td>
</tr>
<tr>
<td>Poly(CPH)</td>
<td>48</td>
<td>143</td>
<td>100:0</td>
<td>14,300</td>
<td>2.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>No T<sub>g</sub> is observed above room temperature.

#### 5.3.2 SAXS experiments

Samples were prepared for SAXS by melting the polymer into DSC aluminum sample pans (Perkin Elmer). These make ideal sample holders because the aluminum is fairly transparent to the high-brilliance X-rays used in this study, and the rigid pans maintain the sample thickness when the polymer is melted in the vertically mounted SAXS sample chamber. The thickness of each sample was accurately determined using a micrometer and the values were between 500 and 1000 μm. The samples were subsequently annealed in a DSC (DSC7, Perkin Elmer) by heating above the melting temperature for five minutes, and then rapidly quenched to below the T<sub>g</sub>. The SAXS experiments were conducted at Beamline 12-ID at the synchrotron beam source at Argonne National Laboratory. A custom heating furnace was used which permits accurate temperature control without interfering with the beam, thus permitting in situ data collection. The incident beam energy was 12 keV (λ = 1.035 Å). A 15 cm × 15 cm CCD detector, at a distance of 2 m from the sample, was used to measure the intensity of scattering and the transmitted beam intensity was measured using
photodiode on the beam stop. The scattering data were appropriately corrected and azimuthally averaged to obtain the one-dimensional intensity data, $I'(q)$, as a function of scattering vector, $q$ ($q = 4\pi \sin(\theta/2)/\lambda$; $\theta$ = scattering angle; $\lambda$ = incident radiation wavelength). The resulting intensity data were corrected for the scattering from the blank aluminum sample pan. Data were collected at five-second, ten-second and 30-second intervals during isothermal crystallization at two temperatures (using separate samples) for each polymer.

### 5.4 SAXS Analysis

Several additional corrections to the raw data must be made prior to extracting the morphological parameters of interest. Noise reduction, particularly in the high-$q$ region, is essential to obtain suitable signal-to-noise ratios. This was accomplished by the data smoothing technique proposed by Hsiao and Verma\(^{35}\).

$$I_i = \frac{I_i-n + I_{i-n+1} + \ldots + I_{i-1} + I_i + I_{i+1} + I_{i+2} + \ldots + I_{n}}{2n+1} \quad (5.1)$$

Here $I_i$ represents the smoothed data and $I'_i$ represents the raw data. This method is very effective at eliminating noise. The parameter $n$ was set to zero at low $q$ and increased linearly with the scattering vector to account for the higher signal-to-noise ratio at higher $q$.

The finite $q$-range over which data is collected requires extrapolation in both the high-$q$ and low-$q$ regions\(^{36}\). The Porod law\(^{35}\) was used to extrapolate the scattering at high-$q$ and to simultaneously correct for background scattering. This was done by fitting the high-$q$ region to\(^{35}\)

$$\lim_{q \to \infty} (I_{corr} q^4) = \lim_{q \to \infty} [(I - I_b) q^4] = K_p \quad (5.2)$$
Here $I_{\text{corr}}$ is the corrected intensity, $I_b$ is the background scattering, and $K_p$ is the Porod constant. The background scattering was subtracted to obtain $I_{\text{corr}}$ over the entire $q$ range and the high $q$ range decays as $K_p q^{-4}$. Extrapolation to zero scattering vector in the region of the beam stop was accomplished by linear extrapolation of the log of the intensity:

$$\ln(I_{\text{corr}}) = aq + b \quad (5.3)$$

The resulting intensity profiles were then modified via the Lorentz correction to obtain $I_l(s)$ as

$$I_l(s) = 4\pi s^2 I(s) \quad (5.4)$$

Here $s = q/2\pi$. Bragg's law was used to find the long period ($L_B$) directly from the position of the first maximum in the Lorentz-corrected intensity profile, $s^*$.

$$L_B = \frac{1}{s^*} \quad (5.5)$$

The one-dimensional correlation function, $\gamma(r)$, offers more details of the microstructure\textsuperscript{36} and is found by taking the Fourier cosine transform of $I_l(s)$.

$$\gamma(r) = \frac{1}{Q_1} \int_0^\infty I_l(s) \cos(2\pi sr) ds \quad (5.6)$$
Here $Q_1$ is the invariant of the first file. The invariant was computed for each file as the area under the Lorentz-corrected intensity profile.

$$Q = \int I_i(s)ds$$

(5.7)

From the one-dimensional correlation function, the long period is given by the position of the first maximum, $r^*$. The degree of crystallinity, $\alpha_y$, is obtained as

$$\alpha_y = \begin{cases} \frac{A}{Q + A}; & \alpha_y < 0.5 \\ 1 - \frac{A}{Q + A}; & \alpha_y > 0.5 \end{cases}$$

(5.8)

Here $A$ is the absolute value of the correlation function at the first minimum. The ambiguity in equation 5.8 arises from the fact that the scattered intensity of a sample with crystallinity $\alpha_y$ is identical to that with a crystallinity of $1 - \alpha_y$, necessitating additional information in order to absolutely determine the crystallinity. This ambiguity is dealt with in the next section. The electron density contrast between the crystalline and the amorphous domains can be obtained\(^{36}\) from

$$\left( \rho_c - \rho_a \right)^2 = \frac{A' + Q'}{\alpha_y}$$

(5.9)

Here $(\rho_c - \rho_a)^2$ represents the electron density contrast (scattering cross sectional area per squared unit volume) between the crystalline and amorphous phases. $A'$ and $Q'$ represent the
values of $A$ and $Q$ normalized to an absolute intensity scale. Normalization is performed with reference to a polyethylene standard as previously described$^{25}$.

The interface distribution function, $g(r)$, can also be used to obtain morphological data$^{36}$. The interface distribution function is obtained by taking the Fourier transform of the interference function.

$$g(r) = 8\pi^2 \int_0^\infty [I_p - s^2 I_1(s)] \cos(2\pi sr) ds \quad (5.10)$$

$$I_p = \lim_{s \to \infty} s^3 I_1(s) \quad (5.11)$$

For a lamellar system, the position of the first maximum in $g(r)$, $r_1$, gives the length scale of the smallest dimension. The second maximum (which is usually obscured by the nearby local minimum) indicates the length scale of the larger dimension. The long period is indicated by the position of the first minimum ($r_3$). The crystallinity, $\alpha_g$, can be obtained as

$$\alpha_g = \begin{cases} 
\frac{r_1}{r_3} & \alpha_g \leq 0.5 \\
1 - \frac{r_1}{r_3} & \alpha_g \geq 0.5 
\end{cases} \quad (5.12)$$

By analyzing all three of these profiles, $I_l$, $\gamma$, and $g$, a comprehensive picture of the lamellar structure is obtained. Others have observed the trend $L_B > r^* > r_3^{28,35,37}$. It has been shown that the long period represented by $L_B$ is close to the weighted average, while that given by the interface distribution function, $r_3$, is least affected by the distribution of sizes and is therefore closer to the number average$^{37,39}$. It has also been shown that the crystallinities
obtained from these analyses typically follow the trend $\alpha_{\gamma} < \alpha < \alpha_{g}$, though most authors report only $\alpha_{\gamma}$. We compute both $\alpha_{\gamma}$ and $\alpha_{g}$ for comparison and use $\alpha_{\gamma}$ to determine the crystallization kinetics. These trends will be discussed in the next section.

The crystallization kinetics were fit to the Avrami equation

$$
\frac{\alpha_{\gamma}(t)}{\alpha_{\gamma}(t = \infty)} = 1 - \exp\left[ -Z(t - \tau)^n \right]
$$

(5.13)

Here $\alpha_{\gamma}(t = \infty)$ is the equilibrium degree of crystallinity obtained from the one-dimensional correlation function. $Z$ and $n$ are geometric constants for the Avrami equation. $\tau$ is an induction time required for the nucleation of crystals.

5.5 Results and Discussion

Plots of the smoothed and extrapolated intensity profiles for each polymer at each temperature are shown in Figure 5.2. The file number indicates the progression in time. As the experiment progressed the interval between files was increased from five seconds to ten seconds to 30 seconds. Figure 5.3 shows the Lorentz-corrected intensities obtained in each experiment. For all of the experiments conducted on polymers with SA content of 50% or greater, some residual crystallinity exists at the start of the experiment, despite the annealing during the sample preparation. This effect is most pronounced in the 50:50 copolymer. This is attributed to crystals nucleating during the quenching and the brief period of time required to heat the sample to the crystallization temperature. For the 50:50 copolymer, the glass transition is below room temperature, so the crystallinity has essentially equilibrated prior to the experiment. For the 80:20 copolymer and the poly(CPH) there is a significant induction time before any crystallization occurs. This is consistent with the observation that the melt-
quench procedure was sufficient to eliminate the crystals. The temporal variation of the long period ($L_B$) obtained from the Lorentz-corrected data in each experiment is plotted in Figure 5.6.

**Figure 5.2.** Corrected and extrapolated intensity profiles for: a. poly(SA) at 67 °C, b. poly(SA) at 73 °C, c. 20:80 (CPH:SA) at 54 °C, d. 20:80 (CPH:SA) at 59 °C, e. 50:50 (CPH:SA) at 37 °C, f. 50:50 (CPH:SA) at 42 °C, g. 80:20 (CPH:SA) at 75 °C, h. 80:20 (CPH:SA) at 100 °C, i. poly(CPH) at 100 °C, and j. poly(CPH) at 122 °C.
Figure 5.2. (Continued)

Figure 5.3. Lorentz-corrected intensity profiles for: a. poly(SA) at 67 °C, b. poly(SA) at 73 °C, c. 20:80 (CPH:SA) at 54 °C, d. 20:80 (CPH:SA) at 59 °C, e. 50:50 (CPH:SA) at 37 °C, f. 50:50 (CPH:SA) at 42 °C, g. 80:20 (CPH:SA) at 75 °C, h. 80:20 (CPH:SA) at 100 °C, i. poly(CPH) at 100 °C, and j. poly(CPH) at 122 °C.
Figure 5.3. (Continued)
Figures 5.4 and 5.5 show the one-dimensional correlation functions and the interface distribution functions respectively. The morphological parameters obtained from these two sets of data ($r^*$, $r_1$, $r_2$, and $Q$) are shown in Figure 5.6. For the poly(SA) and the 20:80 copolymer, the long period increases initially (for about the first 250 seconds). In the
experiments for which the crystallization was allowed to continue beyond this time (poly(SA) at 67 °C and 20:80 copolymer at 59 °C), the long period decreases, but the
lamellar thickness remains constant. This decrease in the long period is also evident in the
studies with all of the other copolymer compositions, and is attributed to secondary
crystallization in the interlamellar space. It is also noted that for all of the experiments the
trend $L_B > r^* > r_3$ is observed, which is consistent with the observations of others noted
earlier. Correlating $L_B$ to the weight average long period and $r_3$ to the number average long
period, it is apparent that the compositions rich in SA have a wider distribution of lamellar
sizes than the 80:20 copolymer and the poly(CPH). This can be understood by the
observation that for the 80:20 copolymer and poly(CPH), the long induction time for
nucleation essentially results in the formation of lamellae at the crystallization temperature.
However, in the SA-rich polymers, the residual crystallinity that remained after the
quenching procedure contributes to the wider distribution of lamellar sizes. In all of the
experiments, the invariant, $Q$, increases with time. Others have used the invariant to
characterize the kinetics of crystallization when $\alpha_g > 0.5$. 

Figure 5.4. One-dimensional correlation function profiles for: a. poly(SA) at 67 °C, b. poly(SA) at 73 °C, c. 20:80 (CPH:SA) at 54 °C, d. 20:80 (CPH:SA) at 59 °C, e. 50:50 (CPH:SA) at 37 °C, f. 50:50 (CPH:SA) at 42 °C, g. 80:20 (CPH:SA) at 75 °C, h. 80:20 (CPH:SA) at 100 °C, i. poly(CPH) at 100 °C, and j. poly(CPH) at 122 °C.
Figure 5.4. (Continued)
Figure 5.4. (Continued)

Figure 5.5. Interface distribution function profiles for: a. poly(SA) at 67 °C, b. poly(SA) at 73 °C, c. 20:80 (CPH:SA) at 54 °C, d. 20:80 (CPH:SA) at 59 °C, e. 50:50 (CPH:SA) at 37 °C, f. 50:50 (CPH:SA) at 42 °C, g. 80:20 (CPH:SA) at 75 °C, h. 80:20 (CPH:SA) at 100 °C, i. poly(CPH) at 100 °C, and j. poly(CPH) at 122 °C.
Figure 5.5. (Continued)
Figure 5.5. (Continued)
Figure 5.6. Morphological parameters for: a. poly(SA) at 67 °C, b. poly(SA) at 73 °C, c. 20:80 (CPH:SA) at 54 °C, d. 20:80 (CPH:SA) at 59 °C, e. 50:50 (CPH:SA) at 37 °C, f. 50:50 (CPH:SA) at 42 °C, g. 80:20 (CPH:SA) at 75 °C, h. 80:20 (CPH:SA) at 100 °C, i. poly(CPH) at 100 °C, and j. poly(CPH) at 122 °C. L_B = •; r^1 = ■; r_3 = ▲; r_1 = Δ; Q = □.
Figure 5.6. (Continued)
For each experiment, the crystallinity is computed from both the one-dimensional correlation function ($\alpha_{c}\gamma$) and the interface distribution function ($\alpha_{a}$) via equations 5.8 and 5.12 respectively. The temporal evolution of the crystallinity for each experiment is shown in Figure 5.7. For the poly(SA) the crystallinity equilibrates in about 100 seconds at both temperatures. The 20:80 and 50:50 copolymers show little change in the crystallinity with time, indicating that the crystallinity has already approached equilibrium prior to the start of the experiments. This is not surprising for the 50:50 copolymer since its glass transition is below room temperature, as noted earlier. For the 20:80 copolymer, apparently the crystals formed very rapidly during the quenching and the brief period required to heat the sample to the crystallization temperature. Consequently, for these three compositions, the structural changes observed in the experiments represent primarily changes in the crystal morphology, rather than changes in the crystallinity.
Figure 5.7. Crystallinity for: a. poly(SA) at 67 °C, b. poly(SA) at 73 °C, c. 20:80 (CPH:SA) at 54 °C, d. 20:80 (CPH:SA) at 59 °C, e. 50:50 (CPH:SA) at 37 °C, f. 50:50 (CPH:SA) at 42 °C, g. 80:20 (CPH:SA) at 75 °C, h. 80:20 (CPH:SA) at 100 °C, i. poly(CPH) at 100 °C, and j. poly(CPH) at 122 °C. \( \alpha_y = \bullet \); \( \alpha_g = \triangle \).
Figure 5.7. (Continued)
For the poly(SA), the 20:80 copolymer, and the 50:50 copolymer, the most notable morphological change that is observed occurs in the long period. In the case of the poly(SA) crystallized at 67 °C and the 20:80 copolymer crystallized at 59 °C, the long period rises initially and then decays slowly at longer times. In both cases, changes in the crystallinity and the invariant are associated with the initial change in the long period. The subsequent decay in the long period must therefore correspond to a rearrangement of the crystals. Larger lamellae are melting while smaller ones are growing. The 20:80 copolymer crystallized at 54°C exhibits the initial rise in the long period, though it is less pronounced, and the subsequent decay is not exhibited. For the poly(SA) crystallized at 73 °C, data were not collected at longer times, so the decay is not observed. For the 50:50 copolymer, it is interesting to note that the lamellar thickness and the long period are significantly higher for the experiment conducted at 42 °C than for the experiment conducted at 35 °C. This general trend is noted in all of the compositions (higher temperature corresponds to higher values of
the long period and lamellar thickness), but the difference seems excessive in the case of the 50:50 copolymer. As the long period decays slightly in the 50:50 copolymer crystallized at 42 °C, the degree of crystallinity increases, so it follows that a different equilibrium for the microstructure is obtained at the higher temperature than at the lower temperature, whereas the equilibrium morphology for the lower temperature is very similar to that already obtained prior to the experiment.

The 80:20 copolymer and the poly(CPH) do crystallize during the experiment. For both of these polymers, the increase in the crystallinity corresponds to a decrease in the long period despite the relatively constant lamellar thickness. This indicates that the crystallization is occurring via a lamellar insertion mechanism, that is some lamellae are growing in the interlamellar space. For both the poly(CPH) and the 80:20 copolymer, the crystallization occurs more slowly at the higher temperature. This is due to the fact that the higher temperature is approaching the melting temperature, although the chains are more mobile, the free-energy benefit of crystallization is lower due to the increase in entropy with increased temperature.

In all of the experiments, we have chosen to define \( \alpha_t \) as \( A/(Q+A) \). There is no question that this is the correct assignment in the case of the 80:20 copolymer and the poly(CPH) as it is obvious from the intensity plots (figures 5.2 and 5.3) that no crystallinity exists at the start of the experiment and the alternative assignment for \( \alpha_t \) would result in a very high degree of crystallinity initially. A similar argument can be made for the poly(SA) and the 20:80 copolymer. Based on the intensity evolution, we expect the crystallinity of these polymers to grow with time. The crystallinity for the 50:50 copolymer is more difficult to assign. Here we have relied on our previous experiments (DSC and WAXD) that indicate a crystallinity for the 50:50 copolymer that is less than 50%. Similar lines of reasoning were
used for the assignment of $\alpha_g$, noting that for the poly(SA), $\alpha_g$ crosses the 0.5 mark at some point during the experiment. At this point the alternative equation is used. With respect to the crystallinity, we make one final note that the trend $\alpha_g > \alpha_r$ is consistent for all of the experiments. The lamellar thickness, the long period, the degree of crystallinity, and the electron density contrast of each of the equilibrated structures are compared in Table 5.2. As noted earlier, the morphological parameters increase with increasing temperature for each polymer. For each experiment exhibiting suitable changes in $\alpha_r$, the crystallization kinetics are fit to the Avrami equation. The constants are listed in Table 5.3. The parameter $\tau$ is negative for the 20:80 and 50:50 copolymers, and represents the progress of the crystallization prior to the start of the experiment, so these values are not reported. It is notable that the 50:50 copolymer exhibits the highest value of the rate constant $Z$. This follows from the fact that the glass transition for this polymer is lowest, and the polymer chains are therefore most mobile. Also, note that the rate constant increases with increasing temperature for the 80:20 copolymer, also indicating increasing mobility of the polymer. The increase in the induction time with increasing temperature for the 80:20 copolymer is attributed to the increase in entropy at increasing temperature, which lowers the free-energy benefit obtained upon crystallization. The Avrami exponent, $n$, is essentially unity for all of the experiments. This indicates that the nuclei do not sporadically form, but are predetermined, and that crystallization is restricted to a single dimension. This observation is consistent with the previous comments on the longer induction time for nucleation for the CPH-rich polymers and the wide distribution of lamellar sizes due to pre-existing lamellae in the SA-rich polymers.
Table 5.2. Comparison of morphological characteristics obtained at equilibrium for each experiment.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>T (°C)</th>
<th>Lamellar thickness (Å)</th>
<th>Long period (Å)</th>
<th>Degree of crystallinity</th>
<th>Density contrast (Å$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(SA)</td>
<td>67</td>
<td>28</td>
<td>52</td>
<td>136 126 101</td>
<td>0.22 0.54 1.06</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>38</td>
<td>54</td>
<td>154 137 100</td>
<td>0.29 0.52 0.85</td>
</tr>
<tr>
<td>20:80 (CPH:SA)</td>
<td>54</td>
<td>40</td>
<td>42</td>
<td>123 119 96</td>
<td>0.34 0.45 0.92</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>42</td>
<td>47</td>
<td>137 136 114</td>
<td>0.31 0.37 1.4</td>
</tr>
<tr>
<td>50:50 (CPH:SA)</td>
<td>37</td>
<td>40</td>
<td>46</td>
<td>144 133 107</td>
<td>0.30 0.42 0.83</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>48</td>
<td>54</td>
<td>203 193 143</td>
<td>0.25 0.36 0.78</td>
</tr>
<tr>
<td>80:20 (CPH:SA)</td>
<td>75</td>
<td>35</td>
<td>52</td>
<td>168 158 143</td>
<td>0.22 0.36 1.03</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>40</td>
<td>62</td>
<td>186 173 164</td>
<td>0.24 0.38 1.05</td>
</tr>
<tr>
<td>Poly(CPH)</td>
<td>100</td>
<td>35</td>
<td>57</td>
<td>143 133 125</td>
<td>0.26 0.55 0.77</td>
</tr>
<tr>
<td></td>
<td>122</td>
<td>47</td>
<td>71</td>
<td>166 158 142</td>
<td>0.30 0.50 0.64</td>
</tr>
</tbody>
</table>
Table 5.3. Avrami constants for each experiment determined from the evolution of $\alpha_p$.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>T (°C)</th>
<th>$Z$ (sec$^{-1}$)</th>
<th>$\tau$ (sec)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPH:SA (20:80)</td>
<td>59</td>
<td>0.00037</td>
<td>-</td>
<td>1.11</td>
</tr>
<tr>
<td>CPH:SA (50:50)</td>
<td>42</td>
<td>0.00221</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>CPH:SA (80:20)</td>
<td>75</td>
<td>0.000223</td>
<td>600</td>
<td>0.991</td>
</tr>
<tr>
<td>CPH:SA (80:20)</td>
<td>100</td>
<td>0.00205</td>
<td>845</td>
<td>0.984</td>
</tr>
<tr>
<td>Poly(CPH)</td>
<td>122</td>
<td>0.000980</td>
<td>637</td>
<td>1.08</td>
</tr>
</tbody>
</table>

As researchers attempt to design controlled release devices from polyanhydrides, it has become apparent that the microstructure of the polymer plays a very important role in determining the drug release kinetics. In addition to discerning the kinetics of the crystallization, which may be used to control crystallinity during device fabrication, these results are essential to a comprehensive understanding of the morphological changes that occur during polyanhydride erosion. Previous work with polyanhydrides has demonstrated that the amorphous domains erode faster than crystalline domains and that the heterogeneous erosion results in a porous microstructure$^{19,20,24}$. The evolution of the porous erosion zone affects the overall erosion kinetics. Additionally, drug release kinetics are also closely linked to the porosity, when drugs dissolve and diffuse through the pores. Because these pores are initially formed primarily from the erosion of the amorphous regions, this detailed description of the morphology is essential for understanding the erosion and drug release kinetics.
Our future investigations of the microstructure of polyanhydrides for drug delivery will focus on the effects of dissolved drugs on the crystallization kinetics and crystal morphology.

5.6 Conclusions

Morphological changes during the isothermal crystallization of polyanhydride copolymers can be accurately observed via in situ SAXS. The Avrami equation can be used to design fabrication processes for drug release devices, such that the crystallinity is accurately controlled. This study reveals that although it would be difficult to control the crystallinity in poly(SA), the 20:80 copolymer, and the 50:50 copolymer, the crystallinity in the 80:20 copolymer and poly(CPH) can be accurately controlled by controlling the thermal history. At equilibrium, the lamellar thickness for all polymers studied was between 30 and 70 Å. Additionally, for all of the systems studied, this work provides accurate structural description of the lamellar thickness and degree of crystallinity. This microstructural characterization is an essential element of accurate erosion and drug release models.

5.7 References


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CHAPTER 6
NANOSCALE MORPHOLOGY OF POLYANHYDRIDE COPOLYMERS
CHARACTERIZED BY SOLID STATE NMR AND SAXS


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P. Thiyagarajan\textsuperscript{6}, Klaus Schmidt-Rohr\textsuperscript{3,7} and Balaji Narasimhan\textsuperscript{1,8}

6.1 Abstract

The microphase separation of random polyanhydride copolymers composed of 1,6-bis-(p-carboxyphenoxy)hexane and sebacic acid is described. Though the copolymers are random, the monomers are sufficiently long and the segment-segment interaction parameter is sufficiently high to support microphase separation when the composition is rich in one component. Solid state NMR spin diffusion experiments and synchrotron small-angle X-ray scattering are used to discern length scales of the microphase separation. Both techniques reveal a microstructure with domain sizes less than 25Å. This nanostructure is compared to

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\textsuperscript{6} Intense Pulsed Neutron Source, Argonne National Laboratory. Assisted with interpretation of SAXS data.
\textsuperscript{7} Helped perform and interpret NMR data.
\textsuperscript{8} Major professor, corresponding author
approximate calculations of chain dimensions based on a random coil model and discussed in the context of the use of these materials for drug delivery applications.

6.2 Introduction

Polyanhydrides are a class of surface-erodible polymers that have been investigated as potential vehicles for drug delivery and other biomedical applications$^{1-6}$. The erosion kinetics of polyanhydride copolymers can be modulated by altering the copolymer composition, when the two constituent polymers have different erosion rates$^{6,7}$. This strategy has proven effective for controlled release applications. In our previous work, we have demonstrated that some polyanhydride copolymers exhibit microphase separation, and that this microphase separation affects the mechanism of erosion and drug release kinetics when the relative erosion rates of the two constituent phases are different$^{7-10}$. Rational design of controlled release formulations requires a detailed description of polymer microstructure in order to predict drug release kinetics.

We have focused on copolymers based on 1,6-bis(p-carboxyphenoxy)hexane (CPH) and sebacic acid (SA). Previously, we have described the crystallinity and crystallization kinetics of these copolymers by wide-angle X-ray scattering (WAXS), differential scanning calorimetry (DSC), and small angle X-ray scattering (SAXS)$^{8,11}$. We have quantitatively studied the phase behavior of the poly(CPH)/poly(SA) blend system by SAXS, atomic force microscopy (AFM), optical microscopy, and molecular simulation in order to determine the segmental interaction parameter ($\chi$)$^{12}$ as a function of temperature (based on the volume of an SA monomer).

\[ \chi = -2.04 + \frac{802}{T} \]  

(6.1)
We have also qualitatively described the microphase separation in CPH:SA copolymers by AFM and demonstrated its effects on drug release kinetics\textsuperscript{7,9}.

It is important to note that the copolymers we describe in this study are random copolymers. Their molecular architecture has been previously described by \textsuperscript{1}H NMR\textsuperscript{7}. However, since the monomer units are sufficiently long, and the segment-segment interaction parameter is sufficiently high, even relatively short sequence lengths (<15) support microphase separation at the nanometer length scale. Thus, we refer to these copolymers as "weakly segregated, block-like copolymers." Not all compositions of these copolymers exhibit such behavior. The synthesis route employed, melt polycondensation, results in high polydispersity (in chain length and presumably in sequence length). Thus, compositions containing nearly equal amounts of the two constituent monomers (e.g. poly(CPH:SA) (50:50)) do not demonstrate microphase separation. However, compositions relatively rich in one component (e.g. 80:20 poly(CPH:SA) (80:20) and 20:80 poly(CPH:SA) (20:80)) have molecular architecture characterized by relatively long segments (10 to 15) of the majority monomer punctuated by short sequences (2 to 4) of the minority monomer. A sequence of 10 SA monomers contains 110 backbone bonds and a sequence of 10 CPH monomers contains 190 backbone bonds.

Our hypothesis of the microphase separation is predicated upon detailed studies of the erosion and drug release kinetics from these copolymers and upon characterization of the copolymer microstructure by atomic force microscopy\textsuperscript{7,9} and solution NMR. In these studies we noted that as the polymer degrades, the two monomers are not released at the same rate. Additionally, in drug-loaded copolymers, the drug release kinetics is highly correlated to one of the individual monomer release profiles, depending on the monomer-drug compatibility.
We conclude from these observations that the copolymers have a microphase-separated structure that permits partitioning of the drug between the two phases. We have successfully modeled the erosion and drug release kinetics by assuming a microphase separated structure, but we have not described that structure in detail.\(^{10}\) We further hypothesize that the microphase-separated domains are very small—on the order of a few nanometers in size, based on the average sequence lengths. In the current study, we use two experimental techniques that provide nanometer length scale resolution, solid state NMR and SAXS, to explore the morphology of these block-like copolymers.

6.3 Experimental

6.3.1 Polymer synthesis and characterization

Details of the polymer synthesis and characterization are reported elsewhere\(^{11}\). The poly(CPH:SA) (20:80) has an \(M_n\) of 9600 and a PDI of 2.2. The poly(CPH:SA) (80:20) has an \(M_n\) of 9700 and a PDI of 2.1. The degree of polymerization is 45 and 31 for the 20:80 and 80:20 copolymers respectively. \(^1\)H NMR of the copolymers revealed that the actual compositions were 17:83 and 78:22, but we refer to them here by their nominal compositions, 20:80 and 80:20 respectively. The homopolymers were also synthesized and characterized as controls. The characterization of these materials is also reported elsewhere\(^{11}\).

6.3.2 Solid state NMR

Solid state NMR spin diffusion experiments for probing microphase separation in copolymers are conducted by first establishing a \(^1\)H magnetization gradient that is based on some difference between the characteristic spectra of the two phases. Next a series of spectra
at different mixing times are taken that permits observation of the return to equilibrium magnetization. In our experiments a chemical shift filter is used to suppress the magnetization of the aliphatic nuclei in the SA monomers. Magnetization of the aromatic nuclei in the CPH monomers is passed to the aliphatic nuclei via cross polarization of $^1\text{H}$ with $^{13}\text{C}$ in the process of “spin-diffusion.” The kinetics of spin diffusion reveal details about the microstructure. Specifically, the time required to reach equilibrium is related to the domain size by

$$d_{\text{A,NMR}} = \frac{\varepsilon}{f_{\text{B}}} \sqrt{\frac{4D_s}{\pi}}$$  \hspace{2cm} (6.2)

Here, $d_{\text{A,NMR}}$ is the characteristic diameter of phase A (the magnetization donor), $f_{\text{B}}$ is the proton density of the B phase (the magnetization receiver), $D$ is the spin diffusion coefficient, and $\varepsilon$ is a parameter determined by the geometry. In this study, cylindrical domains are assumed ($\varepsilon = 2$).

6.3.3 Small-angle X-ray scattering

Samples were prepared for SAXS by melting the polymer into DSC aluminum sample pans (Perkin Elmer). As we have reported previously, these make ideal sample holders because the aluminum is fairly transparent to the high-brilliance X-rays used in this study, and they allow us to use the DSC to control the thermal history of the samples prior to the SAXS experiments. The thickness of each sample was accurately determined using a micrometer and the values were between 700 and 800 $\mu$m. Prior to the SAXS analysis, the samples were annealed in a DSC (DSC7, Perkin Elmer) by heating above the melting temperature for five minutes, and then rapidly quenched to below the $T_g$. The SAXS experiments were conducted at Beamline 12-ID at the synchrotron beam source at Argonne
National Laboratory\textsuperscript{15}. The incident beam energy was 12 keV ($\lambda = 1.035$ Å). A 15 cm $\times$ 15 cm CCD detector, at a distance of 0.805 m from the sample, was used to measure the intensity of scattering and the transmitted beam intensity was measured using a photodiode on the beam stop. The scattering data were appropriately corrected and azimuthally averaged to obtain the one-dimensional intensity data, $I(q)$, as a function of scattering vector, $q$ ($q=4\pi \sin(\theta/2)/\lambda$; $\theta$ = scattering angle; $\lambda$ = incident radiation wavelength). Five sets of data were collected and averaged for each sample. The resulting intensity data were corrected for the scattering from the blank aluminum sample pan.

The SAXS data of interest in this study corresponds to very small length scales (~1 nm) and relatively high values of $q$. In this region there is a significant contribution due to density fluctuations within the individual phases, which must be subtracted in order to obtain the correct absolute intensity. This background scattering is approximated by the sum of the scattering from the two homopolymers, weighted by their respective volume fractions in the copolymer\textsuperscript{15}.

6.4 Results and Discussion

6.4.1 Solid state NMR

The spectra for the poly(CPH:SA) (80:20) are shown in Figure 6.1. The peak intensity at 34 ppm representing aliphatic nuclei in the SA is used to characterize the spin diffusion. After correction for the $t_1$ relaxation, the normalized intensities as a function of mixing time ($t_m$) are plotted in Figure 6.2, along with a fit to equation 6.2. Assuming cylindrical domains ($e = 2$) and a diffusion coefficient of 0.3 nm$^2$/ms results in the values for the characteristic diameters shown in Table 6.1. The same procedure was used to extract domain sizes from the poly(CPH:SA) (20:80) NMR spectra (not shown).
6.1. Spectra for the poly(CPH:SA) (80:20) at different mixing times.
Figure 6.2. Intensities at 34 ppm from the poly(CPH:SA) (80:20) spectra plotted in Figure 6.1.

Table 6.1. Domain sizes obtained from solid state NMR and SAXS.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$d_{\text{CPH,NMR}}$ (Å)</th>
<th>$d_{\text{SA,NMR}}$ (Å)</th>
<th>$d_{\text{CPH,SAXS}}$ (Å)</th>
<th>$d_{\text{SA,SAXS}}$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CPH:SA) (20:80)</td>
<td>16</td>
<td>14</td>
<td>10.0</td>
<td>-</td>
</tr>
<tr>
<td>Poly(CPH:SA) (80:20)</td>
<td>20</td>
<td>10</td>
<td>-</td>
<td>9.1</td>
</tr>
</tbody>
</table>

6.4.2 SAXS

The SAXS data, shown in Figure 6.3, exhibit a peak corresponding to the periodicity of the microphase separation. This characteristic length scale is computed from Bragg’s law.

$$d = \frac{2\pi}{q^*} \quad (6.3)$$
Here, \( q^* \) is the position of the peak. The length scale associated with the microphase separation from the SAXS profiles is also shown in Table 6.1.

Comparing the results from the SAXS and the solid state NMR, we find that the domain diameters observed in the NMR experiments are close to those indicated by the SAXS data (see Table 6.1). Both techniques indicate length scales smaller than 25\( \text{Å} \) for the microphase separation. This small discrepancy is attributed to the approximations necessary for interpretation of the NMR data (e.g., cylindrical domains, value for \( D \)).

We interpret the significance of these results by comparison to calculations of random coil chain conformations. Table 6.2 shows the number average sequence lengths obtained from solution state NMR and reported previously, and the radius of gyration of the longer block-like sequence, \( R_g \).

Figure 6.3. SAXS intensity profiles for poly(CPH:SA) (20:80) (\( \bigcirc \)) and poly(CPH:SA) (80:20) (\( \square \)).
Here, $C_\infty$ is the characteristic ratio, $n$ is the number of bonds in the block-like sequence, and $l$ is the average bond length. We have previously estimated the characteristic ratios for poly(CPH) and poly(SA) as 6.8 and 4.8, respectively, from molecular dynamics simulations\textsuperscript{12}. The values of $n/l^2$ for poly(CPH) and poly(SA) were obtained in the same studies as 43.7 Å$^2$ and 23.8 Å$^2$, respectively. The radius of gyration of a block can be used to estimate the size of a domain of the majority component. For the minority component, the random coil approximation is not valid because of the relatively small number of bonds, so an estimate of the length scale associated with the minority component can be obtained by considering the contour length of a monomer, $nl$. Figure 6.4 is a schematic of our molecular description of the microphase separation in these weakly segregated block-like copolymers.

\begin{equation}
R_g = \left( \frac{C_\infty n l^2}{6} \right)^{0.5}
\end{equation}

**Figure 6.4.** Schematic of microphase separation in a weakly segregated block-like copolymer. The dimensions of the microphase separated domains can be approximated by the radius of gyration for the majority component, $R_{g,1}$, (black) and the monomer contour length for the minority phase, $nl_2$ (gray).
Table 6.2. Number average sequence lengths computed from $^1$H NMR and the calculated radius of gyration for the longer block-like sequence for the copolymers used in this study.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$L_{CPH}$</th>
<th>$L_{SA}$</th>
<th>$R_{g,1}$ (Å)</th>
<th>$nl_2$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CPH:SA) (20:80)</td>
<td>2.3</td>
<td>12.0</td>
<td>15.1</td>
<td>24.6</td>
</tr>
<tr>
<td>Poly(CPH:SA) (80:20)</td>
<td>10.4</td>
<td>1.7</td>
<td>22.7</td>
<td>15.1</td>
</tr>
</tbody>
</table>

These computed radii of gyration are on the same order of magnitude as the domain sizes computed from the solid state NMR and the SAXS (see Table 6.1). These data support our hypothesis of a weakly segregated block-like nanostructure in these copolymers. The structure results from the block-like nature of the random copolymers coupled with the propensity for these materials to phase separate. Comparing the radii of gyration approximated from the random coil approximation to the domain sizes computed from SAXS and solid state NMR, we are led to the conclusion that in the bulk polymer, the blocks of CPH and SA can segregate to form the nanostructure without conformational entropic penalties. This work shows, for the first time, the nanometer length scale of the weakly segregated domains.

6.5 Conclusions

The solid state NMR and SAXS experiments offer nanoscale resolution of the microphase separation in weakly segregated block-like polyanhydride copolymers. These concentration fluctuations are characterized by length scales smaller than 25Å. Based on the relatively large sizes of the monomers in these polymers, we conclude that the microphase-separated domains contain very few monomers and may be formed from a single block or a small number of blocks. This knowledge of the copolymer microstructure, coupled with
accurate erosion and drug release models, will help guide the design of these materials for
drug delivery as we pursue the synthesis of polyanhydride block copolymers.

6.6 References


CHAPTER 7
DESIGN OF AN INJECTABLE SYSTEM BASED ON BIOERODIBLE POLYANHYDRITE MICROSPHERES FOR SUSTAINED DRUG DELIVERY

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Matt J. Kipper¹,², Elizabeth Shen³,⁴, Amy Determan¹,⁵, and Balaji Narasimhan¹,⁶

7.1 Abstract
The fabrication, morphological characterization, and drug release kinetics from microspheres of three bioerodible polyanhydrides, poly[1,6-bis(p-carboxyphenoxy)hexane] (poly(CPH)), poly(sebacic anhydride) (poly(SA)), and the copolymer poly(CPH-co-SA) 50:50 (CPH-SA 50:50) is reported. The fabrication technique yields microspheres with different morphologies for each of the three polymers studied, ranging from very smooth exterior surfaces for poly(CPH) to coarse surface roughness with large pores for poly(SA). Release profiles for the model drug, p-nitroaniline are also different for each polymer. The release profile from poly(CPH) has a large initial burst and shows little additional release after two days. The release from poly(SA) is nearly zero-order and lasts for about 8 days. The release profile from CPH-SA 50:50 shows a relatively small burst and then exhibits zero-order release for about 1 month. The different release profiles are attributed to both polymer erosion rates and drug distribution characteristics of the microspheres. Tailored

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release profiles of a burst followed by zero-order release are obtained by appropriately combining the microspheres. This technique enables independent modulation of both the burst and the zero-order release rate by varying the number of poly(CPH) and poly(SA) microspheres respectively. Additionally, the zero-order release can be extended from about a week to a month by including CPH-SA 50:50 microspheres.

Keywords: Polyanhydrides, microspheres, drug delivery, controlled release.

### 7.2 Introduction

Bioerodible polymers show great potential as vehicles for drug delivery, offering significant improvements over conventional drug delivery methods. For example, devices made from bioerodible polymers have been used to increase the stability of macromolecular drugs [1], target specific organs and tissues [2], and achieve sustained and controlled drug release profiles [3-9].

Langer, Mathiowitz, and co-workers have shown that polyanhydrides are a particularly promising class of polymers for drug delivery, due to their chemistry [3-7, 10-14] and biocompatibility [15, 16]. Polyanhydrides of aliphatic and aromatic dicarboxylic acids have hydrophobic regions separated by relatively hydrophilic acid anhydride bonds. The anhydride bonds are hydrolyzed under physiological conditions, resulting in polymer degradation and subsequent erosion. However, water does not penetrate into the bulk of the hydrophobic polymer [17]. Thus, degradation and erosion occur at the surface, rather than in the bulk. Surface eroding devices are particularly well suited for sustained release and drug stabilization. Additionally, polyanhydrides of varying hydrophobicity have erosion rates that span several orders of magnitude [6]. The potential for not only sustaining the release of a
drug, but also achieving desirable release profiles can be realized by combining polyanhydrides with differing erosion rates in an advantageous way.

Bioerodible polymer microspheres as drug delivery vehicles offer the advantage of not requiring surgical implantation, since they can be injected in suspension; they also do not require surgical removal. Also, since the drug loaded in a microsphere remains separated from that in other microspheres, a further advantage is the potential to administer multiple drugs in a single injection, that for compatibility reasons would otherwise need to be separated.

The effectiveness of a drug is dependent upon its concentration being within a specific therapeutic range, above which toxicity or other side effects dominate and below which concentrations are too small to provide significant benefit. However, maintaining drug concentrations is complicated by metabolism or consumption of the drug. Therapeutic levels are often maintained by multiple administrations (e.g. pain relievers, antibiotics, antidepressants). The faster a drug is metabolized or consumed, the more difficult maintaining therapeutic concentrations by multiple administrations becomes. All of these issues can be resolved by a single dose, which provides an initial burst of drug to reach a therapeutic concentration, followed by a zero-order release of drug that maintains the therapeutic level by compensating for metabolic loss.

We propose a drug delivery system composed of polyanhydride microspheres with varying composition to realize a tailored drug release profile. The system of interest in this work consists of two anhydride monomers 1,6-bis(p-carboxyphenoxy)hexane, CPH, and sebacic acid, SA. The homopolymers of these monomers are shown in Figure 7.1. Poly(CPH) is aromatic and degrades on a time scale of over a year, whereas the aliphatic poly(SA)
degrades in a few days [18]. Random copolymers of SA and CPH have intermediate
degradation times depending on the copolymer composition [19].

![Chemical structures of CPH (top) and SA (bottom) repeat units.](image)

**Figure 7.1.** Chemical structures of CPH (top) and SA (bottom) repeat units.

We have previously examined the microstructure of these copolymers using
differential scanning calorimetry (DSC), wide-angle X-ray diffraction (WAXD), and atomic
force microscopy (AFM) [19]. This study showed that copolymers rich in one component (≥
80 mol%) exhibit microphase separation forming two amorphous phases with different
compositions at equilibrium. It was also shown that the 50:50 copolymer does not exhibit
microphase separation. Because the copolymers are random, the 50:50 copolymer has short
number average sequence lengths, but the 20:80 and 80:20 CPH:SA copolymers have weakly
seggregated block-like structures with relatively high number average sequence lengths of SA
and CPH respectively [19, 21]. These sequence characteristics combined with the difference
in hydrophobicity of the monomer units results in the observed phase behavior. In the same
study [19], DSC and WAXD showed that hydrophobic model drugs loaded into the
semicrystalline polymer, poly(SA), lowered the crystalline melting point. The disruption of the lamellar structure suggests that the drug forms a solid solution with the polymer.

We have also studied dissolution and drug release kinetics of homogeneous and microphase-separated copolymers of CPH and SA in tablet form [22]. In this study, it was found that when compatible drugs (i.e., drugs capable of forming a solid solution with the polymer) were dissolved in homogeneous polymer matrices (e.g., poly(SA) and 50:50 CPH:SA) the drug release profile was dictated by the degradation rate of the polymer. We also showed that the presence of incompatible (i.e., sparingly soluble) drug and its interaction with the polymer had dramatic effects on the polymer erosion rate. For example, when hydrophobic p-nitroaniline (PNA) was incorporated into poly(SA) and 50:50 CPH:SA the drug and monomer release profiles were identical. Additionally, PNA did not significantly alter the erosion rates of either polymer. However when PNA is incorporated into microphase-separated 20:80 and 80:20 CPH:SA copolymers, its release is strongly correlated to the erosion of the CPH-rich phase. From these observations we concluded that PNA release from polymers with homogeneous microstructure follows polymer erosion without significantly affecting the erosion rate. Further, we also showed by atomistic simulations that the PNA is more compatible with poly(CPH) than with poly(SA).

In this work, we discuss the fabrication and characterization of microspheres of poly(CPH), poly(SA) and CPH-SA 50:50. These polymers were chosen because they do not microphase-separate into multiple amorphous phases with different compositions. Thus, drug release kinetics is not affected by partitioning into equilibrium phases. A solvent removal technique employing an oil/water emulsion is used to produce drug-loaded microspheres. We also present release data of p-nitroaniline (PNA) from drug-loaded microspheres, showing how the release profile can be altered. Microspheres with different
time scales of erosion are made by selecting polymers with different copolymer compositions. A “cocktail” of such microspheres with different release kinetics is used to obtain the desired release profile: a burst followed by zero order release.

7.3 Materials and Methods

7.3.1 Materials

Sebacic acid (99%) and p-carboxy benzoic acid (99+%) were purchased from Aldrich (Milwaukee, WI), 1,6-dibromohexane (98%) and poly(vinyl alcohol) (99-100% hydrolyzed) were purchased from Acros (Fairlawn, NJ), p-nitroaniline and sebacic acid were purchased from Sigma (St. Louis, MO), acetic anhydride, chloroform, Histo-Prep tissue embedding media and methylene chloride were purchased from Fisher (Fairlawn, NJ), deuterated chloroform was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA), petroleum ether (hexanes, 55% n-hexane) was purchased from Fisher and dried and distilled over sodium and benzophenone (Fisher) before use.

7.3.2 Polymer synthesis

CPH diacid was synthesized by a method similar to that described by Conix for 1,3-bis(p-carboxyphenoxy) propane[23]. CPH and SA diacids were acetylated to form the prepolymer by the method reported by Shen et. al. [19]. Briefly, diacid was refluxed in excess acetic anhydride for 30 minutes (SA) or 60 minutes (CPH) under dry nitrogen sweep. Unreacted diacid was removed by filtration while the mixture was still warm. The SA prepolymer was isolated by evaporating the solution to dryness at 50°C under vacuum and purified by dissolution in chloroform, filtration, and subsequent precipitation in a 1:1 mixture of dry ethyl ether and dry petroleum ether. The precipitate was filtered and dried under
vacuum overnight. CPH prepolymer was crystallized from the filtrate by evaporating the solution down to a volume of about 150 ml and storing under refrigeration overnight. The crystals were obtained by filtration, washed with ethyl ether and dried overnight. Crude CPH prepolymer was re-dissolved in chloroform and filtered again to remove impurities. The chloroform solution was evaporated and dried overnight under vacuum.

$^1$H NMR was used to determine the degree of polymerization for each of the prepolymers. NMR spectra were obtained on a Varian VXR 300 MHz spectrometer (Varian Inc. Palo Alto, CA). Prepolymers were stored desiccated under dry argon to prevent hydrolysis and $^1$H NMR was used periodically to monitor degree of hydrolysis.

Homopolymers and 50:50 copolymer were synthesized by melt polycondensation of the prepolymer at 180°C under vacuum (<0.5 mmHg) for 90 minutes[13]. About 2ml of acetic anhydride was added to 4g of prepolymer prior to polymerization to ensure complete acetylation. The polymers were desiccated under dry argon to prevent degradation.

7.3.3 Polymer characterization

Neat polymers were characterized by $^1$H NMR in deuterated chloroform (99.8% atom-$d$). Chemical shifts were calibrated to the chloroform peak. Gel permeation chromatography (GPC) was used to determine molecular weights. Samples were dissolved in chloroform and separation was done using PL Gel columns from Polymer Laboratories (Amherst, MA) on a Waters GPC system (Milford, MA). Elution times were compared to poly(methyl methacrylate) standards from Fluka (Milwaukee, WI). Differential scanning calorimetry (DSC) (DSC7, Perkin Elmer, Shelton, CT) was used to verify that crystalline melting points were the same as reported by Shen et. al[19].
7.3.4 Microsphere fabrication

A solvent removal technique employing an oil/water emulsion was used to fabricate microspheres. An aqueous non-solvent phase was chosen to prevent hydrophobic drugs from diffusing out of the polymer solution during fabrication. Polymer (200mg) and PNA (30mg) were dissolved in methylene chloride (2 to 4ml). This solution was added to a 1% (w/v) aqueous solution (200ml) of 99% hydrolyzed poly(vinyl alcohol) (PVA) and immediately dispersed by agitation at 20,000rpm with a handheld homogenizer (Tissue-Tearor™, Biospec Products Inc., Bartlesville, OK) for 1 minute. The water/oil emulsion was stirred for 2h at 300rpm in a 400ml Berzelius beaker using a Caframo overhead stirrer with a 3-inch impeller (Wiarton, Ontario). The microspheres were obtained by centrifugation for 5min at 375xg using an Eppendorf Centrifuge 5403 (Westbury, NY). The PVA solution was decanted off and fresh de-ionized water was added. The centrifugation and decantation was performed at least four times to remove as much of the PVA and undissolved drug as possible. The microspheres were re-suspended in less than 20ml of de-ionized water, flash frozen with dry ice and acetone, and lyophilized overnight. Finally, the microspheres were sieved to eliminate the particles that were larger than 53μm. The fabrication technique was slightly altered for the different polymers. For the 50:50 copolymer, the precipitation was performed in an ice water bath, so that the precipitated polymer would be below its glass transition temperature of about 10°C. For the higher molecular weight poly(SA) a larger volume of methylene chloride was used (up to 4ml).

7.3.5 Microsphere characterization

The yield of microspheres was calculated as the mass of microspheres recovered per mass of polymer used. Size distributions were obtained on a Malvern Mastersizer E particle
size analyzer (Southborough, MA). The morphology of the microspheres was investigated using scanning electron microscopy (SEM) (Hitachi S-2460N, San Jose, CA). Microspheres were sectioned by embedding in Histo-Prep tissue embedding media, followed by microtoming at \(-20^\circ\text{C}\), and lyophilization. Sections were also imaged using SEM. \(^1\text{H}\) NMR (as described in section 7.3.3. for the polymer) was used to quantify the degree of hydrolysis that resulted from the aqueous microsphere fabrication. Detailed discussions of this procedure are in the literature[18, 21]. GPC (as described in section 7.3.3 for the polymer) was used to discern the loss of molecular weight. Drug loading levels (mass of drug loaded per mass of microspheres) were obtained by dissolving the microspheres in pH 7.4 phosphate buffer solution at 80°C and performing UV spectroscopy at 381 nm. The results are reported in Table 7.1.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>(M_w/M_n) of neat polymer</th>
<th>(M_w/M_n) of microspheres</th>
<th>(M_n) loss (%)</th>
<th>Yield (%)</th>
<th>Loading ((\mu\text{g PNA/mg microspheres}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(CPH)</td>
<td>7100 / 4100</td>
<td>7100 / 4000</td>
<td>2</td>
<td>58</td>
<td>5.3</td>
</tr>
<tr>
<td>CPH:SA 50:50</td>
<td>8400 / 4100</td>
<td>7000 / 3400</td>
<td>17</td>
<td>50</td>
<td>4.9</td>
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<td>Poly(SA)</td>
<td>33000 / 9000</td>
<td>20000 / 6000</td>
<td>33</td>
<td>53</td>
<td>7.2</td>
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</tbody>
</table>

7.3.6 Release studies

Microspheres (2 to 10mg) were suspended in 1ml of phosphate buffer (pH 7.4) and incubated at 37°C with gentle agitation (100rpm). Samples were collected by centrifuging the microsphere suspensions (for 20 min at 4150xg), and removing a fraction of the supernatant. Supernatant was replaced with fresh buffer. The sample was diluted with fresh
buffer to increase the volume. UV spectroscopy using a Shimadzu 1601 UV-Visible Spectrophotometer (Columbia, MD), at a wavelength of 381 nm was used to measure the concentration of PNA in the supernatant.

7.4 Results and Discussion

7.4.1 Microsphere characterization

7.4.1.1 Molecular weight loss and yield. Because the polyanhydrides are hydrolytically degraded it is necessary to quantify the molecular weight loss that occurs during the (aqueous) microsphere fabrication procedure. The number-average and weight-average molecular weights of the polymers before and after microsphere fabrication are reported in Table 7.1. The molecular weight loss increases (from 2% for poly(CPH) to 33% for poly(SA)) as the polymer hydrophobicity decreases. Table 7.1 also shows the yields of each type of microsphere. Typically, the yields varied from 50-60%.

7.4.1.2 Particle size distribution. The solvent removal process produces a size distribution of microspheres. The particle size distributions (in terms of surface area fraction) for the poly(CPH) and the poly(SA) microspheres are shown in Figure 7.2 (data for CPH-SA 50:50 copolymer is not shown). The distributions for all three polymers are essentially the same, showing that microspheres with diameters of about 20µm make up the largest surface area fraction of the total microspheres. The data also show that a significant fraction of the surface area is represented by very small microspheres (<2µm). These phenomena can also be observed qualitatively from the SEM micrographs shown in Figure 7.3.
Figure 7.2. Particle size distributions for PNA-loaded poly(CPH) (a) and poly(SA) (b) microspheres plotted as surface area fractions.
7.4.1.3. Morphology. Scanning electron micrographs of microspheres from each type of polymer are shown in Figure 7.3. Three distinct morphologies are represented. Figure 7.3a shows that the poly(CPH) microspheres are very spherical and have smooth external surfaces. The CPH-SA 50:50 microspheres shown in Figure 7.3b have mostly smooth outer surfaces as well, but some are deformed. Many have dents and some are ellipsoidal instead of spherical. The glass transition temperature of CPH-SA 50:50 is 10°C[24]. The deformations are attributed to the rubbery nature of the polymer. Some surface roughness is also observed. Finally, the poly(SA) microspheres in Figure 7.3c show a very different surface morphology. Some of these microspheres have a macroporous surface. These observations are qualitatively consistent with the work of Mathiowitz et al.[5]. Surface roughness may be due to the partial degradation that occurs during microsphere fabrication (see MW loss in Table 7.1).

All three types of microspheres had solid, non-porous internal structures. Figure 7.3d shows a SEM micrograph of a cross section of a poly(CPH) microsphere.

7.4.2 Release kinetics

7.4.2.1. PNA release from poly(SA), CPH-SA 50:50, and poly(CPH) microspheres. The release of PNA from microspheres of each of the three polymers studied shows unique characteristics. The release of PNA from poly(SA) is approximately zero-order for about eight days, after which time the release rate drops off precipitously (Figure 7.4). PNA release from CPH-SA 50:50 microspheres shows a small burst and then releases with a zero-order profile for longer than two weeks (Figure 7.4). The release rate is slower than that for poly(SA) microspheres, and continues for a month. (Data shown is only for the first two weeks. Extended release is evidenced by the data for “cocktails” in section 7.4.2.2.)
difference in release rate for these two polymers is attributed to the difference in relative polymer erosion rates. Also, since erosion occurs at the surface, the increased surface area of the poly(SA) microspheres due to their rough surface morphology (Figure 7.3c), enhances their degradation rate.

**Figure 7.3.** Scanning electron micrographs of PNA-loaded (a) poly(CPH) microspheres, (b) 50:50 CPH-SA microspheres, (c) poly(SA) microspheres showing characteristic sizes and surface morphologies obtained and (d) poly(CPH) microspheres showing solid internal structure.
In contrast to poly(SA) and 50:50 CPH-SA microspheres, the PNA release profile from poly(CPH) microspheres is not dominated by polymer degradation rate. Poly(CPH) has the lowest degradation rate of the three polymers studied. Also, PNA has a higher affinity for poly(CPH) than for poly(SA). Considering only these two characteristics of the system, one would anticipate a very slow PNA release from poly(CPH) microspheres. Instead, the release profile shown in Figure 7.4 is fast. A high rate of release is observed for less than two days. Any subsequent release occurs so slowly that it is not accurately detected.

We have shown that the microspheres have a non-porous internal structure (Figure 7.3d) and verified via SEM that the poly(CPH) microspheres erode only at the surface (micrographs not shown). Since our data clearly show that the microspheres are surface-erodible and non-porous, we hypothesize that the release profile for poly(CPH) is the result
of non-uniform distribution of PNA within the poly(CPH) matrix. One possible reason for the non-uniform distribution is the polarity of PNA, which may cause it to behave as a surfactant during methylene chloride removal. Thus, the PNA concentrates near the surface of the poly(CPH) microspheres leading to the initial burst. Another possible factor that could alter the release kinetics is the diffusion of PNA through the poly(CPH) during release. We do not believe that this is the dominant mechanism of release since the concentration gradients are small and the polymer is glassy at 37°C. One would expect that diffusion, if it were significant, would be evident in the release data from the 50:50 CPH-SA copolymer since its glass transition occurs at about 10°C. The very slow release from the poly(CPH:SA) 50:50 suggests that diffusion is not important.

The burst effect due to non-uniform distribution of the PNA is much less pronounced in the 50:50 CPH-SA copolymer microspheres and not detected at all in the less hydrophobic poly(SA) microspheres. We attribute this to the kinetics of the precipitation during microsphere formation. Since poly(SA) and 50:50 CPH-SA copolymer have higher molecular weights (see Table 7.1), it is reasonable to expect that they precipitate from solution faster than the poly(CPH). This faster precipitation may not allow enough time for PNA to migrate to the surface.

Thus, each polymer shows a distinct characteristic release profile. The release profiles from poly(SA) and 50:50 CPH-SA are nearly zero-order with the release rate dictated by the degradation rate of the polymer. The release rate from poly(CPH) exhibits a burst over a short period of time, presumably due to non-uniform distribution of PNA within the microspheres.
6.4.2.2. PNA release from microsphere "cocktails." The release profiles from poly(CPH) and poly(SA) are of particular interest because the poly(SA) profile shows nearly zero-order release (without a burst) while the poly(CPH) profile exhibits a large burst. This suggests that "cocktails" of poly(CPH) and poly(SA) microspheres could be used to tailor release profiles in which a burst followed by zero-order release is obtained. The magnitude of the burst provided by the poly(CPH) microspheres can be modulated without affecting the zero-order release rate provided by the poly(SA) and *vice versa*. The only limitation is the length of time over which the poly(SA) microspheres release, which is fixed at a little longer than one week. However, additional flexibility can be realized by introducing CPH-SA 50:50 microspheres, which have a longer release time than that of poly(SA).

Release data from three cocktail formulations are presented in Figure 7.5. Cocktail I is made up of 80% poly(CPH) microspheres and 20% poly(SA) microspheres. The release profile for Cocktail I shown in Figure 7.5a shows a large burst (due to the high number of CPH microspheres) in the first six hours followed by a period of zero-order release that lasts until the end of day one. After this, a second period of zero-order release begins, which lasts until about day 8. The poly(CPH) microspheres continue to release at a slow rate for about one day. Thus the first region of zero-order release has a rate that is the sum of the release from the poly(CPH) and the poly(SA) microspheres. Once all of the PNA has been released from the poly(CPH) microspheres, the release rate falls to that of the poly(SA) microspheres. This release rate is very slow due to the small number of poly(SA) microspheres used.
Figure 7.5. Mass of PNA (in μg) released from microsphere “cocktails” made up of (a) 80% poly(CPH) and 20% poly(SA), (b) 50% poly(SA) and 50% CPH-SA 50:50 and (c) 38% poly(CPH), 51% poly(SA), and 11% CPH-SA 50:50.
Cocktail III is made up of 38% poly(CPH) microspheres, 51% poly(SA) microspheres, and 11% CPH-SA 50:50 microspheres (Figure 7.5c). The burst is evident in the first eight hours followed by sustained, zero-order release lasting for nearly one month. This profile also shows that CPH-SA 50:50 microspheres release PNA for at least 27 days.

7.5 Conclusions

We have shown that the release of PNA from microspheres of poly(CPH), poly(SA), and CPH-SA (50:50) is a function of polymer erosion rate and interactions of the drug with the polymer. Each polymer studied has a unique characteristic release profile. Release from poly(SA) microspheres is nearly zero-order for about eight days. The release from poly(CPH) microspheres exhibits a burst and shows almost complete release in about two days. The CPH-SA 50:50 microspheres show a small burst followed by zero-order release that lasts for about a month.
Release profiles can be tailored by combining microspheres in “cocktails” such that the sum of the release profiles from each type of microsphere yields the desired profile. Profiles containing a burst followed by zero-order release are desirable for many drug delivery applications. This drug release profile can maintain therapeutic drug concentrations while obviating the need for multiple administrations. The system presented here offers this capability. The magnitude of the burst can be varied independently (by changing the amount of poly(CPH) microspheres used) of the subsequent zero-order release rate (obtained from the poly(SA)), rendering the system flexible. Additional flexibility is introduced by the incorporation of CPH-SA 50:50 microspheres, which release drug for over a month. The continued study of drug release from these bioerodible systems will lead to the development of safer and more effective methods of delivering injectable therapeutic formulations.

7.6 Acknowledgements

We express our gratitude to the Whitaker Foundation, the Roy S. Carver Trust, and the Iowa State University Office of Biotechnology for their funding of this research. We would also like to thank Quentin Leigh, our undergraduate research assistant at Iowa State University. Finally, we would like to thank Warren Straszheim for his expertise with the scanning electron microscope.

7.7 References


CHAPTER 8
SINGLE-DOSE TETANUS VACCINE BASED ON BIOERODIBLE POLYANHYDRIDE MICROSPHERES CAN MODULATE IMMUNE RESPONSE MECHANISM

Matt J. Kipper\textsuperscript{1,2}, Jennifer Wilson\textsuperscript{3,4}, Michael Wannemuehler\textsuperscript{3,5}, and Balaji Narasimhan\textsuperscript{1,6}

8.1 Abstract

The development of a single-dose tetanus toxoid (TT) vaccine based on polyanhydride copolymer microspheres composed of 1,6-bis(p-carboxyphenoxy)hexane (CPH) and sebacic acid (SA) is reported. The release kinetics can be modulated by altering the copolymer composition, which allows unique immunization regimens to be developed. \textit{In vivo} studies in mice demonstrate that the encapsulation procedure preserves the immunogenicity of the TT. The polymer itself has an adjuvant effect, enhancing the immune response to a small dose of TT, but as the dose of polymer is increased, a localized, dose-dependent inhibition is observed. The sustained release of the antigenic/immunogenic protein is essential for the efficacy of a single-dose vaccine. TT released from the microspheres maintains its immunogenicity and antigenicity and the microspheres provide a

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\textsuperscript{5} Major Professor to Jennifer Wilson
\textsuperscript{6} Major professor to Matt Kipper, corresponding author
prolonged exposure to TT sufficient to induce the secondary immune response (i.e., isotype switching and high titers) without requiring an additional administration. In addition to providing an immune response with a single immunizing dose, the microsphere delivery vehicle offers the opportunity to select the preferred immune response pathway. While TT administered in buffer alone is known to induce a Th2 immune response, we demonstrate that TT-loaded microspheres preferentially induce both the Th1 and Th2 immune response pathways as evidenced by the IgG2a and IgG1 antibody responses, respectively, when injected intramuscularly into mice. Though the Th2 immune response is higher for some formulations, it can be selectively inhibited by altering the vaccine formulation. The ability to induce immune deviation by utilizing the microsphere delivery system may allow for induction of immune responses that are more effective for some viral and other intracellular pathogens. We present a strategy for modulating the immune response by delivering the microspheres along with a small bolus of unencapsulated TT. This ability to tune the immune response without the administration of additional cytokines or noxious adjuvants is a unique and valuable feature of this delivery vehicle that may make it an excellent candidate for many vaccines.

8.2 Introduction

The National Institutes of Health identified the development of single-dose vaccines as one of 14 grand challenges in global health in 2003 (1). Even diseases for which effective vaccines exist, many remain a threat to public health because patient dropout rates after initial vaccine doses reach as high as 70% in developing countries (2). Improved vaccine delivery techniques that require only a single dose to confer protective immunity against childhood diseases would help make mass immunization programs successful. For instance,
tetanus is responsible for over 700,000 neonatal deaths annually, half of which could be prevented by immunization alone (3). Additionally, the ability to purposefully modulate the immune response to more effectively protect the host from a particular disease state would improve the effectiveness of vaccines in combating worldwide disease. While traditional alum-based vaccines, incorporating tetanus toxoid (TT) in particular, initiate primarily a T helper type 2 (Th2) response (4, 5), a T helper type 1 (Th1) response may be more effective for protection from or treating cancers and infections due to intracellular pathogens (6). The Th2 (humoral) response, characterized by the activation of B cells that secrete antibodies, is effective at neutralizing extracellular pathogens and toxins, and the Th1 (cell-mediated) response, involving the activation of cytotoxic T cells and macrophages, is effective for fighting intracellular pathogens (7). Induction of the appropriate immune response is essential to the safety and efficacy of vaccines (7, 8). However, the mechanisms governing the type of immune response are complex and not well understood (8, 9).

The goal of this study was to develop a single dose vaccine utilizing bioerodible polyanhydride microspheres that offers the potential to preferentially induce a Th1 immune response, using TT as a model antigen. Single-dose vaccines must provide prolonged exposure to an antigen so that the secondary immune response occurs without the necessity of a second administration. Consequently, the protein must also be stabilized so that an immunogenic/antigenic form is released. Finally, we seek to target delivery of the protein to phagocytes of the immune system to take advantage of their ability to shape the nature of the adaptive immune response.

Our previous work has characterized the erosion kinetics of and the drug release kinetics from polyanhydride copolymers composed of 1,3-bis(\(p\)-carboxyphenoxy)hexane (CPH) and sebacic acid (SA) (10, 11). These polymers are shown in Figure 8.1. We have
also reported the fabrication of microspheres and the controlled release of small molecular mass compounds (12, 13). A key feature of these materials is that their performance in controlled release applications is enhanced by their hydrophobicity. Commonly used polyesters such as poly(lactide) (PLA) and poly(lactide-co-glycolide) (PLGA) have been studied for single-dose vaccines (14, 15). Unlike these polyesters, the polyanhydrides used in this study do not swell in the presence of water. This hydrophobic property of the microspheres results in the release of immunogen by a process of surface erosion. More importantly, the exclusion of water from the microsphere may aid the stabilization and prolonged immunogenicity of encapsulated proteins (16, 17). It has been shown that high-moisture environments can cause proteins such as TT and diphtheria toxoid to form insoluble aggregates, losing about 75% of their solubility, and thus their immunogenicity, within one week (17). Another important feature of polyanhydrides is that the degrading microsphere does not form an acidic microenvironment as extreme as that formed by PLA and PLGA (17-19). This is due in part to the limited solubility of the monomeric dicarboxylic acids released during erosion. We have previously described the stabilization of proteins in poly(CPH-SA) microspheres (20) and the ability to purposefully modulate the release profile, by changing the copolymer composition, and thus the hydrophobicity (10-12).

![Figure 8.1. Poly(CPH) (top) and poly(SA) (bottom).](image-url)
Here we report the fabrication of TT-loaded polyanhydride microspheres, their in vitro antigen release kinetics, and the in vivo ability to induce an antigen-specific immune response. We observed a dose-dependent inhibition of the immune response by the polymer at high polymer doses, but as the polymer dose is reduced, the inhibition is eliminated and a stimulatory adjuvant effect is observed. No other adverse effects were observed, even when the immune response was inhibited. We also discuss the mechanism(s) that may govern the deviation of the immune response (T\textsubscript{H}1 v. T\textsubscript{H}2) as defined by changes in the TT specific IgG1 and IgG2a antibody responses. The microspheres are capable of inducing a combined T\textsubscript{H}1/T\textsubscript{H}2 immune response when injected intramuscularly, rather than the T\textsubscript{H}2 immune response that is typical of alum-based vaccines (4) and TT in particular (5). By injecting the microspheres along with a small bolus of unencapsulated TT, the T\textsubscript{H}2 immune response can be selectively inhibited without reducing the overall TT specific antibody production. The bolus alone is not sufficient to induce a measurable immune response. We discuss possible mechanisms involved in shaping the immune response.

8.3 Materials and Methods

8.3.1 Polymer synthesis and characterization

Poly(CPH-SA) (20:80) and poly(CPH-SA) (50:50) were synthesized by melt polycondensation from acetylated prepolymer as described previously (12). Gel permeation chromatography was performed on a Waters GPC system (Milford, MA) using PL Gel columns (Polymer Laboratories, Amherst, MA). The 20:80 copolymer had a weight average molecular weight (M\textsubscript{w}) of 21,000 and a polydispersity index (PDI) of 2.2. The 50:50 copolymer had an M\textsubscript{w} of 13,000 and a PDI of 2.0. Polymers were stored desiccated under dry argon.
8.3.2 Microsphere fabrication

Purified TT (1.5mg/ml, 490Lf/ml) was purchased from University of Massachusetts Biologic Laboratories (Jamaica Plain, MA). Protein was dialyzed against de-ionized water for 48 hours and lyophilized before encapsulation. TT-loaded microspheres were fabricated by a water/oil/oil double emulsion similar to the method reported by Esparza and Kissel for poly(D,L-lactide-co-glycolide) microspheres (21). Polymer (100 mg) was dissolved in methylene chloride (4 ml). Protein (4 mg) was dissolved in nanopure water (100 μl). The protein solution was added to the polymer solution in a 50 ml centrifuge tube and immediately emulsified by agitation at 15,000 rpm with a handheld homogenizer (Tissue-Tearor™, Biospec Products Inc., Bartlesville, OK) for one minute. While still homogenizing, 4 ml of Dow Corning Fluid, saturated with methylene chloride, was added drop wise to form the microspheres. Homogenization was continued for an additional minute. To precipitate the microspheres, the double emulsion was transferred to a 400 ml Berzelius beaker containing 300 ml n-heptane on an ice water bath. The heptane was stirred at 300 rpm using a Caframo overhead stirrer (Warrington, Ontario) with a three-inch impeller for three hours to extract the methylene chloride. Heptane was periodically added during the solvent removal to replace the volume lost due to evaporation. The microspheres were isolated by filtration using Whatman #50 filter paper. The beaker and impeller were rinsed several times with fresh heptane to maximize recovery. The microspheres were washed at least three times with 50 ml of heptane to rinse off residual Dow Corning fluid, and dried for 24 hours under vacuum. This procedure yielded a free-flowing powder with about 80% of the polymer mass being recovered. Blank microspheres were also fabricated by a similar technique that contained no inner water emulsion.
8.3.3  *In vitro TT release kinetics*

Microspheres (3 to 6 mg) were suspended in 2 to 3 ml of 0.1 M phosphate buffer (pH 7.4). Samples of the release buffer (100 μl) were taken periodically and the sample volume was replaced with fresh buffer. Release kinetics experiments were conducted in duplicate. Concentrations in the buffer solution were assessed by micro BCA Protein Assay (Pierce, Rockford, IL).

8.3.4  *In vivo inhibition of immune response*

Mice (3 C3H/HeOuJ mice per group, at least 8 weeks of age) were injected intramuscularly in the right quadriceps with unencapsulated TT and blank 20:80 poly(CPH-SA) microspheres both suspended in 50% cottonseed oil/saline emulsion. Doses for each group of mice are listed in Table 8.1. Groups I, II, and III received the same amount of TT (3 μg), and 3, 1, or 0.5 mg of polymer, respectively. Group IV received its TT injection (3 μg) in the same site as the microsphere injection (3 mg) three days later. Group V received only TT (3 μg) in the right leg and blank microspheres (3 mg) in left leg. Group VI received only TT (3 μg). Mice were sampled weekly by collecting 100 μl of blood from the saphenous vein. Serum was separated by centrifugation. TT specific antibody responses were assessed by enzyme-linked immunosorbant assay (ELISA) (see Section 8.3.6 below). Samples were collected from all three mice at each time point.
Table 8.1. Experimental groups in this study.

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</tr>
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</tr>
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Antibody response assay

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8.3.5 *In vivo antibody response to vaccine formulations*

Mice (4 to 8 C3H/HeOuJ mice per group, 8 weeks of age) were injected intramuscularly in the right quadriceps with either TT-loaded microspheres, TT-loaded microspheres plus a 0.5 μg bolus of TT, blank microspheres, or blank microspheres plus a 0.5 μg bolus of TT suspended in sterile saline containing 0.5% fetal calf serum (FCS). The treatments for each group are listed in Table 8.1. As controls, mice in group XV received a 0.5 μg bolus injection of TT without any microspheres. Mice were sampled weekly by collecting 100 μl of blood from the saphenous vein. Serum was separated by centrifugation. Antibody titers were determined by ELISA (see Section 8.3.6 below). Samples were collected from all mice at each time point.

8.3.6 *ELISA*

The ELISA for antibody titers was performed in 96-well format. Immulon II high binding plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated overnight with 100 μl of 1μg/ml TT in phosphate buffered saline (pH 7.4, PBS). Plates were washed with PBS containing 0.05% Tween 20 (PBST) and then blocked for two hours at room temperature with PBST containing 2% gelatin and 2% FCS. Serum samples from the individual mice were serially diluted in PBST with 2% FCS. Detection antibody was alkaline phosphatase-labeled affinity purified antibody to mouse IgG (H&L) (0.5 μg/ml) (KPL, Gaithersburg, MD). Phosphatase substrate (Sigma 104, phosphatase substrate, p-nitrophenyl phosphate, Sigma-Aldrich, Saint Louis, MO) was diluted to 1 mg/ml in sodium carbonate buffer (pH 9.3) and allowed to react for 1 hour. Plates were read using Spectramax 190 Plate Reader (Molecular Devices, Sunnyvale CA). ELISA to determine IgG2a and IgG1 antibody titers was
performed similarly. Detection antibodies were goat anti-mouse IgG1-AP and goat antimagne IgG2a-AP (Southern Biotechnology Associates, Birmingham, AL).

8.4 Results and Discussion

The TT-loaded microspheres had sizes ranging from 10 to 50 μm. Scanning electron micrographs of the TT-loaded microspheres are shown in Figure 8.2. The TT-loaded poly(CPH-SA) 20:80 microspheres have small (<1μm) particles flocculated on their surfaces. Blank microspheres did not exhibit this surface characteristic, nor did the TT-loaded poly(CPH-SA) 50:50 microspheres. We hypothesize that these surface particles are composed primarily of poly(CPH-SA) 20:80 polymer rather than protein as no significant burst is observed in the protein release profiles. The surfaces of some of the poly(CPH-SA) 50:50 microspheres have large circular divots, as observed in Figure 8.2. These are probably formed when microspheres fuse together during the solvent removal and subsequently break apart after drying. Poly(CPH-SA) 50:50 microspheres fused together in small clusters were observed by the scanning electron microscope (not shown). Other than these characteristics, all formulations resulted in microspheres with smooth surfaces, free from pores and cracks.

The in vitro release profiles for TT-loaded microspheres are shown in Figure 8.3. The loading efficiency was calculated from the total mass of protein released. The loading efficiency is 34% for the 20:80 copolymer and 51% for the 50:50 copolymer, leading to loading values of 1.4% and 2%, respectively. As anticipated, increasing the hydrophobicity of the polymer by increasing the CPH mole fraction decreases the release rate. Zero-order (uniform) release of the protein is obtained in both cases. The poly(CPH-SA) 20:80 copolymer releases greater than 90% of the protein in one week, while the poly(CPH-SA) 50:50 copolymer releases all the protein over a period of about 19 days.
Figure 8.2. TT-Loaded poly(CPH-SA) microspheres. 20:80 (top) and 50:50 (bottom).
Figure 8.3. *In vitro* TT release profiles for TT-loaded poly(CPH-SA) 20:80 (●) and 50:50 (▲) microspheres.

Figure 8.4 shows the results from the inhibition assay. Groups I, II, and IV show significant inhibition compared to groups III, V, and VI. This demonstrates that the polymer induces a localized, dose-dependent inhibition. When the polymer dose is reduced to 0.5 mg the local inhibition is eliminated. Note that when polymer and TT are delivered in separate injection sites (group V) the inhibition is nearly eliminated. This indicates that the inhibition is primarily local, rather than systemic, and may involve the recruitment of antigen presenting cells to the injection site. The antibody response at week two for group III is the same as that for group V at the same time point, and by week four is not significantly different from the group that received no polymer at all (group VI). Based on these results, 0.5 mg of polymer was chosen as the maximum dose for a single injection. This is also the dose extrapolated on an anticipated polyanhydride polymer weight per kilogram body weight
dose for human drug delivery (22). While several studies have been conducted to evaluate the effect of polyanhydrides on various tissues for therapeutic drug delivery (22-26), their effects on and engagement of the immune system have not yet been evaluated.

The secondary immune response is evaluated by ELISA detecting TT-specific IgG. Figure 8.5 shows the antibody titers for groups VII through XV over the twelve weeks of this study. All of the mice given only blank microspheres (groups VII and XI, open circles) show no significant response. When a 0.5 μg bolus of TT is delivered along with blank microspheres (groups VIII and XII, filled circles) a small response is observed, but in both cases is much greater than the response induced by the bolus alone (group XV, crosses). Thus, the polymer itself offers an adjuvant effect, provided it is delivered in small doses. Group XII exhibited a greater response than group VIII. The maximum titer for group XII was 8100 (week 6) and the maximum titer for group VIII was 2600 (week 7). We hypothesize that since the poly(CPH-SA) 50:50 microspheres likely remain at the injection site for a longer time (due to the increased hydrophobicity), the adjuvanticity of the polymer is enhanced. All of the mice receiving the TT-loaded microspheres (groups IX, X, XIII, and XIV, open and filled squares) exhibit a response. The responses of groups IX and XIII indicate that the protein is released from the microspheres in an immunogenic form. The prolonged exposure to immunogenic TT provided by the microspheres is sufficient to stimulate both a primary and secondary immune response, which is sustained over the 12 weeks of the study. Groups XIII and XIV have titers between 20,000 and 60,000 over the last five weeks and groups IX and X have titer values between 50,000 and 110,000 over the last five weeks.
Figure 8.4. Optical densities from ELISA at 1:400 dilution for inhibition assay at week 2 (a) and week 4 (b). Error bars indicate standard error. All groups received the same dose of unencapsulated TT (0.5 µg). Groups I, II, and III received 3, 1 and 0.5 mg of poly(CPH-SA) 20:80 blank microspheres, respectively, at the same injection site. Group IV received 3 mg of blank microspheres three days prior to the TT dose at the same injection site. Group V received 3 mg of microspheres in the opposite leg as the TT injection. Group VI received no microspheres. The treatments for each group are detailed in Table 8.1.
Figure 8.5. Average antibody titers for (a) groups VII (○) VIII (●) IX (□) and X (■), which received poly(CPH-SA) 20:80 microspheres, and (b) groups XI (○) XII (●) XIII (□) and XIV (■), which received poly(CPH-SA) (50:50) microspheres. Group XV, which received only a 0.5 µg bolus of unencapsulated TT, is shown in both plots (+). In both plots ○ represents blank microspheres only, ● represents blank microspheres plus the bolus of unencapsulated TT, □ represents the TT-loaded microspheres, and ■ represents the TT-loaded micropsheres plus the bolus of unencapsulated TT. Treatments for each group are detailed in Table 8.1.
The 0.5μg bolus of TT added to the treatments for groups X and XIV does not significantly affect the overall TT-specific IgG antibody titer compared to groups IX and XIII, respectively. However, it does alter the nature of the immune response in the case of group X. In order to evaluate the nature of the immune response, IgG1 and IgG2a antibody isotypes were assayed by ELISA at weeks zero, four, eight, and twelve for these four groups. Figure 8.6 shows the TT-specific antibody titers for IgG1 and IgG2a isotypes for groups IX, X, XIII, and XIV at four, eight and twelve weeks. For group IX, which received only TT-loaded poly(CPH-SA) 20:80 microspheres, a preference for IgG1 production is observed, indicating a Th2 dominant response. However, when the 0.5 μg bolus is delivered along with the microspheres (group X) the IgG1 production is inhibited. At week 12, the IgG1 antibody titer for group X is 76% lower than that for group IX at the same time point. The IgG2a antibody titer is not significantly altered by the addition of the bolus. In this case, the addition of the bolus has altered the nature of the immune response from a Th2 dominant response to a balanced Th2/Th1 response (i.e., Th0). Despite the reduction in IgG1 production observed for group X, the overall TT-specific IgG is not significantly altered by the addition of the bolus (Figure 8.5a). A hypothesis for the mechanism by which the immune response is altered is given below. Most vaccines currently approved for human use contain an alum-based adjuvant (27), which typically induces a Th2 dominant response (4). The Th1 immune response is a beneficial response for enhanced immunity to viral or other intracellular pathogens and the Th2 immune response has been implicated in the development of allergies. Thus, the ability to preferentially inhibit the Th2 immune response is a valuable and unique feature of this delivery vehicle.
Figure 8.6. TT-specific IgG1 and IgG2a antibody titers for (a) groups IX and X, which received poly(CPH-SA) (20:80) microspheres and (b) groups XIII and XIV, which received poly(CPH-SA) (50:50) microspheres. Groups IX and XIII received only TT-loaded microspheres. Groups X and XIV received TT-loaded microspheres with a 0.5 μg bolus of unencapsulated TT. Treatments for each group are detailed in Table 8.1.
Groups XIII and XIV, which received the poly(CPH-SA) 50:50 microspheres, without and with the 0.5 μg bolus, respectively, show significantly weaker IgG1 and IgG2a production compared to groups IX and X, which is consistent with the total TT-specific IgG titers shown in Figure 8.5. The bolus administered to group XIV does not significantly alter the nature of the immune response as it does in the case of group X.

The exact mechanism by which the bolus affects the nature of the immune response for group X is not clearly understood. Figure 8.7 illustrates possible differences in the immune response for groups IX, X, and mice receiving an equivalent dose of unencapsulated TT. Immediately following the administration of any of the three immunization regimen, inflammatory chemokines are produced at the injection site as a result of the injected materials (i.e., physical trauma), which results in the recruitment of macrophages, neutrophils, and dendritic cells. Dendritic cells can phagocytose microspheres (shown in Figure 8.7a. and b.) and pinocytose unencapsulated TT (shown in Figure 8.7b and c). When only microspheres are phagocytosed in the absence of unencapsulated TT (Figure 8.7a), the migration of the dendritic cells to the lymph node is delayed because the microspheres are processed as particulate antigen (28). But when unencapsulated TT is present (Figure 8.7b and c) the dendritic cells migrate to the lymph node more rapidly. When the dendritic cells reach the lymph node they stimulate either T<sub>H1</sub> cells, T<sub>H2</sub> cells, or both. When only microspheres are delivered and the migration to the lymph node is delayed, the microspheres are allowed to degrade so that some dendritic cells either pick up released protein or have phagocytosed microspheres that subsequently degrade prior to the complete maturation of the dendritic cell. Delay in the migration of the dendritic cells also permits the inflammatory response to wane, thus hindering DC activation and migration. When the dendritic cell reaches the lymph node, the type of T helper cell stimulated is primarily antigen driven, as
most of the microspheres have degraded. Since TT preferentially induces the T_{H2} immune response, the production of IgG1 dominates. When only unencapsulated TT is administered (Figure 8.7c), the migration of the dendritic cells is not delayed, and again, the immune response is antigen driven leading to a T_{H2} dominant immune response, which we and others have observed (data not shown) (5). However, when both unencapsulated TT and TT-loaded microspheres are delivered, the migration of the dendritic cells to the lymph node is uninhibited because some dendritic cells have pinocytosed unencapsulated antigen. In this case, some dendritic cells reach the lymph node with intact microspheres. These dendritic cells process the TT in the context of particulate antigen and produce interleukin 12 (IL-12). IL-12 preferentially stimulates T_{H1} cells that produce interferon γ (INF-γ) in sufficient quantity to suppress IgG1 production. In this case, both IgG1 (not shown in Figure 8.7b) and IgG2a are produced and neither isotype dominates, leading to a T_{H0} phenotype.

Based on the results shown in Figure 8.6b, we hypothesize that the 50:50 microspheres do not release sufficient immunogenic protein in a short enough time span to induce a strong T-cell response. The weak T-cell response is characterized by no preference for IgG1 or IgG2a production and weak affinity antibody. This would be consistent with the lower antibody titers depicted in Figures 8.5 and 8.6 for groups XIII and XIV (i.e., the groups receiving the poly(CPH-SA) 50:50 microspheres) compared to groups IX and X (i.e., the groups receiving the poly(CPH-SA 20:80 microspheres).
Figure 8.7. Hypothesized immune response mechanism for (a) TT-loaded poly(CPH-SA) 20:80 microspheres, (b) TT-loaded poly(CPH-SA) microspheres with a bolus of unencapsulated TT, and (c) unencapsulated TT alone.
Migration to lymph node enabled as microspheres degrade

Figure 8.7. (Continued.)
8.5 Conclusions

Tetanus toxoid was successfully encapsulated in and released from polyanhydride microspheres. Smooth microspheres in the form of a free flowing powder are obtained from a water/oil/oil double emulsion technique. Altering the copolymer composition modulates the in vitro release kinetics and protein that is released maintains its immunogenicity and antigenicity. The polymer itself has an adjuvant effect, enhancing the immune response to a small dose of TT, but as the dose of polymer is increased a localized, dose-dependent inhibition is observed. The sustained release of the antigenic protein is essential for the efficacy of a single-dose vaccine. The microspheres prolonged the exposure to immunogenic/antigenic TT sufficiently to induce the secondary immune response, without requiring an additional administration of the vaccine.

In addition to providing an effective single dose vaccination regimen, the microsphere delivery vehicle offers the opportunity to select the preferred immune response pathway. The poly(CPH:SA) 20:80 microsphere formulations induce both the Th1 and Th2 immune response pathways. When the microspheres are delivered alone, the immune response appears to be antigen driven, leading to a Th2 dominant response for TT. However, when the microspheres are delivered along with a small bolus of unencapsulated TT, the Th2 immune response is selectively inhibited, leading to a combined Th rather than the Th2 immune response that is typical of alum based vaccines and of TT in particular. The overall TT-specific IgG is unaffected by the isotype switch. The preferential reduction of the Th2 immune response and the ability to induce a balanced immune response is a unique and valuable feature of this delivery vehicle that may make it an excellent candidate for developing vaccines to intracellular pathogens including HIV/AIDS. Though it is not clear exactly how the antigen presenting cells are processing the encapsulated antigen resulting in
different immune responses, the immune response may be governed by the combination of
the microsphere release kinetics and the propensity for dendritic cell migration from the
injection site to be delayed in the context of particulate antigen.

The poly(CPH-SA) 50:50 microspheres induce a weak T-cell response, characterized
by no preference for IgG1 or IgG2a production and weak affinity antibody. This could either
be due to poor stabilization of the antigen, or the prolonged slower release leading to the
induction of tolerance.

Further investigation of the interactions between polyanhydride microspheres and
lymphocytes will improve our understanding of the immune response mechanism, and guide
the design of controlled release vaccines that selectively induce the desired immune
response.

8.6 References
1. Foundation for the National Institutes of Health, *Grand Challenges in Global Health*


CHAPTER 9
MECHANISTIC UNDERSTANDING OF DEGRADATION IN BIOERODIBLE POLYMERS FOR DRUG DELIVERY


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9.1 Abstract

A new model has been developed to understand the mechanism of erosion in bioerodible polymers. A fundamental understanding of the mechanism of erosion in these polymers is essential to accurately predict drug release and precisely design controlled release devices. This model takes into account the phenomenon of microphase separation that has been observed for polyanhydrides of certain copolymer compositions. The model assumes that erosion is dominated by degradation and thus, in a system with a fast eroding and a slow eroding species, two rate constants, one for each species, essentially control the evolution of the polymer microstructure. Expressions have been derived for the fraction of each monomer released as well as for the porosity in the system. A partition coefficient accounts for thermodynamic partitioning of drug into the micro-domains. The solutions of the model equations have been fitted to experimental data on monomer release kinetics from two polyanhydride systems to obtain the erosion rate constants. Drug release kinetics

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experiments are compared to the model solution for drug release and the partition coefficient of the drug is obtained from the fits. The comparisons to the data are promising, while pointing out the limitations of the model. The model does not account for oligomer formation prior to monomer release or for the dependence of the rate constants on parameters such as the degree of crystallinity, the local pH, and the polymer molecular weight.

Keywords: Degradation, Bioerodible, Mathematical Model, Polyanhydrides, Drug Release

9.2 Introduction

The use of biodegradable polymers as carriers for controlled release systems has been the object of research over the past two decades due to their advantages over other competing systems. The biodegradable structure of the polymer obviates the need to surgically remove the device. Biocompatibility of the degradation products has also been established. For example, the FDA has approved the use of the copolymer, poly[1,3-bis(p-carboxyphenoxy)propane-co-sebacic anhydride], p(CPP-SA) 20:80, for human use, and the delivery of anti-cancer proteins has been reported (Dang et al., 1996).

For clarity, it is important to distinguish between the terms “degradation” and “erosion”. The term “degradation” refers to the chain scission process by which polymer chains are cleaved into oligomer or monomer units. The term “erosion” refers to mass loss from the bulk polymer. In other words, erosion could be considered as the sum of several elementary processes, one of which is degradation.

The pioneering work of Langer, Mathiowitz, and co-workers (Leong et al., 1985; Leong et al., 1986; Mathiowitz and Langer, 1987; Chasin et al., 1988; Mathiowitz et al., 1988; Mathiowitz et al., 1990a; Mathiowitz et al., 1990b; Tamada and Langer, 1992; Shieh
et al., 1994; Goepferich and Langer, 1995) has established the potential of biodegradable polyanhydrides based on aromatic and aliphatic dicarboxylic acids as carriers for therapeutic compounds. This class of polyanhydrides consists of a backbone of monomeric carboxylic diacids joined by anhydride linkages; these bonds are hydrolyzed upon exposure to an aqueous environment, forming water-soluble monomeric degradation products. In spite of the hydrophilicity of the characteristic anhydride functional group, the presence of aromatic and aliphatic groups in the monomer confers a high hydrophobicity to the polymer. As a consequence, water is prevented from penetrating into the bulk of the polymeric device causing the erosion process to occur on the surface of the device (Goepferich, 1996a; Goepferich, 1996b). This is in contrast to “bulk-erodible” polymers such as poly(lactic acid), poly(glycolic acid), and their copolymers, in which water penetrates into the bulk of the polymer (Langer, 1990; Peppas and Langer, 1994). The polyanhydride system is extremely versatile due to the differences in the homopolymer erosion rates; in fact, by simply employing different co-monomer ratios, the erosion times can be varied from a few weeks to a few years (Leong et al., 1985).

Several studies report in vitro erosion of copolymeric polyanhydrides. Some of the earlier work with this system reported only total mass loss (Leong et al., 1985; Mathiowitz and Langer, 1987; Chasin et al., 1988; Mathiowitz et al., 1988; Mathiowitz et al., 1990a; Tamada and Langer, 1992; Shieh et al., 1994); but, more recently, individual monomer release has been reported because this information provides a more detailed view of the mechanism of degradation (Tamada and Langer, 1990; Shen et al., 2002). Recent studies conducted by our group have shown that depending on the copolymer composition, the system based on sebacic anhydride (SA) and 1,6-bis(p-carboxyphenoxy)hexane (CPH) (see Figure 9.1) undergoes microphase separation (Shen et al., 2001). For compositions that
contain one component in excess of ~80 mol%, we have shown earlier, using a combination of nuclear magnetic resonance (NMR), atomic force microscopy (AFM), differential scanning calorimetry (DSC), and wide angle X-ray diffraction (WAXD), that the polymer microstructure exhibits microphase separation. The presence of microphase separation in such systems is due to a combination of two factors. First, the reactivity ratio of the co-monomers, SA and CPH, are both close to unity (Ron et al., 1991; Shen et al., 2002), and hence, if there is an excess of the SA component, SA-SA bonds are more abundant than SA-CPH or CPH-CPH bonds. Second, the relative hydrophobicity of the two co-monomers are different (manifested by their vastly differing degradation rates), and thus in situations where, for example, when SA-SA bonds are numerous, the fewer CPH moieties thermodynamically prefer to form "micro-domains" within a matrix of SA. Thus, on a microscopic scale, two equilibrium phases are formed with each phase containing predominantly one constituent. This structure is represented schematically in Figure 9.2.

Figure 9.1. Chemical structures of CPH (top) and SA (bottom) repeat units.
Furthermore, it can be argued that if there is microphase separation in polyanhydrides of certain copolymer composition, drugs loaded into such polymers may thermodynamically partition into one phase or the other, depending on the compatibility of the drug with the constituent monomers. Careful examination of the literature on *in vitro* drug release from polyanhydrides of specific compositions reveals that drug release profiles (Mathiowitz and Langer, 1987; Chasin et al., 1988) do not match polymer erosion profiles over the *entire duration* of release. In fact, drug release profiles match individual monomer release profiles depending on their compatibility to the monomer phase. Thus for example, the release of *p*-nitroaniline (PNA), which is mainly dissolved in the slowly eroding phase (CPP or CPH), mimics the erosion profile of that phase once the fast eroding phase (SA) is dissolved (Shen et al., 2002). Qualitatively similar inferences can be drawn by observation of release data of other compounds loaded into copolymers of the same family (Leong et al., 1985; Mathiowitz and Langer, 1987; Chasin et al., 1988; Mathiowitz et al., 1988; Mathiowitz et al., 1990a;
Mathiowitz et al., 1992; Shieh et al., 1994; Chickering et al., 1996; Olivi et al., 1996; Kuntz and Saltzman, 1997; Park et al., 1997; Park et al., 1998).

A fundamental understanding of the mechanism of degradation is essential to accurately predict drug release and precisely design controlled release devices. The development of a preliminary model that accounts for both microphase separation and drug partitioning is the main subject of this paper. The next section provides a brief summary of current models that describe polymer degradation and drug release. The following two sections present the assumptions and the salient features of the new model, and discuss the estimation of key model parameters from comparisons to experimental data, limitations of the model, and suggestions for improvements. The last section highlights the main conclusions of this work.

9.3 Previous Models

One of the first mathematical models describing the diffusive release of a drug from a polymeric device undergoing surface erosion was developed by Thombre and Himmelstein (Thombre and Himmelstein, 1984). They assumed that the erosion front moves with a zero-order velocity and that Fickian diffusion occurs in the non-eroded zone under pseudo-steady state conditions. Consequently, two boundaries simultaneously move from the surface of the device to its center. This analysis, which was also extended to similar devices with a secondary membrane, fundamentally aimed to characterize the concentration profile of the drug inside the device and was able to predict fractional mass loss based on the drug concentration profile within the polymer. The same authors (Thombre and Himmelstein, 1985) have developed another model, which accounts for diffusion of all the species involved in the process. In addition, autocatalytic reaction of the polymer during degradation is taken
into account. The system studied was made of a poly(orthoester) matrix in which an acid anhydride and a bioactive agent are uniformly distributed. The presence of the acid anhydride catalyzed degradation of the polymer matrix according to an elementary three-step reaction, which ultimately hydrolyzed the polymer.

A different approach to understand the mechanism of degradation was proposed by Zygourakis (Zygourakis, 1990; Zygourakis and Markenscoff, 1996). He used the cellular automata method, first discretizing the system into cells, each one defined as either polymer or drug. Furthermore, each type of cell was characterized by a time constant for dissolution upon exposure to solvent. Dissolution time steps were then iterated to compute the temporal evolution of the erosion front and drug release. The resulting profiles showed almost linear fractional mass release profiles in contrast with the sigmoidal release that many experimental data show (Chasin et al., 1988; Mathiowitz et al., 1988; Shieh et al., 1994; Park et al., 1998; Shen et al., 2002).

Goepferich and Langer (Goepferich and Langer, 1993) developed a stochastic model using Monte Carlo methods that considers the presence of multiple phases in the polymer matrix, which was otherwise similar to the cellular automata approach of Zygourakis. An important similarity between the models of Zygourakis and Goepferich and Langer is that erosion is considered an elementary process. The models differ in that Goepferich and Langer assumed finite probabilities for each erosion event, instead of assigning a constant velocity for erosion, and did not consider drug loading. Their model was applied to polyanhydrides using two types of polymer cells with different characteristic erosion times corresponding to crystalline and amorphous zones. The time constants were evaluated by fitting to experimental in vitro erosion data, and not explicitly correlated to physical processes such as hydrolysis, dissolution, and subsequent diffusion. Goepferich and Langer
further developed this model (Goepferich and Langer, 1995) to include monomer diffusion through the resulting porous network.

More recently, a quantitative model was developed by Batycky et al. (Batycky et al., 1997) to predict mass loss, molecular weight change, and macromolecular drug release as a function of time for the case of bulk eroding microspheres. In this model, the two mechanisms of chemical degradation, *i.e.*, random chain scission and end scission, were combined to obtain a more accurate prediction of the observed mass and molecular weight evolution. Two (coupled) drug release processes are accounted for: desorption from the surface in contact with the buffer solution, and Fickian diffusion through mesopores. The desorption leads to a burst release from the initially exposed surface in contact with the buffer solution. A subsequent lag time in the drug release is attributed to the induction time associated with the growth of micropores and their coalescence to form mesopores sufficiently large for release of additional macromolecular drug.

It is worthwhile to note that all the models described above were developed for polymers with homogenous composition; thus, the drug is uniformly distributed throughout the polymer matrix, and there is no thermodynamic partitioning. One model that does account for partitioning was developed by Varelas et al. (Varelas et al., 1995a; Varelas et al., 1995b). In this model, drug is encapsulated in a polymeric dispersed phase and diffuses through a continuous phase. The continuous phase is a hydrogel in which the drug is only slightly soluble. Thus, the dispersed phase acts as a reservoir, a pseudo-steady state is obtained during release, and release is zero-order. The device is not bioerodible.

The existence of microphase separation in polymers and consequent partitioning of drugs into the micro-domains necessitates the development of new models that incorporate these phenomena into the analysis. This is the main goal of the current work. In addition,
physical significance will be assigned to model parameters by linking them to elementary physical processes. Furthermore, the parameters will be compared to independent experiments to verify the validity of the assumptions intrinsic to the model.

9.4 Model Development

In general, polymer erosion is a complex phenomenon that depends on many factors such as: polymer degradation, polymer chemistry and composition, crystallinity, hydrophobicity, polymer molecular weight, and diffusivity of monomers and oligomers. Each of these factors plays a different role in determining the rate of erosion. Schematically the erosion process is composed of the following elementary steps:

1) Water ingress: Initially the polymeric device is exposed to an aqueous buffer solution, which leads to water ingress. Two kinds of degradation mechanisms can be distinguished: bulk and surface, depending on the polymer hydrophobicity.

2) Chemical degradation: The water randomly breaks the chemical bonds in the polymer and produces the constituent monomers and oligomers.

3) Dissolution: Finally, the monomers dissolve in the buffer and diffuse through the device into the bulk.

Thus, the erosion process could be viewed as the sum of different elementary processes, each characterized by a time constant. The analysis of these processes with the associated time constants allows us to discern which ones might be rate limiting and, consequently, which could be ignored in order to achieve a simplified model.
A new model has been developed that describes the surface erosion of biodegradable polymers comprising of microphase-separated domains. We have chosen the copolymeric polyanhydride system based on SA and CPP (or CPH) as a starting point. In this system, microphase separation occurs when one component of the copolymer is present in excess (i.e., > 80 mol%) (Shen et al., 2001).

We have recently performed experiments (Shen et al., 2002) in which we analyzed the release of each the constituent monomers of the CPH-SA system, in addition to the overall polymer erosion. The system consisted of a tablet whose thickness was small compared to the diameter (aspect ratio ~10), so that one-dimensional transport could be assumed. The coordinate system of choice has its origin at the center of the tablet of total thickness 2L. Symmetry enables the development of model equations for 0 ≤ x ≤ L. From these experiments, we know that:

1) the thickness of the matrices prepared from these copolymers does not change for several days during erosion;
2) the erosion front that separates the eroding and the non-eroded zones always moves parallel to the polymer surface that is exposed to the buffer solution (see Figure 9.3).
3) the hydrophobic nature of the copolymers does not allow water to penetrate into the core of the tablet; thus, degradation occurs on the surface.

Additionally, the rate of scission of the bonds for the copolymer p(SA-CPP) was recently evaluated using NMR spectroscopy (Heatley et al., 1998; McCann et al., 1999). The results showed that the degradation rate of the SA-SA bond is of the same order of magnitude as that of the CPP-SA bond and two orders of magnitude less than that of the CPP-CPP bond.
Starting from these considerations it is clear that the reaction/transport process that takes place within the copolymer is essentially governed by two different time constants, one associated with SA erosion ("fast" eroding species) and the other with CPP or CPH erosion ("slow" eroding species). For the sake of simplicity, we will refer to the fast eroding species (SA monomer) as A and the slowly eroding species (CPH or CPP monomer) as B.

As reported previously (Goepferich and Langer, 1995; Goepferich, 1996a; Goepferich, 1996b), after a short period of time referred to as the "induction period," which is attributed to several phenomena (the most important of which is the initial appearance of the monomer), the erosion of A proceeds at constant velocity, independent of the A concentration outside the tablet. This indicates that the diffusion rate of A through and the dissolution rate of A within the eroding zone of the tablet may play a minor role in determining the overall erosion kinetics. From these observations, we assume as a first
approximation that the erosion process of each constituent of the copolymer is controlled by the degradation of the corresponding monomer-monomer bond. We note however, that Goepferich and Langer also showed considerable build-up of monomer crystals near the surface of the polymer during erosion, indicating that polymer degradation and monomer dissolution may not occur simultaneously (Goepferich and Langer, 1993). If the drug release kinetics can be well correlated to monomer release, the need to treat monomer crystallization and dissolution in the foregoing model is obviated. Thus, these effects can be essentially lumped into an erosion rate constant. The erosion of species A can be described by a surface zero-order reaction:

\[
\frac{dm_A}{dt} = M_A S' \phi_{A0} (1 - p) k'_A
\]

In equation 9.1, \( m_A \) is the mass of species A lost from the tablet; \( M_A \) is the molecular weight of species A; \( S' \) is the effective surface area in contact with the buffer solution; \( \phi_{A0} \) is the surface fraction of A domains; \( k'_A \) is the molar average surface erosion rate; and \( p \) is the mole fraction of B present in the A domains. The erosion rate constant, \( k'_A \), is assumed to be constant, though slow diffusion of polymer degradation products may have a considerable effect on the local pH as reported by Mäder et al., accelerating erosion (Mäder et al., 1997).

Previous studies using atomic force microscopy (AFM) are in agreement with the result predicted from theoretical calculations of the phase diagram that a small fraction of the constituent of the dispersed phase may be present within the matrix phase (Shen et al., 2001). In the absence of experimental data, the parameter \( \phi_{A0} \) can be estimated as the volume fraction of the A domains and \( p \) can be estimated by considering the probability of finding an A-B bond in a randomly distributed medium of A-A, A-B, and B-B bonds.
In order to model the polymer surface, we hypothesize that $S'$ is the product of the cross sectional area $S$ and a constant term $r$ that accounts for the roughness of the surface. In general, the surface roughness changes continuously during erosion so the assumption is only valid in the limit of short induction periods and for pseudo-steady state conditions. Since $S'$ is written as $Sr$ and the term $r$ is independent of the geometrical configuration of the tablet, it can be combined with the $k_A'$ constant, leading to a single constant $k_A$ that contains all the information about the erosion process (i.e., $k_A = k_A' r$). Thus, we can define the molar average surface erosion rate, $k_A$, as the rate at which the erosion front $L$ is moving inside the tablet. Hence, we have:

$$k_A = \left[ (1-p) \frac{\rho_A}{M_A} + p \frac{\rho_B}{M_B} \right] \frac{dL}{dt} = \overline{\rho} \frac{dL}{dt} \quad (9.2)$$

Here $\overline{\rho}$ is the average molar density of the A domains, and $\rho_A$ and $\rho_B$ are the mass densities of A and B respectively. (The units of $k_A$ are moles per area per time.) By integration of equation 9.2 with the condition that at $t = 0$, $L = L_0$, the position of the erosion front as function of time (for constant $k_A$) is:

$$L = L_0 - \frac{k_A}{\overline{\rho}} t \quad (9.3)$$

This equation is valid for $0 \leq t \leq t_A$, where $t_A$ is the time required for all of the A phase (the fast-eroding phase) to erode. This is the time at which the erosion front reaches the center of the tablet. This time constant $t_A$ can be calculated by setting $L = 0$ in equation 9.3.

$$t_A = \frac{L_0 \overline{\rho}}{k_A} \quad (9.4)$$
Integrating equation 9.1, the mass of A lost during erosion of the A domains is:

\[ m_A = M_A S \phi_{A0} (1-p) k_A t \]  \hspace{1cm} (9.5)

The corresponding transport equation that describes the release of species B from the A domains is:

\[ m_B = M_B S \phi_{A0} p k_A t \]  \hspace{1cm} (9.6)

Analogous to the model equations for the fast eroding zone, transport equations can be written for the slowly eroding zone, considering a zero order reaction for the degradation of B. In this case, the disappearance of the A domains and the shrinking of the B domains induces a change in the B surface area exposed to the buffer; therefore, the porosity of the tablet \( \varepsilon(x,t) \) continuously varies with both time and position (i.e., along the tablet thickness). To model the porosity, a mass balance for species B in a differential element \( dx \) positioned at distance \( x \) from the center of the tablet is written:

\[ \varepsilon(x,t) = \phi_{A0} + \int_{(L_0-x)/2}^{(L_0-x)/2} \frac{k_B M_B}{\rho_B} \sigma(x,t') \cdot dt' \]  \hspace{1cm} (9.7)

Here \( k_B \) is the surface erosion rate associated with the scission of B-B bonds and dissolution of the B monomer and \( \sigma(x,t) \) represents the surface area of B in contact with the buffer per
unit volume. To find an expression for \( \sigma(x,t) \), the B phase is modeled as spheres with an average diameter \( d_0 \) (note that this is a surface-area average), for each infinitesimal volume \( Sdx \). It is important to recognize that these spheres could be interconnected or disconnected. If they are interconnected, the device retains its mechanical integrity; if not, the spheres erode away into the surrounding buffer, which acts as an infinite medium, and degrade. Then, \( \sigma(x,t) \) becomes a function of time through the cube root of the porosity and is given by:

\[
\sigma = \frac{6\phi_{A0}^{1/3}}{d_0} \varepsilon^{2/3}(1-\varepsilon) \quad (9.8)
\]

Applying the Leibniz rule to integrate equation 9.7 and imposing the condition that at time \( t = 0+ \) the porosity in the infinitesimal volume \( Sdx \) is equal to the volume fraction of A domains, we obtain an analytical expression for the porosity \( \varepsilon(x,t) \):

\[
\varepsilon(x,t) = 1 - \phi_{B0} \left[ 1 - \alpha \left( t - \frac{(L_0 - x)\rho}{k_A} \right) \right]^{3} \quad (9.9)
\]

It is important to note that the functionality for \( \varepsilon \) with respect to the spatial dimension \( x \) is obtained considering that A domains between \( x = L_0 \) and the surface at \( x \) must completely degrade for the surface at \( x \) to be exposed to the water. Here, \( \alpha \) is defined as:

\[
\alpha = \frac{2k_B M_B}{d_0\rho_B} \quad (9.10)
\]

---

\( ^7 \) For spherical domains, \( \sigma = \frac{6}{d} \) and \( \frac{d}{d_0} = \left( \frac{1-\varepsilon}{\varepsilon_{B0}} \right)^{1/3} \) where subscript 0 indicates initial condition.
The term $\alpha$ represents a time constant for the degradation of phase B with dimensions of reciprocal time. As a consequence, equation 9.9 is valid for values of $x$ and $t$ such that $\varepsilon$ is always greater than zero. In other words, the B domains present in each of the infinitesimal volumes of the tablet erode after being in contact with water for a time equal to $t_B$. Additionally, $t_B$ can be viewed as the time at which the tablet starts to shrink, i.e., when $x = L_0$, the time at which $\varepsilon = 1$ is $t_B$ and is given by $t_B = 1/\alpha$. Thus, the total time required for the complete erosion of the tablet is the sum of the two time constants, $t_A$ and $t_B$.

It is instructive to recognize that the condition $t_A > t_B$ could also occur in the systems under consideration. For this condition to occur, $d_0$ must be decreased or $L_0$ must be increased. Decreasing $d_0$ causes the spherical domains to be unconnected, thus compromising the mechanical integrity of the device; increasing $L_0$ may violate the assumptions of no diffusion control and one-dimensional transport; hence, this case is not considered here. Thus, $t_A$ is always less than $t_B$ in this work.

### 9.5 Results and Discussion

#### 9.5.1 Model solution

When $t_A < t_B$, three different regimes $[0, t_A] \ [t_A, t_B] \ [t_A, t_A + t_B]$ may be distinguished. For each of these regimes, the mass of B eroded can be calculated by integration with respect to position ($x \in [0, L_0]$) and time ($t \in [0, t]$) of the mass balance of B species in B domains.

\[
m_B = \frac{\beta t}{3 \alpha} - \frac{\beta}{12 \alpha^2} \left[1 - (1 - \alpha t)^4 \right] \quad t \leq t_A
\]  

(9.11)

\[
m_B = \frac{\beta t}{3 \alpha} - \frac{\beta}{12 \alpha^2} \left[(1 - \alpha (t - t_A))^4 - (1 - \alpha t)^4 \right] \quad t_A \leq t \leq t_B
\]  

(9.12)
Adding the mass of B lost from the A domains and normalizing with respect to the initial mass of B in the tablet, the mass fraction of B eroded can be obtained. Finally, the mass fractions of A \( x_A \) and B monomer \( x_B \) released from the tablet during the three regimes are:

\[
x_A = \frac{m_A}{m_{A0}} = \frac{t}{t_A} \quad t \leq t_A
\]

\[
x_B = \frac{m_B}{m_{B0}} = \frac{t}{t_A} - \delta \cdot \left[ 1 - (1 - \alpha)^t \right] \quad t \leq t_B
\]

\[
x_B = \frac{m_B}{m_{B0}} = 1 - \delta \cdot \left[ (1 - \alpha(t - t_A))^{t_A} - (1 - \alpha)^t \right] \quad t_A \leq t \leq t_B
\]

\[
x_B = \frac{m_B}{m_{B0}} = 1 - \delta \cdot \left[ 1 - \alpha(t - t_A)^t \right] \quad t_A \leq t \leq t_B + t_A
\]

\[
\delta = \frac{\beta}{12\alpha^2}
\]

From the above equations, it is clear that in the first regime, the erosion rate of B increases with the exposed surface, and reaches a maximum by the time all of the A is eroded. In the second regime, the erosion rate of B decreases as consequence of the decreasing exposed surface; and, at the end, in the third regime the mass fraction of B eroded approaches unity with zero slope.
9.5.2 CPP-SA and CPH-SA 20:80 erosion

Equations 9.15-9.18 can be used to fit monomer release data from CPP-SA 20:80 (or CPH-SA 20:80) copolymers. From the fit of equation 9.15 to SA release data, the SA erosion rate $k_A$ can be obtained. Once $k_A$ is obtained, $t_A$ can be calculated using equation 9.4. Knowing $t_A$, the fit of equation 9.16 to CPP (or CPH) release data in the first regime ($t \leq t_A$) can be used to obtain the CPP (or CPH) erosion rate, $k_B$. Once both $k_A$ and $k_B$ are known, the CPH release profiles in regimes 2 and 3 can be predicted by the model using equations 9.17 and 9.18. The parameters that are known in this analysis are $L_0$, $d_0$, $p$, $\rho_A$, $\rho_B$, $M_A$, $M_B$, and the copolymer composition. From AFM experiments (Shen et al., 2001), the value of $d_0$ is estimated to be of the order of 25nm. The value of $p$ has been chosen to be 0.07 based on image analysis of the surface fraction of SA and CPH domains in the AFM experiments of Shen et al. (Shen et al., 2001). The density values have been taken from Mathiowitz et al. (Mathiowitz et al., 1990b). The values of these parameters for the two experimental systems considered below are shown in Table 9.1.

A combination of UV-Vis spectrophotometry and high pressure liquid chromatography can be used to measure the release of the individual monomers, SA and CPP (or CPH) (Tamada and Langer, 1990). Using these methods, Tamada and Langer have obtained release data for SA and CPP release from CPP-SA 20:80 copolymer tablets degrading in phosphate buffered saline solution (pH = 7.4) at 37°C (Tamada and Langer, 1990; Tamada and Langer, 1992). The SA release data (open circles) is fit with equation 9.15 and is shown in Figure 9.4. The value of $k_A$ obtained from this fit is $1.1 \times 10^{-4}$ mol cm$^{-2}$ day$^{-1}$. Using this value of $k_A$, $t_A$ for this system is calculated from equation 9.4 as 3.3 days. The CPP release data (open squares) for $t \leq 3.3$ days is fit with equation 9.16 and is also
shown in Figure 9.4. The value of $k_B$ obtained from this fit is $2.2 \times 10^{-9} \text{ mol cm}^{-2} \text{ day}^{-1}$. As expected, the SA erosion rate is much higher (~5 orders of magnitude) than the CPP erosion rate. It is instructive to note here that $k_A$ represents the erosion rate of both SA-SA and SA-CPP bonds. Using the values of $k_B$ just obtained, the value of $t_B$ can be calculated using $t_B = 1/\alpha$, and for this system, $t_B = 8.4$ days. Thus, the total time required for erosion of the CPP domains should be $t_A + t_B = 11.7$ days. In addition, the CPP release for regimes 2 and 3 can be predicted using equations 9.17 and 9.18. These predictions are compared to the experimental release data in Figure 9.4 and as shown, the agreement is excellent. It is important to note that the theoretical CPP release profiles in regimes 2 and 3 and the total CPP release time are predicted from the model once $k_A$ and $k_B$ are known.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_0$</td>
<td>0.075cm</td>
</tr>
<tr>
<td>$d_0$</td>
<td>25nm</td>
</tr>
<tr>
<td>$\rho$</td>
<td>0.07</td>
</tr>
<tr>
<td>$\rho_A$</td>
<td>1.1g/cm$^3$</td>
</tr>
<tr>
<td>$\rho_B$</td>
<td>1.1g/cm$^3$</td>
</tr>
<tr>
<td>$M_A$</td>
<td>182</td>
</tr>
<tr>
<td>$M_{B,\text{CPH}}$</td>
<td>342</td>
</tr>
<tr>
<td>$M_{B,\text{CPP}}$</td>
<td>300</td>
</tr>
</tbody>
</table>
Figure 9.4. Comparison of the model with experimental results for CPP (□) and SA (○) monomer release for the erosion of CPP-SA 20:80 tablets (Tamada and Langer, 1990). The solid lines represent model fits to monomer release (equations 9.15-9.18).

We have extended the method developed by Tamada and Langer to monitor individual release of SA and CPH monomer from tablets of CPH-SA 20:80 copolymers (Shen et al., 2002). The details of the experimental techniques are described elsewhere (Shen et al., 2002). The theoretical fits of individual monomer release from the current model and the experimental data are shown in Figure 9.5. The value of $k_A$ obtained from this fit (using equation 9.15) is $2.2 \times 10^{-4}$ mol cm$^{-2}$ day$^{-1}$. Using this value of $k_A$, $t_A$ for this system is calculated from equation 9.3 as 2.0 days. It is instructive to note that the value of $k_A$ obtained from this fit is of the same order of magnitude as that obtained from the SA released in the CPP-SA 20:80 system (see Table 9.2 for a summary of the calculated parameters of the CPP-SA 20:80 and CPH-SA 20:80 systems). The CPH release data (open
squares) for \( t \leq 2.0 \) days is fit with equation 9.16 and is also shown in Figure 9.5. The value of \( k_B \) obtained from this fit is \( 5.2 \times 10^{-10} \text{ mol cm}^{-2} \text{ day}^{-1} \). Once again, the SA erosion rate is much higher (>5 orders of magnitude) than the CPP erosion rate. In addition, the CPH erosion rate is slower than the CPP erosion rate (see Table 9.2). This is expected since CPH is more hydrophobic than CPP (see Figure 9.1). Using the value of \( k_B \) just obtained, the value of \( t_B \) can be calculated using \( t_B = 1/\alpha \), and for the CPH-SA system, \( t_B = 7.7 \) days. Thus, the total time required for erosion of the CPH domains should be \( t_A + t_B = 9.7 \) days. In addition, the CPH release for regimes 2 and 3 can be predicted using equations 9.17 and 9.18. These predictions are compared to the experimental release data in Figure 9.5 and as shown, the model correctly predicts the shape of the release profile. However, the model over-predicts the CPH release in the second and third regimes. The reasons for this discrepancy are discussed later.

Using the values of \( k_A \) and \( k_B \), the porosity, \( \varepsilon \), in the CPH-SA system can be plotted as a function of both position and time using equation 9.9. This porosity profile is shown in Figure 9.6. As expected, \( \varepsilon \) approaches 1 at the surface \( (x = L_0) \) at \( t = t_A \).
Figure 9.5. Comparison of the model with experimental results for the erosion of CPH-SA 20:80 tablets (Shen et al., 2002). The solid lines represent model fits to monomer release (equations 9.15-9.18).

Table 9.2. Model parameters from fits to experimental monomer release data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CPP-SA 20:80</th>
<th>CPH-SA 20:80</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_A$</td>
<td>$1.1 \times 10^{-4}$ mol cm$^{-2}$ day$^{-1}$</td>
<td>$2.2 \times 10^{-4}$ mol cm$^{-2}$ day$^{-1}$</td>
</tr>
<tr>
<td>$t_A$</td>
<td>3.3 days</td>
<td>2.0 days</td>
</tr>
<tr>
<td>$k_B$</td>
<td>$2.2 \times 10^{-9}$ mol cm$^{-2}$ day$^{-1}$</td>
<td>$5.2 \times 10^{-10}$ mol cm$^{-2}$ day$^{-1}$</td>
</tr>
<tr>
<td>$t_B$</td>
<td>8.4 days</td>
<td>7.7 days</td>
</tr>
</tbody>
</table>
Figure 9.6. Porosity distribution within CPH-SA 20:80 tablet as a function of position and time (equation 9.9).

9.5.3 Drug release from CPH-SA 20:80

Based on experimental data of drug release from polyanhydrides with microphase separation (e.g. CPH-SA 20:80), we have proposed that the compatibility of the polymer and drug drives the thermodynamic partitioning of the drug within the micro-domains of the polymer (Shen et al., 2002). Heterogeneous polymers such as CPH-SA 20:80 and 80:20 provide a microphase-separated environment in which the drug can preferentially partition itself. Drug molecules loaded into such polymers will attempt to distribute themselves into compatible regions until saturation is reached. If very few compatible domains are present,
the excess drug will be forced to distribute itself into less compatible regions, resulting in a pronounced burst effect. Drug solubility also plays a key role in determining the release profile characteristics from bioerodible polymers. In heterogeneous systems, the drug preferentially partitions itself into compatible domains. Once these domains become saturated, the drug attempts to solubilize within the less compatible regions. Any excess drug precipitates out, resulting in an initial burst.

An example of a solute exhibiting this partitioning behavior is \( p \)-nitroaniline (PNA), for which experimental evidence points to the preferential partitioning within the aromatic-rich (CPP- or CPH-rich) domains in CPP-SA or CPH-SA 20:80 copolymers (Mathiowitz and Langer, 1987; Shen et al., 2002). Thus, we define a partition coefficient, \( P \), as the ratio of drug concentration in the B (CPP or CPH) phase to that in the A (SA) phase. The excess drug in the system is released in the form of a burst. It is important to understand that the amount of drug released in the burst is the sum of the amounts released from both phases. However the model in its current form does not distinguish between the drug released during the burst from either phase. Thus, the drug released during the burst is treated only as a consequence of the supersaturation in the polymer. It can be proved that the fraction of drug released, \( x_D \), is given by

\[
x_D = (1-b)x_{D,p} + b
\]  \hspace{1cm} (9.20)

Here \( b \) is the fraction of drug released in the burst and \( x_{D,p} \) is the fraction of drug released that is partitioned within the polymer. The total mass of drug released that is partitioned within the micro-domains of the polymer is given by
Here \( x_A \) is the mass fraction of A released (given by equation 9.15), \( x_B' \) is the mass fraction of B released from the B phase (given by subtracting equation 9.6 from equation 9.11 and normalizing with respect to the total mass of the B phase), \( C_{D,A0} \) is the initial concentration of drug in the A phase, and \( C_{D,B0} \) is the initial concentration of drug in the B phase. The initial mass of drug that is partitioned within the two phases is

\[
m_{D,0} = \left( \phi_{A0} C_{D,A0} + \phi_{B0} C_{D,B0} \right) S L_0
\]  

Using the definition of the partition coefficient, dividing equation 9.21 by equation 9.22, and using equation 9.20, the fractional drug released from the CPH-SA 20:80 polymer can be derived as

\[
x_D = (1-b) \left( \frac{x_A \phi_{A0} + x_B' \phi_{B0} P}{\phi_{A0} + \phi_{B0} P} \right) + b \]  

The burst is modeled as the fraction of drug released in the early stages of release by comparison with the experimental data. Then, the drug release data can be fit to equation 9.23 in each regime of release and \( P \), the partition coefficient can be estimated by fitting equation 9.23 to the first regime. In addition, since experimental data for monomer release from drug-loaded polyanhydrides is available, the monomer release rate constants, \( k_A \) and \( k_B \), can also be estimated using the method described earlier.

Figure 9.7 shows the release of SA, CPH, and PNA from a CPH-SA 20:80 copolymer containing 10%w/w of PNA. The drug-containing copolymer is in the form of 110mg tablet
with a diameter of 10mm and a thickness of 1.5mm. The SA release is fitted to equation 9.15 and the value of $k_A$ obtained from the fit is $1.9 \times 10^{-4}$ mol cm$^{-2}$ day$^{-1}$. This value is relatively unchanged from the value of $k_A$ obtained when no PNA is present (see Table 9.2). This suggests that the presence of PNA does not affect the release of SA or, in other words, PNA does not interact strongly with SA in CPH-SA 20:80. The CPH release during the first regime ($t \leq 2.3$ days) is fitted to equation 9.16 and the value of $k_B$ obtained from the fit is $1.1 \times 10^{-9}$ mol cm$^{-2}$ day$^{-1}$. This value is about 5 times the value of $k_B$ when no PNA is present (see Table 5.2). This suggests that the presence of PNA affects the release of CPH; it accelerates the rate of release of CPH. As before, the CPH release in regimes 2 and 3 is predicted using the value of $k_B$ obtained from the fit of the data in the first regime to the model.

By observation of the PNA release data (filled circles) in Figure 9.7, the value of $b$ is estimated as 0.41. Using equation 9.23, the value of $P$ is estimated from the PNA release data in the first regime ($t \leq 2.3$ days) as 2.55. This value of $P$ indicates that the PNA is 2.55 times more soluble in the CPH phase as it is in the SA phase; thus, the model shows that the PNA partitions into the CPH phase in the CPH-SA 20:80 copolymer. This result from the model is in agreement with several experimental studies (Mathiowitz and Langer, 1987; Shen et al., 2002) of PNA release from p(CPP-SA) 20:80 copolymers that suggest PNA affinity to CPH.
Figure 9.7. Comparison of the model with experimental results for CPH (□), SA (○) and PNA (●) release from PNA-loaded p(CPH-SA) 20:80 tablets. The solid lines represent model fits to monomer release (equations 9.15-9.18) and the dotted line represents the model fit to PNA release (equation 9.23).

9.5.4 CPH-SA 80:20 erosion

As stated earlier, the CPH-SA and CPP-SA systems exhibit microphase separation when one component is present in excess of 80mol%. The preceding discussion was concerned with the case for which the fast eroding component A was present in excess of 80mol%. Now the analysis is extended to the situation when the slowly eroding component B is present in excess of 80mol%. In the former case, it was assumed that the fraction of A within the B domains is negligible; here, we assume that there is no B within the A domains, i.e., p = 0. Once again, the basis of this assumption is the AFM data in Shen et al. (Shen et al., 2001).
In this case, the disappearance of the A domains and the growth of the B domains induces a change in the B surface area exposed to the buffer; therefore, the porosity of the tablet $\varepsilon(x,t)$ continuously varies with both time and position (i.e., along the tablet thickness).

As before, a mass balance for species B in a differential element, $dx$, positioned at distance $x$ from the center of the tablet is written (see equation 9.7 for the integral form). To find an expression for $\sigma(x,t)$, the surface area of B in contact with the buffer per unit volume, the A phase is modeled as spheres with a (surface-area average) diameter $d_0$, for each infinitesimal volume $Sdx$. Then, $\sigma(x,t)$ becomes a function of the porosity, $\varepsilon$, and is given by:

$$\sigma = \frac{6 \phi_{A0} \gamma s}{d_0} \varepsilon^{2/3} (1 - \varepsilon)$$  \hspace{1cm} (9.24)

The factor $(1 - \varepsilon)$ accounts for the decrease in surface area as the micro-domains coalesce. Substituting equation 9.24 in equation 9.7, we obtain an ordinary differential equation for the porosity $\varepsilon(x,t)$ given by:

$$\frac{d\varepsilon}{dt} = - \frac{6 M_B \phi_{A0} \gamma s}{d_0} \varepsilon^{2/3} (1 - \varepsilon)$$  \hspace{1cm} (9.25)

The porosity can be obtained via numerical integration of equation 9.25 once $k_A$ and $k_B$ are known. The expression for the mass of A released is given by equation 9.11. This release of A induces porosity in the CPH-SA 80:20 polymer and thus, the mass of B released as a function of time is given by

$$m_B = \int_0^t M_B (1 - p)(\varepsilon - \phi_{A0}) S k_A \, dt'$$  \hspace{1cm} (9.26)
The total mass of B released is normalized with respect to the initial mass of B in the system to obtain the fraction of B released as a function of time. We have obtained experimental data for SA and CPH release from p(CPH-SA) 80:20 tablets (Shen et al., 2002). This data and the model fits are shown in Figure 9.8. From the fit of the SA release data, the value of $k_A$ is obtained as $1.4 \times 10^{-4}$ mol cm$^{-2}$ day$^{-1}$. This value is of the same order of magnitude as the value obtained for the CPH-SA 20:80 copolymer, which is expected since $k_A$ describes the rate of scission of SA-SA bonds and subsequent dissolution of the SA monomer. Also shown in Figure 9.8 is the fit of the CPH release data for a period of 60 days. During this period, about 30% of the CPH was released. The value of $k_B$ obtained from the fit is $8.0 \times 10^{-11}$ mol cm$^{-2}$ day$^{-1}$. This value is about 6 times slower than the value of $k_B$ obtained for CPH release from CPH-SA 20:80, suggesting that there are attributes of the microstructure in the 80:20 case that are different from that of the 20:80 case. This discrepancy is discussed in the next section.

Using the values of $k_A$ and $k_B$, the porosity, $\varepsilon$, in the CPH-SA system can be plotted as a function of both position and time. This porosity profile for the first 60 days of release is shown in Figure 9.9. It is instructive to note that $\varepsilon$ approaches $\sim 0.33$ after about 60 days of release, in reasonable agreement with $\sim 30\%$ of CPH released after the same time.
Figure 9.8. Comparison of the model with experimental results for CPH (□) and SA (○) with for the erosion of CPH-SA 80:20 tablets (Shen et al., 2002). The solid lines represent model fits to monomer release (equations 9.15 and 9.26).
Figure 9.9. Porosity distribution within CPH-SA 80:20 tablet as a function of position and time (obtained from integration of equation 9.25).

9.5.5 Limitations of the model

The model assumes that $k_A$ and $k_B$ are the rates at which monomers are produced as reaction products when the polymer is exposed to buffer. In reality, when the polymer is exposed to buffer solution, the degradation process that takes place is probabilistic and leads to products with a distribution of chain lengths. In other words, oligomeric species are first produced before monomer is released. This explains the over-prediction observed in Figures 9.5 and 9.7. On the other hand, the model appears to work well for the CPP release from
CPP-SA 20:80. This indicates that apart from the distribution of chain lengths, \( k_A \) and \( k_B \) must depend on other factors. This is discussed next.

It is well known that both the CPP-SA and the CPH-SA systems are semicrystalline (Mathiowitz et al., 1990b). It has also been shown that monomer crystals form during erosion (Goepferich and Langer, 1993). The model does not explicitly treat the effects of crystallinity on erosion. This may also explain the differences between the value of \( k_B \) obtained for CPH-SA 20:80 and 80:20. It has been shown by Mäder et al. that the local pH during erosion of CPP-SA decreases, leading to increased solubility of the degradation products, and thus accelerated erosion (Mäder et al., 1997). We argue that this increase in solubility is compensated for by the formation of monomer crystals during degradation, thus canceling the effect of both phenomena. This effect may be less pronounced in the CPH-SA case since CPH is less crystalline than CPP and degrades much more slowly (Mathiowitz et al., 1990b; McCann et al., 1999).

Another factor that limits the model is the absence of any molecular weight and/or polydispersity dependence of the rate constants. The number average molecular weights for the CPH-SA 20:80 and 80:20 used in the experiments that the model is fitted to are 8,800 and 5,500 respectively (Table 9.2). New expressions for \( k_A \) and \( k_B \) need to be developed that account for the statistical distribution of chain lengths, degree of crystallinity, the local pH, and polymer molecular weight.

Other aspects that currently limit the model include the lack of reliable data for the parameters \( d_0 \) and \( p \) and the absence of polymer-drug interactions. These parameters have been estimated by comparison to AFM data obtained on the CPH-SA system, but more detail on the solid-state microstructure of the copolymers is needed to correctly estimate these parameters. As seen in the data of Figure 9.7, the presence of the drug influences the
degradation rate, suggesting the presence of polymer-drug interactions. The model "lumps" these interactions within the partition coefficient, P, but a more thorough understanding of the nature of the interactions and their effect on the monomer release is needed. Further, a more detailed argument for the burst effect needs to be developed. Refinements of the current model based on these considerations are underway.

9.6 Conclusions

A new model was developed to understand the mechanism of degradation and drug release kinetics of surface-erodible polymers. This model takes into account the phenomenon of microphase separation that has been observed for polyanhydrides of certain copolymer compositions. The model assumes that erosion is dominated by degradation and thus, in a system with a fast eroding and a slow eroding species, two rate constants, one for each species, essentially control the evolution of the polymer microstructure. Expressions were derived for the fraction of each monomer released as well as for the porosity in the system. When drugs are loaded into such heterogeneous polymers, they undergo thermodynamic partitioning depending upon their compatibility with each phase of the copolymer. This aspect has been modeled via a partition coefficient. The solutions of the model equations were fitted to experimental data on monomer release kinetics from two polyanhydride systems to obtain the erosion rate constants. Drug release kinetics experiments were compared to the model solution for drug release and the partition coefficient of the drug is obtained from the fits. The comparisons to the data are promising, while pointing out the limitations of the model. The model does not account for oligomer formation prior to monomer release or for the dependence of the rate constants on parameters
such as the degree of crystallinity, the local pH, and the polymer molecular weight. These limitations will be addressed in a future publication.

9.7 References


CHAPTER 10
A MOLECULAR LEVEL EROSION MODEL FOR SURFACE-ERODIBLE
SEMICRYSTALLINE HOMOPOLYMERS AND COPOLYMERS

A paper to be submitted to Macromolecules, 2004
Matt J. Kipper¹, and Balaji Narasimhan²

10.1 Abstract

A new model for the erosion kinetics of semicrystalline surface erodible homopolymers and copolymers is presented. The model was derived for a class of surface-erodible polyanhydride copolymers, with the goal of describing erosion in terms of fundamental, elementary processes. This model is based on an accurate description of copolymer microstructure and can thereby account for the heterogeneous erosion due to microphase separation and crystallinity. In addition to accurately predicting the overall erosion profile and the release of individual monomer species, several key phenomena that occur during erosion are described. These include precipitation of slightly soluble degradation products inside the pores of the erosion zone and pH changes during erosion due to dissolution of acidic monomers and the consequent changes in monomer solubility. This model also motivates future experiments to investigate predicted phenomena such as the effects due to local changes in pH and degradation rate constants for crystalline and amorphous moieties. The design of biomedical devices such as vehicles for drug delivery

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and scaffolds for tissue engineering will be aided by the application of this model and future extensions of it.

10.2 Introduction

Bioerodible polymers are ideal for a variety of biomedical applications. Their chemistries can be tailored to stabilize macromolecular drugs, their surfaces can be modified to target delivery to specific cells and tissues, and their erosion kinetics can be modulated by a variety of techniques. Bioerodible polymers have been used to fabricate scaffolds for tissue engineering, implants for orthopedic applications, and vehicles for targeted and controlled drug delivery. In all of these applications, the erosion kinetics is key to the performance of the device. The erosion kinetics can be modulated by altering copolymer composition when the two constituent polymers erode at different rates or by changing the crystallinity when crystalline and amorphous domains erode at different rates.

Surface eroding polymers, such as polyanhydrides and poly(orthoesters), are a particularly promising class of bioerodible polymers for many biomedical applications. They do not swell in the presence of water. Rather, their hydrophobicity prevents the ingress of water. This hydrophobic environment may be advantageous for stabilizing macromolecular drugs such as proteins, growth hormones, and vaccines. For controlled release applications, drug release kinetics is controlled by the erosion kinetics, rather than by swelling and diffusion as in some bulk eroding systems. Since the eroding zone is limited to the surface, the bulk polymer maintains its shape and mechanical integrity as the device shrinks, which may be desirable for orthopedic applications.

However, the erosion kinetics is complicated when phases or components are added, as in the case of semicrystalline polymers or microphase-separated copolymers. When the
two phases erode at different rates, surface eroding polymers begin to exhibit some characteristics of bulk eroding polymers. The erosion of a fast eroding phase may leave behind a porous microstructure of the slow eroding phase. Water may then penetrate into the eroding zone, drugs may diffuse out of the material, and the mechanical properties in the eroding zone are subject to significant change. The design of biomedical devices based on surface-eroding phase-separated polymers requires a detailed understanding of the mechanisms of erosion that lead to these phenomena.

Several approaches to understand and model the erosion mechanisms of surface-erodible polymers have appeared in the literature. Thombre and Himmelstein have developed a phenomenological model in which the erosion front progresses through the device while diffusion is permitted in the intact zone. A diffusion barrier representing an external membrane is added. An extension of this model accounts for diffusion of the degradation products and for catalyzed polymer degradation. Zygourakis proposed a cellular automata approach in which individual volume elements are assigned erosion times upon exposure to water. A porous microstructure develops as the polymer erodes. Göpferich and Langer have proposed a similar model. In their model each volume element erodes stochastically and an extension of the model accounts for monomer diffusion through the porous erosion zone. Batycky et al. developed a mechanistic model that explicitly accounts for polymer degradation by both random chain scission and end chain scission rather than simply modeling erosion as an elementary process. Drug release is also accounted for by desorption from the polymer matrix and by diffusion through mesopores. A non-homogeneous distribution of drug is accounted for in a drug release model proposed by Varelas et al., but the polymer matrix does not erode. In this model isolated domains act as reservoirs from which drug diffuses through a polymer matrix. We have developed a
phenomenological erosion and drug release model that accounts for microphase separated domains that erode at different rates and for partitioning of encapsulated drugs within the phase separated domains. However, our previous model assumes that erosion is primarily controlled by degradation and ignores the subsequent mass transfer phenomena of dissolution and diffusion.

10.3 Experimentally Observed Features of Polyanhydride Erosion

Most polyanhydride copolymers that have been synthesized for biomedical applications are semicrystalline. It has been demonstrated experimentally that the amorphous phase of semicrystalline polyanhydrides erodes faster than the crystalline phase. In most cases, the spherulitic structure of the crystalline phase remains intact as the amorphous phase degrades, leaving behind a porous matrix, the morphology of which is defined by the original morphology of the crystalline/amorphous phase separation.

The erosion mechanism of semicrystalline, surface-erodible copolymers is complex and consists of several elementary steps, beginning with degradation. In the case of binary A-B copolymers, three types of bonds are available for degradation: A-A bonds, A-B bonds, and B-B bonds. It has been shown that for copolymers of sebacic anhydride (SA) and 1,3-bis (p-carboxyphenoxy) propane (CPP) the SA-SA bonds and SA-CPP bonds are hydrolyzed at a faster rate than the CPP-CPP bonds. This also leads to the observed heterogeneous release of degraded monomer from these copolymer systems.

In addition to the reactivity of the anhydride bonds, the monomer solubility may also affect polyanhydride erosion kinetics. Göpferich et al. have demonstrated the precipitation of monomers inside the eroding zone of CPP-SA copolymers and fatty acid dimer-SA (FAD-SA) copolymers. This phenomenon occurs because anhydride bonds in the polymer...
backbone degrade, resulting in monomer formation at a rate faster than the rate of monomer dissolution. As the solution inside the porous eroding zone of the polymer approaches saturation, monomer crystals accumulate. Closely coupled to the precipitation and dissolution of the monomers, is their effect on the pH of the microenvironment inside the eroding polymer matrix. This pH has been successfully measured in 20:80 CPP-SA copolymer tablets by Mäder et al. using spectral spatial electron paramagnetic resonance imaging (e.p.r.i.)\textsuperscript{40}. They detected pH levels as low as 4.7 in the eroding zone, which rose as the polymer continued to erode and the monomer diffused from the device. The decrease in pH further limits the monomer solubility as the dicarboxylic acid monomers become much less soluble at low pH\textsuperscript{38}.

The decrease in pH inside the eroding zone may not have a strong effect on the degradation kinetics; the degradation of polyanhydrides is known to be base catalyzed\textsuperscript{41,42}. However, the dissolution kinetics and solubility of the dicarboxylic acid monomers is most likely affected by pH.

Our goal in this paper is to understand the mechanism of polymer erosion at a molecular level and to accurately describe the erosion kinetics of surface-eroding phase-separated copolymers. This work is also motivated by our previous experiments with polyanhydride copolymers composed of 1,6-bis(p-carboxyphenoxy)hexane (CPH) and SA\textsuperscript{13,37,43-46} and on the body of experimental and theoretical work describing this and similar copolymer systems. We would like to point out that this model can be generalized to other surface eroding systems such as poly(ortho esters) and could be modified to describe bulk-eroding systems (such as poly(lactide-co-glycolide) copolymers). Additionally, some of our model predictions motivate additional experiments to measure the effects of various phenomena.
10.4 Model Development

Polymer erosion is the sum of several elementary processes.

1. Polymer degradation (monomer formation)
2. Monomer dissolution
3. Diffusion of monomer from the eroded zone

Our view of this complex erosion process is shown in Fig. 1. This figure shows a tablet of half-thickness L, exposed to water on the right at three stages during erosion.

![Figure 10.1](image)

**Figure 10.1.** Schematic illustrating erosion process of a tablet with half-thickness L, exposed to buffer on the right.

We model step one, monomer formation, as a first order process with respect to the surface area of exposed polymer. The exposed polymer surface area is converted to undissolved monomer. Monomer formed in step one is assumed to be in the form of a monolayer, crystallized on the surface and prevents the underlying polymer from degrading.

Figure 10.1a shows four pores that have formed at the exposed surface of a polymer tablet. Figure 10.1b shows the monolayer of undissolved monomer (thick line). Step two, monomer
dissolution, results in mass loss from the device. As monomer dissolves, it exposes undegraded polymer. Dissolved monomer is represented in Figure 10.1c by the particles inside the pores. Some particles remain undissolved, adsorbed to the surface of the pores. A distinction is made between monomer formed from crystalline polymer and monomer formed from amorphous polymer. Probabilities are assigned for the exposure of amorphous polymer and crystalline polymer as the monomer from each of these phases dissolves. This is illustrated in the inset in Figure 10.1c. Here, the two polymer phases are represented by the light gray and the dark gray regions. The monomers formed from these two phases are represented by the striped blocks. The probability that light gray monomer will dissolve to expose light gray polymer is greater than the probability that light gray monomer will dissolve to expose dark gray polymer. For copolymers, the microphase separation can be accounted for in a similar way. In step three the monomer diffuses out of the eroding zone in response to the concentration gradient formed in step two. Surface area fractions of each type of monomer and each type of polymer are functions of both position and time. Likewise, the pore radius, porosity, specific surface area, and concentration of dissolved monomer are also functions of position and time.

As monomer dissolves, the pH of the solution inside the eroding zone changes, which alters the dissolution kinetics by changing the saturation concentration. We assume no pH dependence of the degradation kinetics as we anticipate only acidic conditions in the eroding zone. This is consistent with experimental observations for polyanhydrides, but could be modified for acid catalyzed degradation as is the case for poly(ortho-esters). Dimensionless model variables and parameters are listed in Tables 10.1 and 10.2, respectively.
Table 10.1. Dimensionless model variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{ma1}$</td>
<td>Surface area fraction of monomer formed from amorphous polymer of type one</td>
</tr>
<tr>
<td>$f_{ma2}$</td>
<td>Surface area fraction of monomer formed from amorphous polymer of type two</td>
</tr>
<tr>
<td>$f_{mc1}$</td>
<td>Surface area fraction of monomer formed from crystalline polymer of type one</td>
</tr>
<tr>
<td>$f_{mc2}$</td>
<td>Surface area fraction of monomer formed from crystalline polymer of type two</td>
</tr>
<tr>
<td>$\kappa_1$</td>
<td>Dissolution rate for monomer one</td>
</tr>
<tr>
<td>$\kappa_2$</td>
<td>Dissolution rate monomer two</td>
</tr>
<tr>
<td>$f_{a1}$</td>
<td>Surface area fraction of amorphous polymer of type one</td>
</tr>
<tr>
<td>$f_{a2}$</td>
<td>Surface area fraction of amorphous polymer of type two</td>
</tr>
<tr>
<td>$f_{c1}$</td>
<td>Surface area fraction of crystalline polymer of type one</td>
</tr>
<tr>
<td>$f_{c2}$</td>
<td>Surface area fraction of crystalline polymer of type two</td>
</tr>
<tr>
<td>$R^*$</td>
<td>Average pore radius</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Porosity</td>
</tr>
<tr>
<td>$\chi_1$</td>
<td>Concentration of dissolved monomer of type one</td>
</tr>
<tr>
<td>$\chi_2$</td>
<td>Concentration of dissolved monomer of type two</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Specific surface area</td>
</tr>
<tr>
<td>$p_{aa}$</td>
<td>Probability of amorphous monomer dissolving to expose amorphous polymer</td>
</tr>
<tr>
<td>$p_{ca}$</td>
<td>Probability of crystalline monomer dissolving to expose amorphous polymer</td>
</tr>
<tr>
<td>$\phi_{a1}$</td>
<td>Fraction of the amorphous polymer represented by polymer one</td>
</tr>
<tr>
<td>$\phi_{c1}$</td>
<td>Fraction of the crystalline polymer represented by polymer one</td>
</tr>
</tbody>
</table>
Table 10.2. Dimensionless model parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>Ratio of degradation rate constants for crystalline polymer of type one to that for amorphous polymer of type one</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Ratio of degradation rate constants for amorphous polymer of type two to that for amorphous polymer of type one</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Ratio of degradation rate constants for crystalline polymer of type two to that for amorphous polymer of type one</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Long period of crystalline lamellae</td>
</tr>
<tr>
<td>$D_1^*$</td>
<td>Diffusion coefficient for monomer of type one</td>
</tr>
<tr>
<td>$D_2^*$</td>
<td>Diffusion coefficient for monomer of type two</td>
</tr>
<tr>
<td>$\xi$</td>
<td>Distance from the surface</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Time</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Degree of crystallinity</td>
</tr>
<tr>
<td>$k_{d1}$</td>
<td>Dissolution rate constant for monomer of type one</td>
</tr>
<tr>
<td>$k_{d2}$</td>
<td>Dissolution rate constant for monomer of type two</td>
</tr>
</tbody>
</table>

The surface of the pores is characterized by the fractional coverage of amorphous polymer of components one and two, $f_{a1}$ and $f_{a2}$, crystalline polymer of components one and two, $f_{c1}$ and $f_{c2}$, and monomer arising from the degradation of each type of polymer, $f_{ma1}$, $f_{ma2}$, $f_{mc1}$, and $f_{mc2}$. Equations 10.1 through 10.4 determine the surface area fraction of each type of monomer on the surface of a pore. These equations are first order in the surface area fraction of polymer and pseudo-first order in the surface area fraction of monomer since the dimensionless dissolution rates, $\kappa_1$ and $\kappa_2$, are functions of concentration.
It is necessary to distinguish between the four types of monomer, because as monomer dissolves, it exposes undegraded polymer, as mentioned earlier and illustrated in the inset of Figure 10.1c. The ratio of the degradation rate constant for the crystalline polymer of type one to that for amorphous polymer of type one is \( \beta \). The degradation rate constants for amorphous polymer of type two and crystalline polymer of type two are normalized similarly to form the ratios \( \gamma \) and \( \delta \), respectively. The dimensionless time parameter, \( \tau \), is normalized by the degradation rate constant for amorphous polymer of type one.

\[
\tau = \frac{k_{a1} t}{\rho y}
\]

Here, \( t \) is time (s), \( k_{a1} \) is the degradation rate constant for amorphous polymer of type one (g cm\(^{-2}\) s\(^{-1}\)), \( \rho \) is the density of the polymer (g cm\(^{-3}\)), and \( y \) is the characteristic length scale (cm) associated with a monolayer of monomer.

Equations 10.6 through 10.9 determine the surface area fraction of each type of polymer. The first term on the right-hand side of each of these equations accounts for
polymer degradation, and the second term accounts for exposure of the undegraded polymer as monomer dissolves.

\[
\frac{\partial f_{a1}}{\partial \tau} = -f_{a1} + \left[ (pa_{am1} + pc_{am1})k_1 + (pa_{am2} + pc_{am2})k_2 \right] f_{a1}
\]  
(10.6)

\[
\frac{\partial f_{a2}}{\partial \tau} = -f_{a2} + \left[ (pa_{am1} + pc_{am1})k_1 + (pa_{am2} + pc_{am2})k_2 \right] (1 - f_{a1})
\]  
(10.7)

\[
\frac{\partial f_{c1}}{\partial \tau} = -\beta f_{c1} + \left[ (1 - pa_{am1} + (1 - pc_{am1})k_1 + (1 - pa_{am2} + (1 - pc_{am2})k_2 \right] f_{c1}
\]  
(10.8)

\[
\frac{\partial f_{c2}}{\partial \tau} = -\beta f_{c2} + \left[ (1 - pa_{am1} + (1 - pc_{am1})k_1 + (1 - pa_{am2} + (1 - pc_{am2})k_2 \right] (1 - f_{c1})
\]  
(10.9)

The probability that monomer from amorphous polymer will expose amorphous polymer upon dissolution is \( p_{aa} \) and the probability that crystalline polymer will expose amorphous polymer is \( p_{ca} \). The estimation of these probabilities is based on the degree of crystallinity, \( \alpha \), and the microstructure. Appendix 10.A.1 describes how these parameters are estimated based on a balance of the total interfacial area initially present. The chemical identity of the monomer is also important since the two types of monomers dissolve at different rates. The fraction of component one in the amorphous and crystalline phases are \( \phi_{a1} \) and \( \phi_{c1} \), respectively.

The average dimensionless radius of a pore, \( R^* \), changes as monomer dissolves (see Figure 10.1b and 10.1c).

\[
\frac{\partial R^*}{\partial \tau} = (f_{ma1} + f_{mc1})k_1 + (f_{ma2} + f_{mc2})k_2
\]  
(10.10)
The porosity, \( \varepsilon \), is the fraction of the total volume that represented by pores. The pore volume is computed assuming cylindrical pores of average dimensionless radius \( R^* \). The number of pores is the initial number of amorphous domains, found by dividing the total volume by the volume associated with a long period for the crystalline lamellae. It is assumed that pores eventually overlap and coalesce. This is accounted for by multiplying the pore volume by the total polymer volume. Thus, as the porosity increases, the probability that a pore will intersect another pore increases. Multiplying by the polymer volume prevents counting the volume of the overlap more than once, via a mean field approximation on the porosity distribution.

\[
\frac{\partial \varepsilon}{\partial \tau} = \frac{8(1-\varepsilon)R^*}{\lambda^2} \frac{\partial R^*}{\partial \tau} \tag{10.11}
\]

Here, \( \lambda \) is the dimensionless long period of the crystalline lamellae and is used to estimate the number of pores.

The dimensionless concentrations of monomer of type one and two, \( \chi_1 \) and \( \chi_2 \), in the erosion zone are given by a diffusion equation with a source term for dissolution from the surfaces of the pores.

\[
\frac{\partial \chi_1}{\partial \tau} = \frac{(f_{m1} + f_{mc1})\kappa_1\sigma}{\varepsilon} + D_1 \cdot \frac{\partial^2 \chi_1}{\partial \xi^2} \tag{10.12}
\]

\[
\frac{\partial \chi_2}{\partial \tau} = \frac{(f_{m2} + f_{mc2})\kappa_2\sigma}{\varepsilon} + D_2 \cdot \frac{\partial^2 \chi_2}{\partial \xi^2} \tag{10.13}
\]

Here, \( \xi \) is the dimensionless position from the original surface of the eroding polymer. \( D_1^* \) and \( D_2^* \) are the dimensionless diffusivities of monomers one and two respectively. Diffusion
only in the direction of propagation of the erosion front is considered. In other words, perfect mixing is assumed in the radial direction inside the pores. Both concentrations are assumed to be zero outside the pores. The dimensionless surface area per unit volume, \( \sigma \), is computed by assuming cylindrical pores and accounting for coalescence of the pores.

\[
\sigma = \frac{2 \varepsilon (1 - \varepsilon)}{R'}
\]  

(10.14)

The dimensionless parameters \( \xi \), \( R^* \), \( D^* \), and \( \chi_n \), are:

\[
\xi = \frac{x}{y}
\]  

(10.15)

\[
R^* = \frac{R}{y}
\]  

(10.16)

\[
D^*_n = \frac{D_n \rho}{k_{s1} y}
\]  

(10.17)

\[
\chi_n = \frac{c_n}{\rho}
\]  

(10.18)

Here, \( x \) is the distance from the original surface of the polymer, \( D_n \) is the diffusivity of the dissolved monomer of type \( n \), and \( c_n \) is the concentration of dissolved monomer of type \( n \). The specific surface area, \( s \), and the long period, \( l \), are also normalized by the length scale \( y \) to give the parameters \( \sigma \) and \( \lambda \) respectively.

\[
\sigma = sy
\]  

(10.19)
The dimensionless dissolution rate for each of the monomers is computed as shown below.

\[ \kappa_n = \frac{k_{dn} (c_{sat,n} - c_n)}{c_{sat,n} k_{sat}} \]  

(10.21)

Here, \( c_n \) and \( c_{sat,n} \) are the concentration of dissolved monomer of type \( n \) and the concentration of dissolved monomer at saturation of monomer type \( n \), respectively, and \( k_{dn} \) is the dissolution rate constant for monomer of type \( n \).

Initial values for the porosity, pore radius, and surface area fractions are all set to zero, with the exception of the surface area fractions of amorphous polymer of types one and two, which are initialized in accordance with the copolymer composition. This assumes that the amorphous polymer preferentially partitions to the surface\textsuperscript{32,33}. Two additional parameters, \( \xi_{ef} \) and \( \xi_{s} \) are the respective positions of the erosion front and the surface. Both of these parameters are initially set to zero, and the erosion front moves through the polymer at the same velocity with which the pores radius grows at the erosion front.

\[ \frac{\partial \xi_{ef}}{\partial \tau} = \frac{\partial R^*}{\partial \tau} \bigg|_{\xi = \xi_{ef}} \]  

(10.22)

The position of the surface is taken as the lowest value of \( \xi \) for which the porosity is less than 1. At \( \xi_{ef} \) an additional source term is added to equations 10.11 and 10.12 to account for the dissolution occurring at the surface normal to the direction of propagation of the erosion.
front. The cumulative fractional mass loss can be computed by integrating the porosity at any time point.

\[
\frac{m(t)}{m_\infty} = \int_0^t \varepsilon(t, \xi) \, d\xi
\]  

(10.23)

Here, \(m_\infty\) is the total mass of the polymer. The cumulative mass loss for each type of polymer is computed by integrating the dissolution rate for the corresponding monomer.

The remaining equations have significant nonlinearities that suggest a modified finite difference solution. The Crank-Nicolson method was used to formulate finite difference equations (FDEs). Thus, solutions to equations 10.1 through 10.4 and 10.6 through 10.13 are obtained implicitly with the exception of the parameters \(\kappa_1, \kappa_2, \phi_1, \phi_2, p_{an},\) and \(p_{ca}\). These parameters are computed from the previous time step. Since the equations may be stiff, depending on the values of the parameters \(\beta, \gamma, \delta, k_{d1},\) and \(k_{d2}\), the second order Gear method was used to integrate the FDEs.

10.5 Model Parameterization

Each of the elementary processes listed at the beginning of section 10.5 that constitute the erosion process has an associated rate constant. The relationships between these rate constants will ultimately determine the nature of the erosion process. Overall erosion rate constants have been reported for several polyanhydrides including copolymers. Diffusion coefficients have been reported for the SA and CPP monomers. Dissolution rate constants for dicarboxylic acid monomers however, are not reported in the literature. To obtain the dissolution rate constants, we melt pressed 10 mm diameter, 100 mg tablets of CPH and SA using a Carver Press (Wabash, Indiana) at 3 metric tons for 5 minutes just above the melting
point of the dicarboxylic acid. Tablets were weighed and allowed to dissolve in 900 ml of 0.1 M phosphate buffer (pH 7.4) at 37 °C. The dissolution media was stirred at 100 rpm. Dissolution experiments were conducted in an SR8-Plus Dissolution Test Station (Hansen Research Inc., Chatsworth, California). The tablets were removed at specified intervals and dried under vacuum for 24 hours. The masses of the tablets were recorded at each of at least 10 time points for each monomer and the experiments were performed in duplicate. Dissolution rate constants are reported in Table 10.3.

Table 10.3. Physical constants for the dicarboxylic acids.

<table>
<thead>
<tr>
<th>Dicarboxylic acid</th>
<th>Dissolution rate constant (g cm(^{-2}) s(^{-1}))</th>
<th>pKa(_1)</th>
<th>pKa(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPH</td>
<td>1.4 x 10(^{-8})</td>
<td>3.7</td>
<td>6.7</td>
</tr>
<tr>
<td>SA</td>
<td>1.4 x 10(^{-6})</td>
<td>4.8</td>
<td>5.6</td>
</tr>
</tbody>
</table>

The saturation concentration at different pH values for each dicarboxylic acid was determined by adding excess dicarboxylic acid to 10ml of 0.1 M phosphate or acetate buffer. Vials were incubated at 37 °C for two days with gentle agitation. Concentrations were determined by UV absorbance at 202 nm for SA and 249 nm for CPH\(^{37}\). For SA the saturation data were also compared to that reported by Göpferich and Langer\(^{38}\). The saturation concentration as a function of pH is shown in Figure 10.2. From these saturation curves, the pKa values for the diacids can be estimated by the positions of the inflection points. These values are also reported in Table 10.3.
The data in Figure 10.2 indicate that the saturation concentration for SA is at least an order of magnitude greater than that for CPH over the pH range from 5.0 to 7.5. Thus, we suggest that the SA concentration is likely to dominate the pH, and propose that the pH inside the eroding zone could be estimated by considering only the SA concentration. If the dissociation of the two carboxylic acids on the SA monomer is considered along with the dissociation of water, a quartic equation for the hydrogen concentration as a function of SA monomer concentration results. Our method for solving this is presented in Appendix 10.A.2. The results in appendix 10.A.2 demonstrate that a pH below about 4.75 is not obtainable, because the concentration of SA required to obtain that pH is above the saturation concentration. This is qualitatively consistent with the experimental findings of Mäder et al.
noted earlier. They observed a pH of about 4.7 inside the eroding zone of SA containing copolymers, which would indicate saturation of the erosion zone.

### 10.6 Model Solution for Poly(SA)

We first investigate the case of semicrystalline poly(SA) homopolymer erosion. For homopolymers, equations 10.2, 10.4, 10.7, 10.9 and 10.13 are unnecessary and equations 10.6, 10.8, and 10.10 are simplified by the absence of the terms related to component two. Additional parameters are listed in Table 10.4. The parameters $k_a$ and $\beta$ were used to fit the model to experimental data for erosion of poly(SA) tablets. The values for $\lambda$ and $\alpha$ were taken from our previous characterization of the crystallinity.

#### Table 10.4. Parameters used to model poly(SA) erosion kinetics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{a1}$</td>
<td>$8.0 \times 10^{-7}$ (g cm$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$\rho$</td>
<td>1.05 (g cm$^{-3}$)</td>
</tr>
<tr>
<td>$k_{d1}$</td>
<td>1.4 $\times 10^{-6}$ (cm s$^{-1}$)</td>
</tr>
<tr>
<td>$D_1$</td>
<td>$6.8 \times 10^{-6}$ (cm$^2$ s$^{-1}$)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.001</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>$2.8 \times 10^{-6}$ (cm)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.6</td>
</tr>
<tr>
<td>$y$</td>
<td>1.0 (nm)</td>
</tr>
</tbody>
</table>

The model was solved with a time step of 0.05 s and a position step of 1 nm. Several reports of experimental results for erosion of poly(SA) are found in the literature. We
compare our model to the experimental results we have previously obtained\textsuperscript{37}. In these experiments, 110 mg tablets, 10mm in diameter and 1.3 mm thick were degraded in 900 ml of pH 7.4 phosphate buffer at 37 °C with agitation at 100 rpm. The cumulative fractional mass loss as a function of time is plotted in Figure 10.3 for the experiment and the model.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure10.3.png}
\caption{Cumulative fractional mass loss for poly(SA) erosion from experiment\textsuperscript{37} (\textbullet{}) and model (line).}
\end{figure}

In addition to accurately predicting the overall erosion profile, the new model offers a detailed description of the erosion process. Of particular interest are the microstructural characteristics that can be obtained, including the ratio of the crystalline to amorphous polymer remaining in the erosion zone, the porosity, and the pH in the erosion zone. Figure 10.4 shows the evolution of the porosity for the first hour of erosion. During the first hour, the erosion front moves 13\textmu m into the tablet, and the porosity at the surface does not go to 1
until 51 minutes after the erosion begins. By this time, the erosion front has moved about 11\textmu m into the tablet. Once the porosity at \( x = 0 \) reaches one, the surface begins to move. At this point, a pseudo steady state develops with the position of the surface and the position of the erosion front moving at the same velocity. The erosion zone at any time consists of the region between the surface, \( x = x_s \), and the position of the erosion front, \( x = x_{ef} \). Since there is a pseudo steady state, the thickness of the erosion zone remains about 11\textmu m until the erosion front reaches the center of the tablet, \( x = x_{\text{max}} \). Note that initially the porosity increases rapidly as the amorphous polymer degrades, leaving behind a network rich in crystalline domains. The progress of erosion at a given position continues to decelerate as the porosity approaches unity.

**Figure 10.4.** Model results for porosity evolution in the first 60 minutes of erosion for poly(SA).
The surface area fractions of crystalline polymer, amorphous polymer, and monomer are plotted in Figure 10.5. Figure 10.5a shows the surface area fractions as a function of the time after the erosion front passes for constant position. Figure 10.5b shows the surface area fractions as a function of position from the erosion front at constant time. Figure 10.5a demonstrates that within about 15 seconds of the erosion front passing through a point, the surface area fractions have reached a steady state, and the exposed surface area is dominated by crystalline polymer. The two types of monomer and the amorphous polymer together make up about 0.1% of the total surface area. Figure 10.5b shows that at a given time, the surface area fractions vary over only a very narrow distance from the position of the erosion front.

The concentration of SA diacid and the resulting pH in the erosion zone is shown as a function of position in Figure 10.6 after the pseudo steady state develops. The concentration reaches the saturation concentration at about 7 μm from the surface. The pH prediction is in good agreement with the experimental results of Mäder et al.⁴⁰.

Although the pore surface within the erosion zone is overwhelmingly dominated by crystalline polymer, a significant fraction of amorphous polymer remains throughout the erosion process. This is evident from the plot shown in Figure 10.7. Here, the crystallinity, α, is plotted as a function of distance from the erosion front. At the erosion front, the crystallinity is 0.6 (the value in the bulk), and just past the erosion front the crystallinity rises sharply to a value of 0.77 inside the erosion zone (inset). Closer to the surface, the crystallinity rises gradually to about 0.82. The region near the erosion front is shown in the inset.
Figure 10.5. Surface area fractions as a function of time at constant position (a) just after the erosion front passes by and as a function of position at constant time (b) near the erosion front.
Figure 10.6. SA concentration (solid line) and the resultant pH (broken line) in the erosion zone.

Figure 10.7. Crystallinity, $\alpha$, as a function of position at a constant time near the erosion front.
The crystallinity plot shown in Figure 10.7 compares well with the experimental results reported in several studies, which indicate that the crystallinity inside the erosion zone is increased over that in the bulk, uneroded polymer\textsuperscript{31-33}.

10.7 Model Solution for Poly(CPH:SA) 20:80

To demonstrate the ability of this model to account for copolymer erosion, we present the solution for poly(CPH:SA) 20:80 erosion. In order to estimate the degradation rate constants for CPH, we compare the molar erosion rate constants for poly(CPH) and poly(SA) reported by Leong \textit{et al.}\textsuperscript{49,50}. The ratio of the erosion rate constants for poly(SA) to that for poly(CPH) is used as a first approximation for the ratio of degradation rate constants for the SA-SA and CPH-CPH bonds, \(k_{\text{SA-SA}}\) and \(k_{\text{CPH-SA}}\), respectively. We then estimate the degradation rate constant for CPH in the copolymer, \(k_{\text{CPH}}\), as:

\[
2k_{\text{CPH}}^{-1} = \frac{(L_{\text{CPH}} - 1)k_{\text{CPH-CPH}}^{-1} + 2k_{\text{CPH-SA}}^{-1}}{1 + L_{\text{CPH}}} \tag{10.23}
\]

Here, \(L_{\text{CPH}}\) is the number average sequence length of CPH in the copolymer reported by Shen \textit{et al.}\textsuperscript{37}, and \(k_{\text{CPH-SA}}\) is the rate constant for the degradation of CPH-SA bonds, approximated here as \(k_{\text{SA-SA}}\). The rate constant for degradation of SA-SA bonds is approximated as \(2k_{\text{SA}}\). The degradation rate constant for SA does not change in the copolymer since \(k_{\text{SA-SA}}\) is equal to \(k_{\text{CPH-SA}}\). This procedure is used to estimate the degradation rate constants for both crystalline and amorphous CPH.

The microphase separation that exists in the copolymer must be accounted for. In this case we account for the microphase separation by setting the parameter \(y\) to 1.5 nm. This is a good approximation for the thickness of a monolayer, as the average monolayer thickness
increases when CPH is added. This is also the approximate length scale associated with the microphase separation. Since our model is discretized at this length, the parameters $\phi_{a1}$ and $\phi_{c1}$ in equations 10.6-10.9 appropriately account for the microphase separation with a mean field approximation. The crystalline/amorphous phase separation is accounted for by the probabilities in equations 10.6-10.9.

The parameters listed in Table 10.4, the parameters used to model the 20:80 copolymer are listed in Table 10.5. The model was solved with a time step of 0.5 s and compared to data for tablet erosion similar to the experiments described above for the poly(SA). The parameter $\beta$ is used to fit the erosion data to the model.

**Table 10.5.** Parameters used to model poly(CPH-SA) 20:80 erosion kinetics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{a1}$</td>
<td>$8.0 \times 10^{-7}$ (g cm$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>$7.8 \times 10^{-9}$ (g cm$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$\rho$</td>
<td>1.05 (g cm$^{-3}$)</td>
</tr>
<tr>
<td>$k_{d1}$</td>
<td>$1.4 \times 10^{-6}$ (cm s$^{-1}$)</td>
</tr>
<tr>
<td>$k_{d2}$</td>
<td>$1.4 \times 10^{-8}$ (cm s$^{-1}$)</td>
</tr>
<tr>
<td>$D_1$</td>
<td>$6.8 \times 10^{-6}$ (cm$^2$ s$^{-1}$)</td>
</tr>
<tr>
<td>$D_2$</td>
<td>$7.2 \times 10^{-6}$ (cm$^2$ s$^{-1}$)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.025</td>
</tr>
<tr>
<td>$\delta$</td>
<td>$2.5 \times 10^{-4}$</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>$5.6 \times 10^{-6}$ (cm)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.467</td>
</tr>
</tbody>
</table>
The overall erosion profile (cumulative fractional mass loss) is shown in Figure 10.8. The erosion profile shows a brief initial lag time of about 4 hours. This is due to inhomogeneous erosion as will be explained shortly.

The porosity evolution for the first 14 hours is shown in Figure 10.9. Similar to the poly(SA), a pseudo steady state develops, wherein the position of the erosion front ($x_{ef}$) and the position of the surface ($x_s$) move through the device with the same velocity. In this case, the erosion zone is about 83 µm thick. Inside the erosion zone, the composition of the surface can be characterized as it was for the poly(SA). As might be expected, the surface is dominated by crystalline poly(CPH), as this is the most slowly degrading species. About 1.3% of the surface inside the erosion zone is occupied by undissolved CPH monomer. The
delay in the erosion profile noted earlier can be associated with the time required for the surface of the tablet to approach a porosity of one.

Figure 10.9. Porosity as a function of time and position for CPH:SA (20:80) copolymer erosion.

The individual monomer release profiles from the model and experiment are shown in Figure 10.9. We can see in Figure 10.9 that the release of CPH monomer lags behind the release of SA monomer. This is consistent with our experimental observations and observations made by others of similar copolymer systems\textsuperscript{36,37}. We also find that similar to the homopolymer case, the erosion zone quickly becomes saturated with SA diacid. The pH inside the pores reached the same value as that for the homopolymer until all of the SA was depleted (data not shown). The pH effect limits the solubility of the CPH diacid inside the pores similar to the effect observed for the SA diacid.
Figure 10.10. Comparison of model to experiment for cumulative fractional SA (□ = experiment, line = model) and CPH (○ = experiment, broken line = model) monomer release from poly(CPH-SA) (20:80) copolymer.

The model accurately predicts the overall erosion kinetics and the CPH monomer release kinetics, but fails to accurately predict the SA monomer release kinetics. It is possible that the SA monomer release kinetics is accelerated by some initial porosity in the system that this model does not account for. We also assumed that the ratio of the degradation rate constants for the crystalline to amorphous polymer was the same for both species, though this need not necessarily be the case. Allowing for \( \gamma \) to be an adjustable parameter, could also improve the fit to experiment. Despite this drawback, the model accurately describes important features of the microenvironment inside the eroding zone including concentrations of dissolved monomer species and the resulting pH. Further, this model motivates additional experiments that could describe the erosion process in more
detail, such as measurements of the degradation rate constants for crystalline and amorphous moieties, and porosity and \( \text{pH} \) within the eroding zone. Though this model was developed for polyanhydride copolymers, it could be used for a variety of surface and bulk eroding systems. Indeed, the results for the copolymer indicate the system is not purely bulk eroding, as the erosion zone represents a significant fraction of the device. Just as the current formulation of the model allows for \( \text{pH} \) dependence of the dissolution kinetics, \( \text{pH} \) dependent polymer degradation could also be accounted for. Further extensions of the model could also account for the release of encapsulated drugs.

### 10.8 Conclusions

The new model for erosion of surface-erodible materials presented here offers the ability to describe many interesting phenomena on a microscopic scale that are difficult to observe directly by experiment. Not only is the erosion kinetics of individual phases discerned, but also the information is resolved at the nanometer length scale. Furthermore, this model can be used to predict phenomena such as monomer precipitation and \( \text{pH} \) inside the pores of the eroding polymer.

The case study results presented here motivate further experimentation to discern additional parameters. Of particular interest are the individual degradation rate constants for crystalline and amorphous polymer. Here we assumed values of the ratios of these rate constants without any direct experimental support. For design of drug-loaded systems for controlled release applications, it is also necessary to have an accurate description of the thermodynamics of the polymer/drug system. Adding a drug may compatibilize the polymer system, affect polymer crystallinity, or form a third phase. Any of these three effects could be dealt with by moderate modifications to the new model.
10.A.1 Appendix. Estimation of Probabilities $p_{aa}$ and $p_{ca}$

In equations six through nine, the crystalline/amorphous phase separation is accounted for by considering the probability that dissolving monomer exposes amorphous polymer or crystalline polymer. The probability depends upon the identity of the monomer element. The probabilities are computed by considering the initial volume associated with the interfacial area. This fractional interfacial volume, $v_{\text{int}}$, is computed according to:

$$v_{\text{int}}(x, t = 0) = \frac{\text{Volume of amorphous polymer at interface}}{\text{Volume of polymer}} = \frac{(1-\alpha)\pi d_{a} y \Delta x}{\frac{\pi d_{a}^{2} \Delta x}{4}} = \frac{(1-\alpha)4}{\lambda}$$

(10.A.1)

Here, $d_{a}$ is the diameter of an amorphous domain and the volume of amorphous polymer at the interface is the interfacial area multiplied by the thickness of a monolayer, $y$. The probabilities are then computed as:

$$p_{aa} = 1 - \frac{v_{\text{int}}}{v_a}$$

(10.A.2)

$$p_{ca} = \frac{v_{\text{int}}}{v_c}$$

(10.A.3)

Here, $v_a$ and $v_c$ are the volume fractions of the crystalline and amorphous phases, respectively. These are obtained by integrating the dissolution rate of each type of monomer at each time step and are functions of position and time.
10.A.2 Appendix. Saturation Concentration and pH Calculations

Equation 21 computes the dissolution rate, $\kappa$, as a function of the saturation concentration, $c_{sat}$. The saturation concentration is a strong function of the pH as illustrated in Figure 10.10. The pH of the microenvironment in the erosion zone is determined by the SA concentration. Five equations are needed to solve for the concentrations of the five species, SA, SA\(^-\), SA\(^{2-}\), H\(^+\), and OH\(^-\). These five equations are the equilibria of SA, SA\(^-\), and water dissociation, the mole balance on the SA derived species, and the charge balance.

\[
K_{a1,SA} = \frac{[H^+][SA^-]}{[SA]} \quad (10.A.4)
\]

\[
K_{a2,SA} = \frac{[H^+][SA^{2-}]}{[SA^-]} \quad (10.A.5)
\]

\[
K_w(37^\circ C) = [H^+][OH^-] = 2.39 \times 10^{-14} \quad (10.A.6)
\]

\[
M_{SA} = [SA] + [SA^-] + [SA^{2-}] \quad (10.A.7)
\]

\[
[H^+] = [SA^-] + 2[SA^{2-}] + [OH] \quad (10.A.8)
\]

The bracketed variables are molarities of the bracketed species and $M_{SA}$ is the total SA molarity. These five equations can be combined to form a quartic equation for $[H^+]$ based on $M_{SA}$.

\[
M_{SA} \left( [H^+]^2 + 2K_{a2,SA} [H^+] \right) = \frac{1}{K_{a1,SA}} [H^+]^4 + [H^+]^3 + \left( K_{a2,SA} + \frac{K_w}{K_{a1,SA}} \right) [H^+]^2 + K_w [H^+] + K_w K_{a2,SA} \quad (10.A.9)
\]
Most of the terms in this equation over at least a portion of the expected pH range remain if the relatively small terms are neglected. Thus, rather than solving the quartic equation at each $x$ and $t$ for the pH and then predicting $c_{\text{sat}}$, we offer empirical equations that fit $c_{\text{sat,CPH}}$ and $c_{\text{sat,SA}}$ as functions of $c_{\text{SA}}$.

\[ c_{\text{sat,SA}} - c_{\text{SA}} = -8.58 \times 10^{-11} \left( \frac{1}{c_{\text{SA}}} \right)^2 + 1.79 \times 10^{-5} \left( \frac{1}{c_{\text{SA}}} \right) - 1.06 \times 10^{-3} \quad (10.A.10) \]

\[ \log_{10} \left( c_{\text{sat,CPH}} \right) = 0.231c^3 + 3.36c^2 + 14.9c + 17.7 \quad (10.A.11) \]

\[ c = \log_{10} \left( c_{\text{sat,SA}} \right) \]

In these equations all of the concentrations have units of g l$^{-1}$. These are plotted in Figure 10.A.1 along with the saturation data. The relationship between $c_{\text{SA}}$ and pH is also fit to an empirical equation

\[ \text{pH} = -0.277 \ln(c_{\text{SA}}) + 3.42 \quad (10.A.12) \]

The solutions to equations 10.A.10 through 10.A.12 are plotted in Figure 10.A.1 along with the saturation data. This plot shows that the minimum pH that can be obtained is about 4.75, as the SA concentration required to obtain lower pH is above the saturation concentration.
Figure 10.A.1. Saturation concentrations of SA (■) and CPH (▲) as a function of pH and concentration of SA that produces the pH according to equation 10.A.9 (+). Solid lines are the model fits, equations 10.A.10, 10.A.11, and 10.A.12 plotted over the ranges that they are used in the model.

10.9 References


CHAPTER 11
CONCLUSIONS AND FUTURE DIRECTIONS

The overall goal of this research is to develop platforms based on polyanhydride copolymers for controlled release applications such as single-dose vaccines. The specific goals of this work are:

SG1. To describe in detail the microstructure of polyanhydride copolymers and the effects of this microstructure on drug/antigen distribution and release from microphase-separated polyanhydrides.

SG2. To design TT-loaded polyanhydride microspheres and perform in vitro and in vivo studies to discern antigen release profiles and antibody production in order to maximize protective immunity.

SG3. To formulate and solve a mathematical model to predict and tailor copolymer microstructure effects on drug/antigen release mechanisms.

Here we have focused on copolymers of 1,6-bis(p-carboxyphenoxy)hexane (CPH) and sebacic acid (SA), because this system offers a range of erosion times and the potential to tailor phase behavior and microstructure to tailor material properties. SG1 is addressed in Chapters 4, 5, and 6. In Chapter 4 the phase behavior of poly(CPH)/poly(SA) blend system is investigated by scattering, microscopy and molecular simulations. The resulting phase diagram exhibits upper critical solution temperature (UCST) behavior. In addition to the
phase diagram, this work yielded the segment-segment interaction parameter, $\chi_{\text{CPH-SA}}$, which could be used to further characterize the phase behavior of copolymer systems. The semicrystalline microstructure and crystallization kinetics of the copolymers and homopolymers is investigated in Chapter 5 by synchrotron small-angle X-ray scattering experiments (SAXS). The crystallization kinetics may be important for determining processing conditions that yield the optimum crystalline microstructure for a given application. We determined that it is difficult to control the crystallinity of the copolymers rich in SA and the poly(SA) homopolymer. Avrami constants for all copolymer compositions studied were reported. In Chapter 6, additional details of the amorphous microphase separation are discerned by SAXS and solid state nuclear magnetic resonance experiments. These experiments provide molecular resolution of the microstructure in these polymers. Random copolymers rich in either CPH or SA showed microphase separation with domain sizes on the order of 1-3nm. Though this microstructure is too small to affect the encapsulation and release of macromolecular drugs, such as vaccines, it can play a key role in determining the performance of controlled release formulations for small molecular weight drugs.

Future investigations of the copolymer microstructure should include studies of how the microstructure changes when solutes are added to the polymer system. Presumably, drugs dissolved in the microphase separated copolymer will either partition into the phase in which they are most soluble, form a third phase, or misciblize the polymer system. Each of these possibilities has the potential to exhibit unique release kinetics that must be understood for the efficient design of controlled release formulations. The microstructure of copolymers with different molecular topologies should also be investigated including block copolymers, ternary copolymers and copolymer blends. These systems have the potential to offer a wide
array of microstructural characteristics that could be of use for biomedical applications. Block copolymers could offer larger microphase separated domains. Blends could be used to create even larger domains or core/shell type microspheres, and ternary systems offer the potential to from more complex phase morphologies that could be used to encapsulate multiple drugs, aid in the stabilization of macromolecular drugs, or produce more complex release profiles.

Chapters 7 and 8 focus on SG 2. In Chapter 7 we demonstrate the fabrication and in vitro release kinetics of polyanhydride microspheres loaded with a model small molecular weight drug. In addition to describing the fabrication of an injectable drug delivery system based on the poly(CPH-SA) system, we demonstrate the ability to tailor the release kinetics by making “cocktails” of microspheres that have different release profiles. Chapter 8 reports the design of tetanus toxoid (TT)-loaded microspheres as single dose vaccines. It is shown that although high doses of the polymer induce a dose-dependent, localized inhibition, small doses have a beneficial adjuvant effect. TT released from the microspheres maintains its immunogenicity and antigenicity and the microspheres provide a prolonged exposure to TT sufficient to induce the secondary immune response without requiring an additional administration of vaccine. In addition to providing an immune response with a single dose, the microsphere delivery vehicle has the capability to modulate the immune response mechanism. It is well known that traditional alum-based vaccines typically induce primarily a T\text{H}2 (humoral) immune response, as does unencapsulated TT. The ability of this delivery vehicle to preferentially induce a balanced (T\text{H}0) response mechanism is a unique and valuable characteristic that may make it valuable for treating a variety of viral and intracellular pathogens. A T\text{H}2 dominant response can also be obtained from the microspheres by altering the formulation.
Future work with this system should involve encapsulation of different small molecules to ascertain the effects of small molecule chemistry on the release kinetics. The release kinetics can be combined with the microstructural characterization to further enhance understanding of the interplay between the macroscopic (release kinetics) and the microscopic (microstructural) features of this polymer system. The results of Chapter 8 motivate several further studies. Additional vaccines should be studied and future work could also draw relationships between release kinetics and immune response. In particular the mechanism of the preferential induction of immune response pathways requires further investigation to understand. The interactions between polyanhydride microspheres and lymphocytes such as dendritic cells could offer insights into the observed phenomenon. Another potential of this system is to provide a low level exposure to an antigen in order to induce tolerance. This has potential for treatment of a variety of disease states, such as autoimmune disorders. Mechanisms of immune tolerance induction are just beginning to be understood and this system could be useful in future studies of tolerance. Controlled release formulations may also be a means of inducing model chronic disease states in otherwise healthy animals.

Chapters 9 and 10 are concerned with SG3. In Chapter 9 an erosion model is introduced that successfully accounts for the proposed microphase separation and partitioning that exists in the copolymers. Comparisons to experiment provide insights into the phenomenon of drug partitioning in microphase separated copolymers and permit the determination of a partition coefficient. This model, like many models in the literature, does not account for the several elementary processes that make up erosion, namely polymer degradation, monomer dissolution, and diffusion. In order to more fully understand the effects of these several phenomena on the erosion mechanism, Chapter 10 offers an improved
model that accounts for these elementary processes. This model is capable of accounting for several key experimental observations that previous models neglect such as the accumulation of undissolved monomer and changes in pH of the microenvironment during erosion. These phenomena may not only affect the erosion kinetics, but may also affect the stability of macromolecular drugs. The model is fit to experimental monomer release kinetics. Detailed descriptions of the microenvironment of the erosion zone are obtained. Parameters such as the pH and concentration of dissolved and undissolved monomers in the erosion zone are difficult to measure experimentally, but are described in detail by this model. This model also motivates additional experiments to ascertain parameters such as the degradation rate constants for amorphous and crystalline polymer and phenomena such as the pH of the microenvironment in an eroding polyanhydride device.

In summary, this work combines materials science theory, device design and fabrication, testing of novel drug delivery devices, and complex kinetic modeling to further the development of controlled release formulations based on polyanhydrides. In addition to the development of a novel vaccine delivery system, this work resulted in the development of two new erosion and drug release models that offer new insights into the mechanisms of erosion, and characterization of a valuable multicomponent polymer system. Additional research is motivated by new questions generated from this research.
BIOGRAPHICAL SKETCH

Matthew J. Kipper was born January 16, 1978 to Dr. Roy C. and Sandra M. Kipper, in Ames, Iowa. He earned his Bachelor's of Science degree in Chemical Engineering from Iowa State University in 2000 and his Ph.D. in Chemical Engineering from Iowa State University in 2004. While an undergraduate Matt worked as a co-op engineer for Equistar Chemicals in Clinton, Iowa. He also worked as an undergraduate research assistant and a tutor for the Department of Chemical Engineering at Iowa State University. Matt received an Honorable Mention from the National Science Foundation Graduate Research Fellowship Program and the Biotechnology Fellowship from the Iowa State University Office of Biotechnology for his Ph.D. research.

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