Biodegradable Filaments for Controlled Ophthalmic Drug Delivery

Thesis by

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Abstract

The focus of this thesis is the wet-spinning, in-vitro characterization, and in-vivo implantation of drug loaded filaments for ophthalmic, controlled-release applications. Filaments of ca. 200-300 μm in diameter are comprised of the copolymer poly(d,l-lactide-co-glycolide), with various lactide to glycolide ratios, and either the antibiotic levofloxacin or the steroid dexamethasone. The objective of this work is to develop implantable filaments that can provide long release of drugs in the eye and then dissolve in order to replace eye drops, since poor patient compliance can limit the utility of drops.

Filament formation by wet-spinning is examined in Chapter 2. Mass transfer during filament coagulation is experimentally probed. The experimental plan explores the effect of drug on the mass flux of solvent and antisolvent. Drug retention during extrusion is examined in the context of mass transport, as well as solid-state and solution-state thermodynamics. Chapter 3 presents data that show how the composition of the filaments affects the thermal, mechanical, and release properties. By manipulating various aspects of filament formulation (drug content, polymer type, etc.), release rate and mechanical properties can be greatly changed. The development of an in-vivo model (rabbit) to verify in-vitro results is described in Chapter 4. Drug release into the tear film and mechanical stability are determined for one filament using three different implantation techniques. Large exposed sections of filament are necessary for drug release into the tear fluid and filament ends must be secured for in-vivo mechanical stability. In-vivo results correlate well to in-vitro results for both drug release and mechanical life span. A method of combining the properties of different single component filaments through side-by-side

multicomponent wet-spinning is described in Chapter 5. Single component properties are maintained by this method, so mechanical and release properties of various monocomponent filaments described in Chapter 3 can be combined into a single filament. This thesis shows that wet-spinning is a versatile method of producing drug loaded filaments with various drug encapsulation and release properties, and that these filaments are well suited for ophthalmic controlled release applications.

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Chapter 1: Introduction

This thesis describes efforts aimed at the production and characterization of a new drug delivery system that is formed by a wet-spinning technique. A filament is created by the coagulation of a polymer solution containing a dissolved or suspended drug. Wet-spinning an old technique used for preparing polymer fibers and filaments, but the addition of drugs to the coagulating system introduces a new variable that required significant investigation. The filaments are designed for use in ophthalmic applications (the primary objective of this work), but they could be used for many other applications. The overall goal of this work is to develop a device that can be implanted into the eye with a standard surgical needle. The filament should maintain mechanical integrity without physically irritating the eye and release drugs over the course of several days to weeks.

1.1. *Ophthalmic Drug Delivery*

Eye drops are the standard way of delivering drugs to the eye but efficacy suffers from poor patient compliance, physical difficulties administering drops, variable drop size, and low residence time in the eye. These problems are often worse in children due to the necessity of adults administering drops to often uncooperative patients. Poor patient compliance compromises pharmaceutical efficacy and compliance is particularly poor for self-administered eye drops[1]. One study of patient compliance using electronic monitoring of drop dispensers found that 41% of patients miss at least 6 doses of drug over 30 days. Another study using self-

reporting found that only 64% of patients used the drops as directed[2]. The main reasons for skipping drug doses are lack of knowledge, lack of interest, and physical inability. These problems are often exacerbated in children[3] and the elderly[2] as their physical limitations often force them to rely of others administering their drops. One study found that 36% of patients missed getting the drop in their eye frequently while 20% had problems squeezing the bottle[2].

To address the problem of poor patient compliance, various controlled release drug delivery strategies have been tried with limited success. The largest commercial success was achieved by the Ocusert, a product developed and sold by ALZA corporation. Ocusert was an unanchored, 5.5 x 13 x 0.5 mm insert containing pilocarpine (an anti-glaucoma drug that requires multiple drop installations each day for efficacy) that was slid into upper or lower cul-de-sac of the eye[4]. Compression by the surrounding tissue would hold the insert in place and drug would release over a week. The Ocusert typically worked well, and reduced intraocular pressure to levels comparable to multiple doses of drop based pilocapine each day. While irritation was reported by some patients, it was not limiting for most. The major drawback to the Ocusert was the tendency of the device to come out of the eye before the appropriate time. A clinical study by Sihvola and Puustjarvi reported that 37% of the devices fell out over the course of a single week and that 41/52 implantations had some problem such as falling out, displacement, distortion, or insufficient effectiveness against glaucoma[4]. While greater instruction on how to use the Ocusert could minimize the risk of device loss, there was no means to reliably protect against elimination.

Implants that require surgical placement have also been studied. Kato et al constructed an episcleral implant made of ethylene vinyl acetate copolymer and polyvinyl alcohol[5]. The episcleral implant required surgery to implant and, since neither polymer is degradable, would require a second surgery to remove. The benefit of such a device is that it can deliver betamethasone at a relatively constant rate for three months since drug release is not dependent on a biodegrading polymer[5]. Intraocular implants that do not degrade have also been studied[6-8]. These implants have essentially the same benefits and drawbacks as the episcleral implant. Since device degradation and morphology are not constraints, traditional techniques of controlling drug release can be used such as coating degradable polymer with non-degrading material[8] or using a membrane[6] to slow delivery. The drawback to these devices will always be that multiple surgeries will be required and patients will not want to go through chronic invasive surgeries for long term treatment. For post-operative therapy, a second surgery several weeks after the first is undesirable from the perspective of the patient. As such, these implants are best suited to non-recurring, acute, and dangerous conditions such as prevention of proliferative vitreoretinopathy after retinal reattachment surgery or treatment of severe keratitis.

Biodegradable implants, both inside the eye and outside the sclera, have also been studied. These implants require only a single surgery since the devices will eventually degrade. Devices to date have tended to be rigid devices made from compression molding, melt extruding, or solvent evaporation techniques[9]. These devices are then placed directly at a site and either secured or allowed to move freely. A notable drug delivery platform is what used to be called the Oculex Drug Delivery System (before Allergan aquired Oculex in 2003)[10, 11]. The form of this device in clinical trials, called Posurdex, releases dexamethasone into the anterior

chamber following cataract surgery[10]. This drug delivery platform has also been used to deliver cyclosporin to the back of the eye. The form of the insert is a 1 x 2 mm rod that is implanted surgically and is made of PLGA. It is implanted immediately after surgery and fully degrades in time. Drugs delivered by this method tend to be hydrophobic and release 0.06 mg of dexamethasone or 0.5 mg of cyclosporin[11]. Other biodegradable implants have been placed outside of surgical sites to prevent wound closure after glaucoma surgery[12]. Biodegradable films for transscleral drug delivery have also been tested in-vitro[13].

In many cases, delivery of drug to the tear film may be desirable or a surgery requiring deep penetration of the tissue may be too extreme. Particularly for antibiotic therapy, devices may be placed outside the front of the eye and contact the tear film directly. One device for this purpose is a bioerodilbe minitablet[14-16], which is made from compressing a powder mixture of binders and ciprofloxacin hydrochloride. This device allowed for the slow release of ciprofloxacin into the tear film by simply dropping the minitablet into the fornix[15]. Initial studies using a fluorescent tracer molecule showed that tear concentration peaked between 2 and 3 hours then fell by over an order of magnitude over the course of the next seven hours[14]. This fall off was relieved somewhat by changing the compression parameters[16], but the goal for this device is approximately 8 hrs of sustained release. Because the minitablet would gel on contact with water, it tended to stay in place over the time course for the experiment (8 hrs), but is unlikely to be a substitute for multiple day therapy. The gelling and dissolving of the tablet also led to reports of irritation and blurred vision in patients with significantly higher lacrimation over eye drops[15]. Another device was described by Pijls et al to release the veterinary antibiotic pradofloxacin into the tear film of dogs. This device consisted of binding polymers and drug

encapsulated in a stainless steel coil and further coated with polymer. The device showed in-vitro release characterized by fast release (with highly device dependent spikes) followed by lower release to two days. In-vivo, the device showed high initial concentrations (30-80 μg/ml depending on the animal), but after 8 hrs most of the tear concentrations fell below 1 μg/ml until the test ended at 16 hrs. It was also observed that most of the devices fall out due to blinking or the third eyelid of the dog dislodging them.

Another category of a polymeric controlled release device is microspheres or nanospheres. Since the microspheres on the eye are easily eliminated by tearing, much as a drop, the microspheres are often administered by sub-conjunctival[17], intra-ocular[18, 19], or periocularl[20] injection. These particles are able to move through the tissue once implanted and are nearly impossible to remove if there is an adverse reaction to the drug or polymer. Since release is usually controlled by the degradation of the polymer, sustained release of several days to weeks is possible. Often these polymers are of the family of polylactides (PLA) or poly(lactide-co-glycolide) (PLGA).

1.2. *Wet-Spinning*

Processing of fibers and filaments from polymer solution was the earliest method of making such devices, and is still used today in a more limited fashion[21]. Polymer is dissolved in an appropriate solvent and extruded into a coagulation bath that contains a nonsolvent that is miscible with the solvent. Wet-spinning has diminished in importance, relative to melt spinning, due to the necessity of removing and disposing of solvent/coagulant mixtures and the problem of retaining solvent in the final product[21]. As a result, wet spinning is typically only used when

the properties of the polymer make melt spinning prohibitively difficult. An example of this is the spinning of Kevlar, Poly-p-paraphenylene terephthalamide, a high-performance fiber composed of aromatic polyamides.

Wet spinning was chosen as the processing technique for this study since our design criteria included making a "soft" device that could deform easily, a desire to process the filaments at or near room temperature, and a size constraint that the filament be implantable by a standard surgical needle. Wet spinning will produce a porous product under many conditions; since the demixing process associated with phase inversion causes polymer lean voids to form in the device structure (see Chapter 3). This porous structure will allow some compliance if the filament is pressed by the surrounding tissue. Wet processing also allows for room temperature conditions during the entire formation as long as solvents are chosen for low temperature dissolution properties. Low temperatures may be important for pharmaceutical processing, as many active species degrade when exposed to high temperatures.

Wet spinning has been used for drug delivery applications, with several examples appearing in the literature in recent years[22-26]. Drug eluting filaments are an exciting platform because the morphology of the device is relevant to many applications. For example, wet processed filaments can be arranged into meshes for tissue engineering or used to directly infiltrate a tumor with chemotherapy agents. For ophthalmic applications, filament morphology allows the mechanical implantation of a device. The filament is "self anchored" so the drug delivering matrix provides its own mechanical stability and could potentially overcome some of the drawbacks of unanchored devices like the Ocusert.

1.3. *Objectives*

The specific objectives of this thesis are as follows:

1. Develop a wet-spinning process for producing drug-loaded filaments out of biocompatible and biodegradable materials.
2. Determine what parameters are important for high drug retention during filament formation.
3. Determine how filament formulation affects thermal properties, mechanical properties, and drug release properties.
4. Examine how filament formulation affects drug release in-vitro and verify release characteristics with in-vivo.
5. Perform in-vitro mechanical properties analysis that mimics in-vivo conditions and provides guidance as to in-vivo filament life span.
6. Enhance the utility of wet-processed filaments through side-by-side, multicomponent spinning and show that single component filament properties can be combined to create a superior product.

1.4. *Materials Selection*

1.4.1 Polymer

Poly(lactide-co-glycolide) (PLGA) (chemical structure shown in Figure 1.1) is probably the most commonly used biodegradable polymer for controlled drug release applications. PLGA is FDA

approved as both suture material and matrix material for controlled release devices. PLGA is also commercially available at many different molar masses and copolymer compositions, so release properties can be tuned.

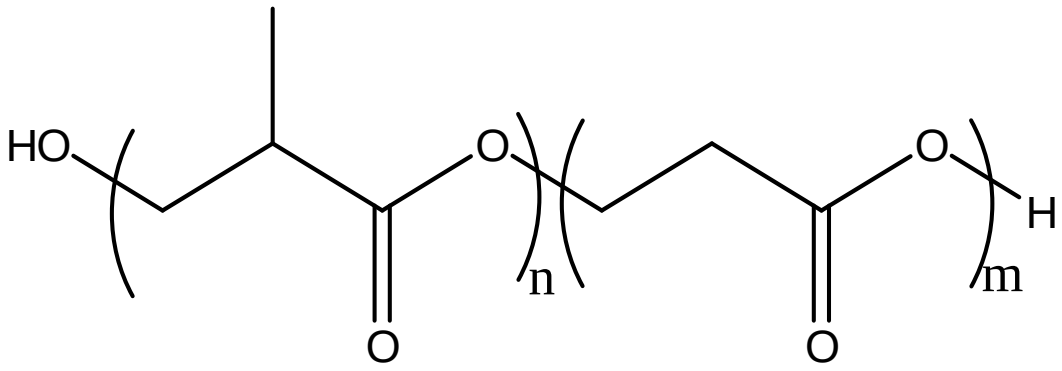

Figure 1.1. Chemical structure of Poly(lactide-co-glycolide) (PLGA).

PLGA is a polyester and degrades by hydrolysis as shown in Figure 1.2. The final degradation products are lactic acid and glycolic acid, both naturally occurring metabolic products. The degradation rate depends on the lactide to glycolide ratio in the copolymer and the rates of degradation for many copolymers are available in the literature[27-29]. Poly(lactide) (100% lactide with no glycolide content) degrades the slowest, with an increasing content of glycolide leading to faster degradation until the lactide to glycolide ratio reaches 50:50. The PLGA that degrades the fastest is the 50:50 copolymer and as the glycolide ratio increases further, degradation slows again.

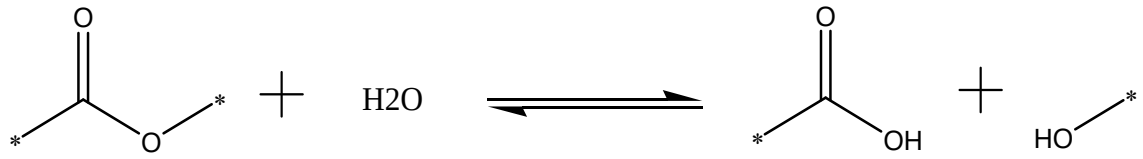

Figure 1.2. Ester hydrolysis in PLGA.

The stereochemistry of the lactide content of PLGA influences degradation rate and physical properties of the polymers[30, 31]. Poly(l-lactide) contains only one enantiomer of lactide, and

results in a semi-crystalline polymer. As glycolide content increases in poly(l-lactide-co-glycolide), crystallinity diminishes and is lost beyond approximately 15-20% glycolide. Crystalline polymers form mechanically strong devices once processed and poly(l-lactide) is one of the most commonly used materials for biodegradable sutures. However, crystalline polymers have limited uses in controlled drug release because of long degradation times and non-homogenous drug release between amorphous and crystalline regions of the polymer[30]. This usually results in a quick release, followed by a very long, slow diffusion-controlled release, and final release due to the eventual degradation of the polymer crystals. This means that drug sequestered in the crystalline regions of the polymer may not be released for months or more. Often, drug release from poly(l-lactide) devices is fully diffusion controlled, with entrapped drug releasing with a constant diffusivity, and polymer degradation is only critical for device elimination. Poly(d,l-lactide) (with approximately equal d and l isomer content) is fully amorphous and is more useful for controlled release applications. Drug is incorporated uniformly through the matrix and degradation happens homogenously. Being amorphous, poly(d,l-lactide) is weaker than poly(l-lactide) and deforms plastically. Amorphous polymers tend to be brittle and are usually not suited to applications requiring mechanical stability under large loads. For our application, we chose poly(d,l-lactide) based PLGA copolymers. Mechanical strength is important for self-anchoring in the eye, but amorphous filaments may irritate less by deforming easily under stress caused by movement of the tissue. More importantly, the need for uniform drug release characteristics and the complete release of drug is of utmost importance when the dimensions of the filaments constrain the total amount of drug that may be released. Many of the techniques described in this thesis should be relevant to a

wide range of polymers, since the overall focus is on the processing technique and in-vivo utility rather than the specific polymer.

1.4.2 Solvent/Non-solvent

When choosing the solvent and non-solvent system for wet spinning a therapeutic loaded filament, the first consideration must be safety. It is unrealistic to expect full elimination of either solvent or coagulant, and certainly not unless the device is exposed to high temperature (undesirable due to polymer and drug considerations) or high vacuum (a limiting process that may end up controlling filament properties). Since residual solvent and non-solvent must be expected, both must be chosen for low toxicity as well as solubility properties.

The U.S. Food and Drug Administration (FDA) characterizes residual solvents in drug formulations by toxicity properties. Class 1 solvents are unacceptably toxic and should be avoided unless there are no other alternatives or there is a significant benefit that justifies the added risk. Class 2 solvents are less toxic, but should be limited in use as much as possible. Class 3 solvents have little toxicity and should be used preferentially over Class 1 or 2 solvents. A list of Class 3 solvents is given in Table 1.1.

Table 1.1. FDA class 3 solvents with Hansen solubility parameters and mixing energetic parameters with PLGA

	Hansen Parameters					REM	
	δ_d	δ_p	δ_h	Ra1	Ra2	Ra1/R	Ra2/R
Acetic Acid	**14.5**	**8**	**13.5**	**6.6**	**6.8**	**0.9**	**0.9**
Acetone	**15.5**	**10.4**	**7**	**5.3**	**7.4**	**0.7**	**1.0**
Anisole	**17.8**	**14.1**	**6.7**	**6.3**	**7.0**	**0.8**	**0.9**
1-Butanol	**16**	**5.7**	**15.8**	**6.9**	**7.6**	**0.9**	**1.0**
2-Butanol	**15.8**	**5.7**	**14.5**	**6.1**	**7.4**	**0.8**	**1.0**
Butyl acetate	**15.8**	**3.7**	**6.3**	**7.6**	**11.6**	**1.0**	**1.6**
tert-Butylmethyl ether	**14.8**	**4.3**	**5**	**9.0**	**12.6**	**1.2**	**1.7**
Cumene	**18.1**	**1.2**	**1.2**	**12.3**	**16.8**	**1.6**	**2.2**
Dimethyl sulfoxide	**18.4**	**16.4**	**10.2**	**7.6**	**5.7**	**1.0**	**0.8**
Ethanol	**15.8**	**8.8**	**19.4**	**9.5**	**7.5**	**1.3**	**1.0**
Ethyl acetate	**15.8**	**5.3**	**7.2**	**6.0**	**9.9**	**0.8**	**1.3**
Ethyl ether	**14.5**	**2.9**	**5.1**	**10.1**	**13.7**	**1.3**	**1.8**
Ethyl formate	**15.5**	**8.4**	**8.4**	**4.4**	**7.2**	**0.6**	**1.0**
Formic acid	**14.3**	**11.9**	**16.6**	**9.1**	**6.3**	**1.2**	**0.8**
Heptane	**15.3**	**0**	**0**	**14.5**	**18.7**	**1.9**	**2.5**
Isobutyl acetate	**15.1**	**3.7**	**6.3**	**8.2**	**12.0**	**1.1**	**1.6**
Isopropyl acetate	**14.9**	**4.5**	**8.2**	**7.2**	**10.5**	**1.0**	**1.4**
Methyl acetate	**15.5**	**7.2**	**7.6**	**5.1**	**8.5**	**0.7**	**1.1**
3-Methyl-1-butanol	**15.4**	**3.3**	**12.3**	**7.3**	**9.9**	**1.0**	**1.3**
Methlyethyl ketone	**16**	**9**	**5.1**	**6.1**	**9.3**	**0.8**	**1.2**
Methylisobutyl ketone	**15.3**	**6.1**	**4.1**	**8.2**	**11.8**	**1.1**	**1.6**
2-Methyl-1-propanol	**15.1**	**5.7**	**15.9**	**7.9**	**8.3**	**1.0**	**1.1**
Pentane	**14.5**	**0**	**0**	**15.1**	**19.1**	**2.0**	**2.5**
1-Pentanol	**15.9**	**5.9**	**13.9**	**5.5**	**7.1**	**0.7**	**0.9**
1-Propanol	**16**	**6.8**	**17.4**	**7.8**	**7.3**	**1.0**	**1.0**
2-Propanol	**15.8**	**6.1**	**16.4**	**7.4**	**7.6**	**1.0**	**1.0**
Propyl acetate	**15.3**	**4.3**	**7.6**	**7.0**	**10.7**	**0.9**	**1.4**
water	**15.5**	**16**	**42.3**	**32.8**	**29.3**	**4.4**	**3.9**

Water was chosen as the non-solvent, due to its non-toxic nature and known ability to precipitate PLGA. Other possible non-solvents (selected from the list of Class 3 solvents) were tried, but usually solvated PLGA too well to be a good coagulant. Some mixtures of water with solvents,

particularly isopropanol and ethanol, may be useful in the future, but the current studies were limited to full solutions of water.

An appropriate solvent for wet spinning must be a good solvent for the polymer, as high concentration solutions are necessary. A convenient initial screen for solubility is to compare the Hansen solubility parameters of the polymer with the solvent. Each species is given three Hansen solubility parameters, with the energy from dispersion bonds (δ_d), hydrogen bonds (δ_h), and polar bonds (δ_p) being reported separately[32]. Similar species will have similar parameters, and be soluble in each other. Experimentally, a polymer can be mapped out in Hansen space and a "solubility sphere", with radius R, which contains all of the solvents that dissolve that particular polymer, can be determined. The distance between parameters (Ra) in Hansen space can be calculated by:

$$Ra = 4\left(\delta_{d1} - \delta_{d2}\right)^2 + \left(\delta_{h1} - \delta_{h2}\right)^2 + \left(\delta_{p1} - \delta_{p2}\right)^2$$

A guideline for whether a particular polymer will be soluble in a particular solvent is the Relative Energy Distance (RED), defined as:

$$RED = \frac{Ra}{R}$$

A RED of less than 1 indicates solubility, while a RED > 1 indicates immiscibility.

The reported solubility parameters of PLGA (50:50 lactide:glycolide) are shown in Table 1.2 reported by Schenderlein et al, who also determined R for PLGA to be 7.5[33]. There are two sets of solubility parameters reflecting differences in the type of experiment used for determination. Comparing these values to the Hansen Parameters of the Class 3 solvents (Table

1.1)[32], Ra for each of the solvents with PLGA is given. Solvents with at least one value of REM less than 1 were considered. Solvents must also be miscible with water in order to be useful in wet-spinning. Restricting the list to only solvents that are fully miscible in water removed many possibilities, including some promising solvents such as 1-butanol, 2-butanol, 1-pentanol, methyl acetate, anisole, and ethyl formate. Acetic and formic acid may be appropriates solvents, but ester cleavage is acid-catalyzed, so both were discounted as possibilities. The remaining solvents with REM < 1 are acetone and dimethyl sulfoxide. DMSO was chosen as our solvent over acetone due to a practical consideration. DMSO is denser than water, so extruded solutions of all concentrations "fell" in the coagulation bath, while acetone solutions would sometimes float and complicate the extrusion. DMSO was also found to be a better solvent for our chosen drugs (it is an especially strong solvent for steroids while acetone is not), so it was decided that DMSO would be the most appropriate solvent.

Table 1.2. Hansen solubility parameters for 50:50 PLGA determined by two methods.

Hansen Solubility Parameters (MPa)		
δ_d	δ_p	δ_h
17.4	9.1	10.5
17	12.6	13.4
16	3.6	8.7

1.4.3 Drugs

The overall application for the filament is the controlled release of antibiotic and steroid to the eye. The requirements of drug loading determine the length of filament that must be implanted, so potent drugs are desired to minimize the length of filament that must be implanted. Many steroids have been formulated for ophthalmic use, including prednisone, prednisolone, dexamethasone, hydrocortisone, and prednisolone acetate. Dexamethasone was chosen for our filament, although all of the above steroids were screened and determined to be good candidates for filament incorporation. Dexamethaone has 5-7 times greater efficacy after systemic administration than prednisolone or hydrocortisone. Dexamethasone has already been tested for use in an ophthalmic implant (Posurdex), showing that the long term administration of dexamethasone to the eye can be safe and effective.

Fluoroquinolones are a potent class of antibiotics that are commonly used for ophthalmic treatment. After testing several fluoroquinolones, including ciprofloxacin, sparfloxacin, ofloxacin, and levofloxacin, we chose levofloxacin as the antibiotic most appropriate for incorporation into a drug eluting filament. Levofloxacin is the effective enantiomer of the racemic ofloxacin, making it twice as potent as ofloxacin. Levofloxacin was found to be potent against a wider range of ocular bacterial isolates than ofloxacin or ciprofloxacin[34, 35]. Levofloxacin had the best solubility in DMSO (~10%), after qualitative dissolution experiments, and also has a relatively high solubility in water (25 g/L[36]). The high solubility in DMSO allowed for significant loading of drug, while the water solubility assured that potent levels of drug could be present in the tear film.

1.5. *Thesis Outline*

This thesis is organized in a manner to lead the reader through the formation, characterization, and in-vivo implantation cycle of single component filaments. Next, the single component work is followed with work that enables the combination of filament properties by side-by-side multicomponent spinning. The presentation sequence is designed to assist in understanding for the reader, but it should be stressed that the actual work was iterative in nature, with problems arising during animal testing often influencing further generations of design. A good example of this iterative process is how the finding on the necessity of tear exposure to the filament (Chapter 4) influenced how much attention was paid to mechanical characterization of the filaments in-vitro (Chapter 3).

Chapter 2 focuses on the formation mechanics of the filaments and describes drug, polymer, solvent, and non-solvent transport during filament formation. The analytical techniques for determining filament composition are described and each individual component of the filament is followed as a function of coagulation time. The phase behaviour of drugs and PLGA in DMSO/water solutions is explored and these studies are complimented by light microscopy experiments to visualize the competing phenomena of drug precipitation and PLGA phase inversion. Significant differences in the precipitation of dexamethasone and levofloxacin lead to very different incorporation profiles in the filament. Drawing on these experiments, and what is known about wet-spinning and similar drug delivery devices, a mechanism is proposed to describe drug incorporation in the filament. The terminology in this section is commonly used in wet-spinning, but may require some brief clarification. The term “dope” is used to describe

the solution from which filaments are made or the uncoagulated solution of polymer and drug. “Protofilament” describes the stream of polymer/drug that is undergoing coagulation but is not yet a fully formed product. The term “filament” is reserved for a formed product that is removed from the coagulation bath and will be exposed to no more processing. This terminology allows for the simple referencing of different states of the same materials (polymer, drug, etc.) during processing. Hopefully, this will avoid confusion by allowing readers to immediately fit a particular reference to a specific point on the extrusion timeline.

Chapter 3 examines the in-vitro physical and release properties of the filaments. A wide range of filaments were produced to explore parameter space, and were characterized by determining the composition, glass transition, mechanical properties, and release properties. Studies explored how the polymer type could be used to control drug release and how the physical state of the drug during formation can affect the subsequent release properties of the drug from the resulting filament. Particular interest is given to early stage release, as this stage of release from filaments has been explored by several other groups with conflicting conclusions drawn from about the importance and mechanism of this release. The physical state of the drug during release is explored to determine if trapped drug is in a crystalline or amorphous state. Thermal and mechanical properties are tested in Chapter 3, with particular attention paid to how water influences the glass transition temperature of PLGA and how that subsequently affects the mechanical properties of the filaments. Water has a significant impact on these properties, which is of note because these values are often reported for dry samples and may not be relevant for in-situ performance. How filaments made of various PLGA copolymers lose mechanical properties with time is explored.

Chapter 4 focuses on in-vivo testing of several filaments and compares in-vitro experimental results to in-vivo performance. An animal model was developed for implantation in New Zealand white rabbits, a common ophthalmic model. Filaments were implanted with a standard surgical needle and tear samples were taken and analyzed for drug content. The implantation technique was explored, and it was discovered that tear film contact with the device was required for successful delivery. A new implantation technique, where a filament could stay implanted until mechanical failure, was developed. The utility of in-vitro models was investigated several ways, including comparing release data for various filaments, comparing mechanical failure times, implanted a filament that had undergone incubation in-vitro, and analyzing filaments that had been sequentially removed from eyes over the period of 7 days.

A method for combining the properties of filaments is described in Chapter 5. A lab scale, side-by-side, multicomponent wet spinning process was developed to allow for the production of layered monofilaments. Each layer can be formulated with a different polymer or drug. Steady drug release over an extended period of time is achieved by combining several components, containing the same drug, but with different types of PLGA. A mechanically stable component, formulated from polycaprolactone, can support a PLGA component that encapsulates drug. It is further demonstrated that the morphology, release properties, and thermal properties of each individual component are retained.

Chapter 6 summarizes the general conclusions of the thesis work and provides suggestions for future work. The further exploration of the wet spinning process, especially the manipulation of

bath-side coagulation conditions, is an interesting avenue for further exploration. As for other applications, filaments may be an excellent platform for the release of anti-glaucoma drugs, especially prostaglandin analogues.

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Chapter 2: Investigation of Formation and Mass Transport during Wet-Spinning for Drug Loaded Filaments

2.1. *Abstract*

The formation process of drug loaded filaments prepared by wet-spinning is investigated, and the results rationalized in terms of the mass transport and thermodynamics. Phase diagrams are created for poly(d,l-lactide-co-glycolide) (PLGA), levofloxacin, and dexamethasone in water/dimethyl sulfoxide (DMSO) mixtures. PLGA has a cloud point at ~7% water over the concentration ranges of interest. At 7% water, levofloxacin is 2.5% soluble while dexamethasone is 35% soluble. Formulations containing PLGA dissolved in DMSO with no drug, 7.0% dexamethasone, 7.0% levofloxacin, or 0.7% levofloxacin, are prepared to study the effects of drug type and amount on mass transfer during coagulation. Light microscopy confirms

that when solutions of 23.3% PLGA, 7.0% drug, and 69.7% DMSO contact water, levofloxacin precipitates before PLGA while dexamethasone remains in solution except in high water content areas (outside of the polymer region). Filaments are prepared by extruding the polymer/drug solutions into a coagulation bath filled with water. The mass transport during filament formation is monitored by compositional analysis of sections of filament after various coagulation times. DMSO is rapidly eliminated in all filaments. Water moves into the filament at the very beginning of the coagulation process, but then flows out of the filament as coagulation proceeds. Drug type or amount has little effect on water uptake or DMSO elimination. A filament formulated with 7% levofloxacin continuously loses drug throughout coagulation, with drug retention ranging from 85% at 15 s to 37% at 63 s. Formulations of 0.7% levofloxacin and a 7% dexamethasone have stable drug retention after a rapid loss of 15-20% of initial drug over the first 20 s of coagulation. Levofloxacin is shown to be 4.4 - 5.5% soluble in PLGA at room temperature while dexamethasone is 12.3 – 14.5 % soluble. The drug levels in filaments exceed solid-state drug solubility significantly for both formulations containing 7% drug, suggesting that solubility of drug in PLGA is not the characteristic determining drug entrapment.

2.2. *Introduction*

Wet-spinning has emerged as an attractive process to make drug-eluting filaments[1-6]. To date, there have been several reported instances of encapsulating therapeutics in biodegradable filaments using wet-spinning, but the mass transfer during formation of wet-spun, drug loaded filaments has not been studied extensively. Here, we look at the formation kinetics of drug-loaded filaments.

For most applications, the preferred method of processing polymer filaments is melt-spinning, with wet-spinning primarily used to create high performance fibers[7]. Wet-spinning is useful for high performance fibers because the polymers used in such filaments typically have very high melting temperatures[8]. For wet-spinning, a polymer is dissolved in an appropriate solvent and then extruded into an antisolvent. The antisolvent is chosen to be miscible with the solvent, but precipitate the polymer. Solvent diffuses out of the polymer matrix while antisolvent diffuses in. Residual solvent is usually present in the filament after processing. Commercially, the application of wet-spinning is limited by the residual solvent in the final product and by disposal considerations for large baths of solvent/antisolvent mixtures[7].

Diffusion of solvent and antisolvent play critical roles during filament extrusion and can affect the morphology and properties of the final product[9]. Polymer precipitation can be facilitated by diffusion of solvent from the filament and/or diffusion of antisolvent into the filament[10]. Coagulation typically proceeds from the outside to the inside, with a skin of precipitated polymer forming around the uncoagulated polymer dope[9, 11]. The skin forms immediately upon

solution contact with antisolvent and acts as a semi-permeable membrane for the rest of the formation process. Small molecular weight substances, like solvent and antisolvent, can pass through the membrane while polymer does not. As solvent diffuses out of the dope, and antisolvent diffuses in, the internal concentrations of solvent, antisolvent, and polymer reach values that cause phase separation, and coagulation occurs.

Solid-state solubility of a drug in a polymer has been used as an indicator of the suitability of a particular drug release matrix[12-14]. Drugs that are more soluble in polymers can maintain high drug loading without affecting controlled release properties. These devices are usually processed by solvent evaporation or dry processes and there have been no studies that indicate that solid-state solubility is the critical design parameter for wet-spun drug containing filaments. Previous studies have also shown the importance of solution thermodynamics to the drug release from in-situ forming depots and other devices similarly processed[15]. It remains an open question as to which solubility is more important for drug encapsulation in a filament processed by wet-spinning, the solubility of drug in polymer or drug in solvent/antisolvent mixture.

While drug containing filaments have been reported, there are no available studies on the mechanisms of coagulation or the methods of drug encapsulation. Final products have been characterized, but how the actual filaments form and how drug is lost during the coagulation process have not been studied. In this chapter, the process of forming a drug loaded filament by wet spinning is explored. Particular consideration is given to how the composition of a filament changes with coagulation time, with drug, solvent, antisolvent, and polymer compositions

independently determined. The mechanism of coagulation and drug encapsulation is also explored.

2.3. *Calculations*

It is convenient to perform a mass balance for the wet-spinning process so that mass losses and gains for solvent, antisolvent, drug, and polymer can be better understood. There are several parameters that are necessary to define a mass balance across a wet-spinning process. The volumetric rate of extrusion (V_E), the linear uptake rate of filament (u), and the radius of the spinneret (r) define the draw ratio (DR) on the filament as follows:

$$DR = \frac{V_E}{\pi r^2 u} \qquad 2.1$$

The draw ratio is the measure of how fast the extrusion is occurring relative to uptake. If the DR<1, then accumulation of the filament in the bath will occur and each section of filament will not be exposed to the same coagulation time. A DR>1 puts a tension on the filament during extrusion that helps to orient the final product. After setting the draw ratio for a particular extrusion, then the initial linear mass of the filament (M, calculated before mass exchange with the bath with dimensions of mass/length) is:

$$M_{TOTin} = \frac{\rho \pi r^2}{DR} \qquad 2.2$$

Where ρ is the density of the polymer solution. With polymer (P), drug (D), and solvent (S) comprising the entire initial mass as follows:

$$M_{TOTin} = M_{Din} + M_{Pin} + M_{Sin} \qquad 2.3$$

And the mass fraction (w) of each component is set:

$$M_{Din} = w_{Din} M_{TOTin} \quad 2.4$$

$$M_{Pin} = w_{Pin} M_{TOTin} \quad 2.5$$

$$M_{Sin} = w_{Sin} M_{TOTin} \quad 2.6$$

Since there is no antisolvent in the spinning dope:

$$M_{ASin} = 0 \quad 2.7$$

During formation of the filament, the composition changes with coagulation time. It is convenient to look at how the composition and mass of the filament change with respect to each component over time, but also interesting to observe how each species present in the extrudate changes with respect to how much is initially present. First, we can define the total linear mass of a filament at any coagulation time to be:

$$M_{TOT}(t) = M_D(t) + M_P(t) + M_S(t) + M_{AS}(t) \quad 2.8$$

The masses of each species can be defined by either the mass fraction in the filament (w) or, for the species present in the extrudate, the fraction of species remaining (f):

$$M_D(t) = w_D(t) M_{TOT}(t) = f_D(t) M_{Din} \quad 2.9$$

$$M_P(t) = w_P(t)M_{TOT}(t) = f_P(t)M_{Pin} \quad 2.10$$

$$M_S(t) = w_S(t)M_{TOT}(t) = f_S(t)M_{Sin} \quad 2.11$$

$$M_{AS}(t) = w_{AS}(t)M_{TOT}(t) \quad 2.12$$

A schematic of this mass balance is shown in Figure 2.1.

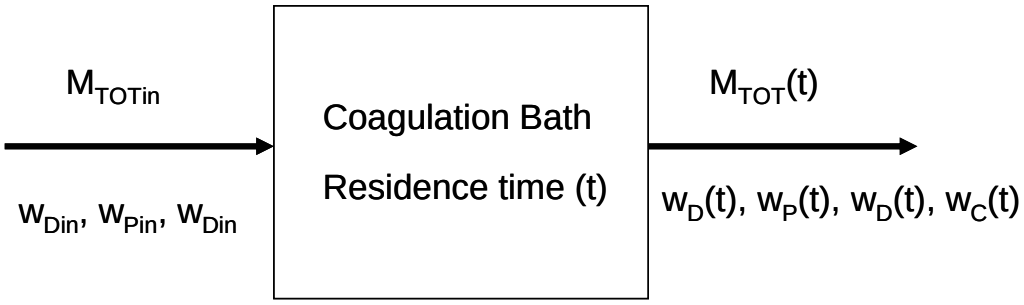


Figure 2.1: Schematic of mass balance over a single filament during formation.

2.4. *Materials and Methods*

2.4.1 Materials

Levofloxacin, dexamethasone, and poly(d,l-lactide) (PDLLA, MW 75,000-120,000) were purchased from Sigma-Aldrich. Poly(d,l-lactide-co-glycolide)'s were obtained from Boehringer Ingleheim. The PLGA's used for this study were Resomer RG 506 (50:50 lactide:glycolide, intrinsic viscosity of 0.82) and Resomer RG 756 (75:25 lactide:glycolide, intrinsic viscosity of 0.80). DMSO, 0.10 N HCl, and 0.10 N NaOH were obtained from VWR.

2.4.2 Phase Diagrams for Dexamethasone and Levofloxacin

Solutions of DMSO and water of various mass ratios were prepared. Drug was added to each solution until in excess. Suspensions of drug were sonicated briefly, and equilibrated overnight at 25°C, vortexing occasionally. Suspensions were centrifuged to a pellet at 100 RPM and the supernatants were passed through a 200 nm syringe filter and the drug content of the resulting translucent solution was analyzed by HPLC.

2.4.3 Phase Diagrams of PLGA

Solutions of PLGA (Resomer RG 506) in DMSO were made with the masses of PLGA and DMSO recorded accurately. These solutions were homogenized, and centrifuged gently to facilitate full mixing and separation of bubbles. Water was added to the solutions in small aliquots (less than 1% by mass addition each time). After water addition, the solutions were homogenized and centrifuged to determine if phase separation was present. When two phases

were observed (one gel-like and rich in polymer, the other liquid with lower viscosity) water addition was stopped and phase separation was reported.

2.4.4 Drug/Polymer Solubility Measurement

Solid-state solubility of a drug in Resomer RG 506 was determined by a semi-quantitative measurement similar to that reported by Panyam et al[12]. Drug solutions of 5 % w/w were made by dissolving levofloxacin in chloroform and dexamethasone in methanol. PLGA was dissolved in chloroform to 2% w/w. Different amounts of drug solution and PLGA solution were mixed and films of ~250 μl were cast on glass microscope slides and allowed to dry. The presence of crystalline of the drug in the film was determined by powder x-ray diffraction.

2.4.5 Formulation

Polymer and drug solutions were made with compositions shown in Table 2.1. Drug was placed in a 4 mL vial and dissolved in DMSO. Polymer was added to the vial and the resulting solution was mixed with a vortexing mixer. The solutions were allowed to settle for 5 hours (voterxing repeatedly to mix).

Table 2.1: Filament formulations

	Formulation Composition		
Drug	**% Drug**	**% PLGA**	**% DMSO**

506-7%Levo	**Levofloxacin**	**7.0**	**23.3**	**69.7**
506-7%Dex	**Dexamethasone**	**7.0**	**23.3**	**69.7**
506-0.7%Levo	**Levofloxacin**	**0.7**	**24.8**	**74.4**
506-25%PLGA	**None**	**0.0**	**25.0**	**75.0**
506-33%PLGA	**None**	**0.0**	**33.0**	**67.0**

2.4.6 Extrusion

A schematic of the wet-spinning process is shown in Figure 2.2. Polymer/drug solutions were loaded into a 5 mL syringe equipped with a 22 gauge flat-tipped needle (Small Parts Inc.) and mounted on a syringe pump. The solution was extruded into a 16 L water bath (22 ± 2°C) and the resulting filament taken up on a 1 in bobbin rotated by a DC gear motor. The pump speed, uptake rate, and path length were chosen to fix a particular coagulation time with a draw ratio of 1.2. Coagulation time was directly measured by mechanically compressing a small section of polymer solution as it exited the syringe and using a stopwatch to measure how long the resulting section of filament took to pass through the bath. After bath residence time was established, approximately 1 m of filament was spun over the course of 2 min. This filament was immediately divided into accurately measured sections and frozen for further analysis.

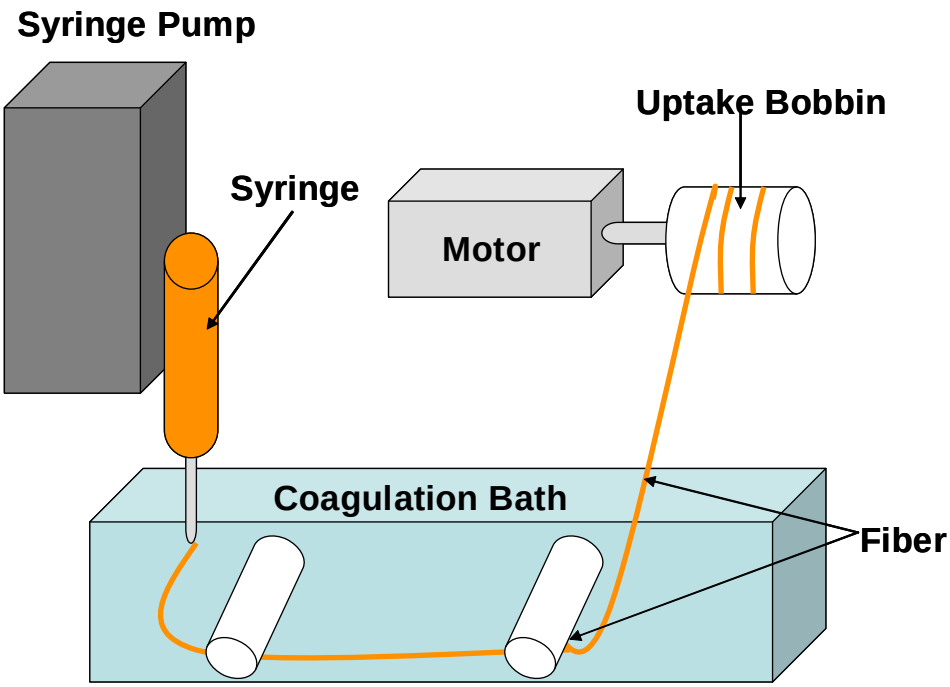


Figure 2.2. Schematic of wet-spinning process.

2.5. *Results*

2.5.1 Phase Diagrams

Levofloxacin and dexamethasone saturation curves are shown in Figure 2.3. Dexamethasone is significantly more soluble in pure DMSO, as well as DMSO/water mixtures. Solid drug particles are in phase equilibrium with the saturated solutions. The points on the curves represent the saturated concentrations of each drug.

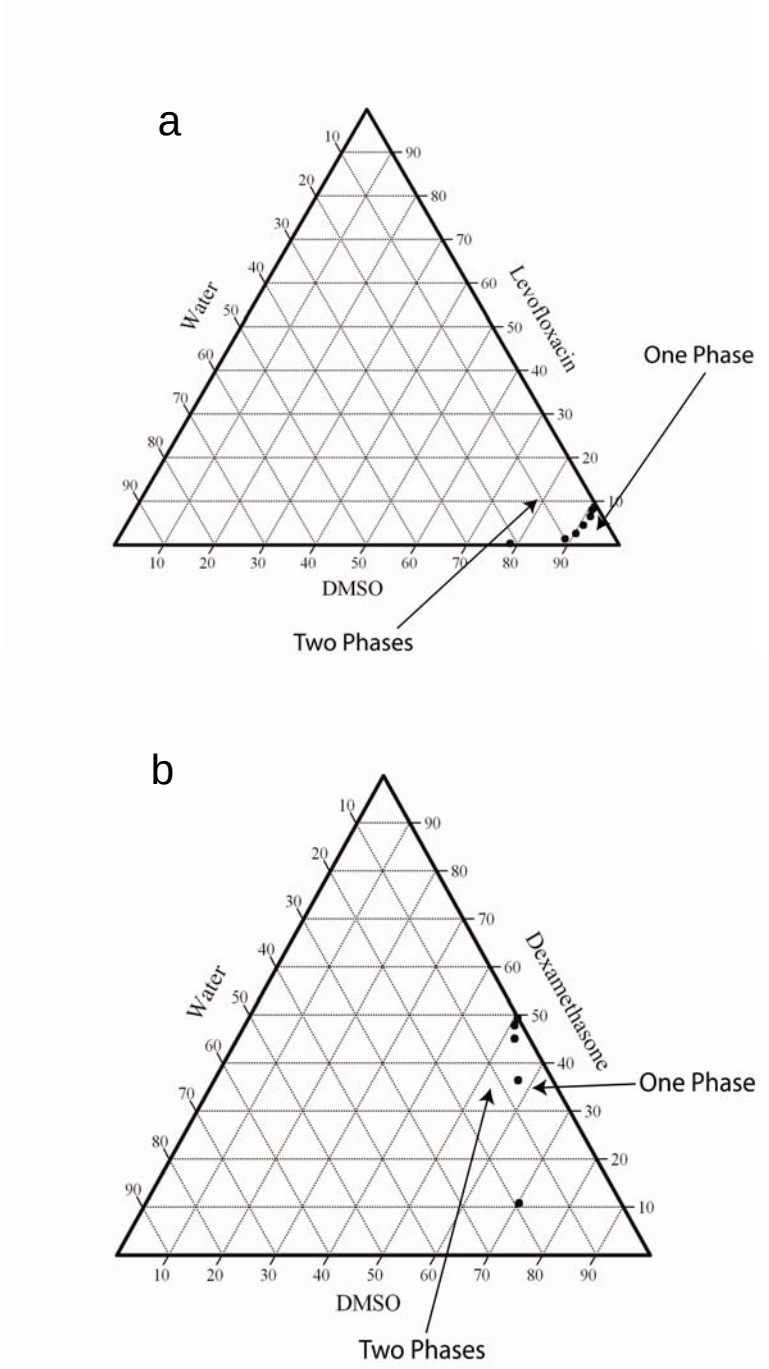


Figure 2.3 Phase diagrams for (a) levofloxacin and (b) dexamethasone.

Figure 2.4 shows the cloud point curve for PLGA, Resomer RG 506, in DMSO and water. The cloud point was determined by dissolving various amounts of PLGA in DMSO and adding water in small amounts. This would cause a small amount of polymer precipitation, which could be re-dissolved if the saturation point had not been reached. PLGA demixes with only a small addition of water, approximately 6-9% over the experiments conducted. The cloud point is the first concentration where a stable, turbid solution is observed. These points were taken at concentrations relevant to filament formation.

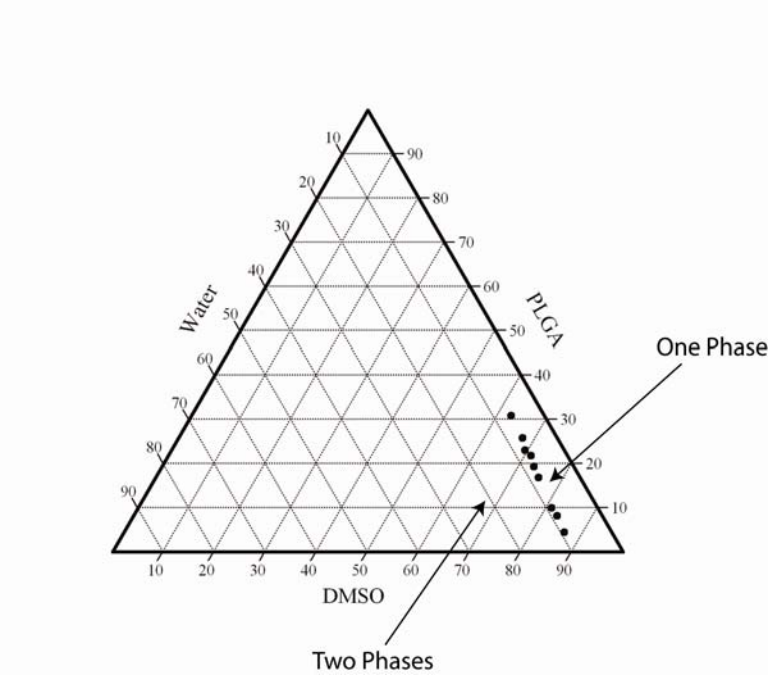


Figure 2.4: Cloud point curve for PLGA.

To better demonstrate the differences in solubility, the above data has been combined and the range has been cropped in Figure 2.5. The percentage of each species (PLGA, Dexamethasone, or Levofloxacin) is plotted against the percentage of water in solution at saturation values. The values of 7.0% and 0.7% are highlighted for clarity, since those are the values at which drug was

formulated for coagulation studies. Of note, the line at 7.0% intersects levofloxacin's curve between 1.3% and 2.4% water, while dexamethasone is still 10% soluble, even at 19% water.

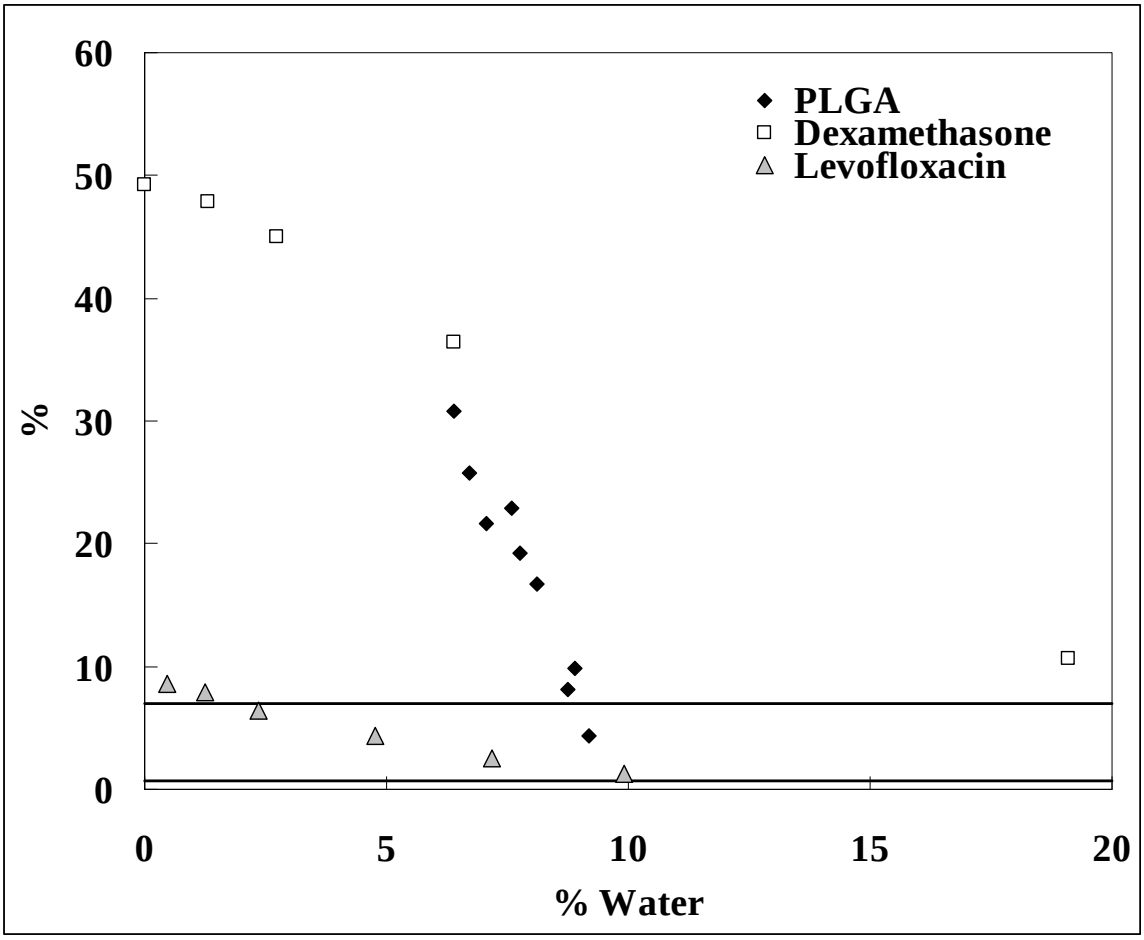


Figure 2.5. Comparative solubility of PLGA, levofloxacin, and dexamethasone in DMSO/water mixtures.
Points represent saturation for particular water content. Lines highlight 7% and 0.7%, concentrations at which levofloxacin was formulated for extrusion studies.

2.5.2 Microscopy

2.5.2.1. 0% Drug

Using light microscopy, the precipitation of drug and polymer can be directly observed. Figure 2.6 shows the interface between a solution of PLGA (P) in DMSO and water (W). A think skins

forms at the interface between the two solutions, allowing water and DMSO exchange. As water diffuses into the polymer solutions, and DMSO diffuses out, the polymer solution undergoes demixing. Long, finger-like structures are observed during coagulation.

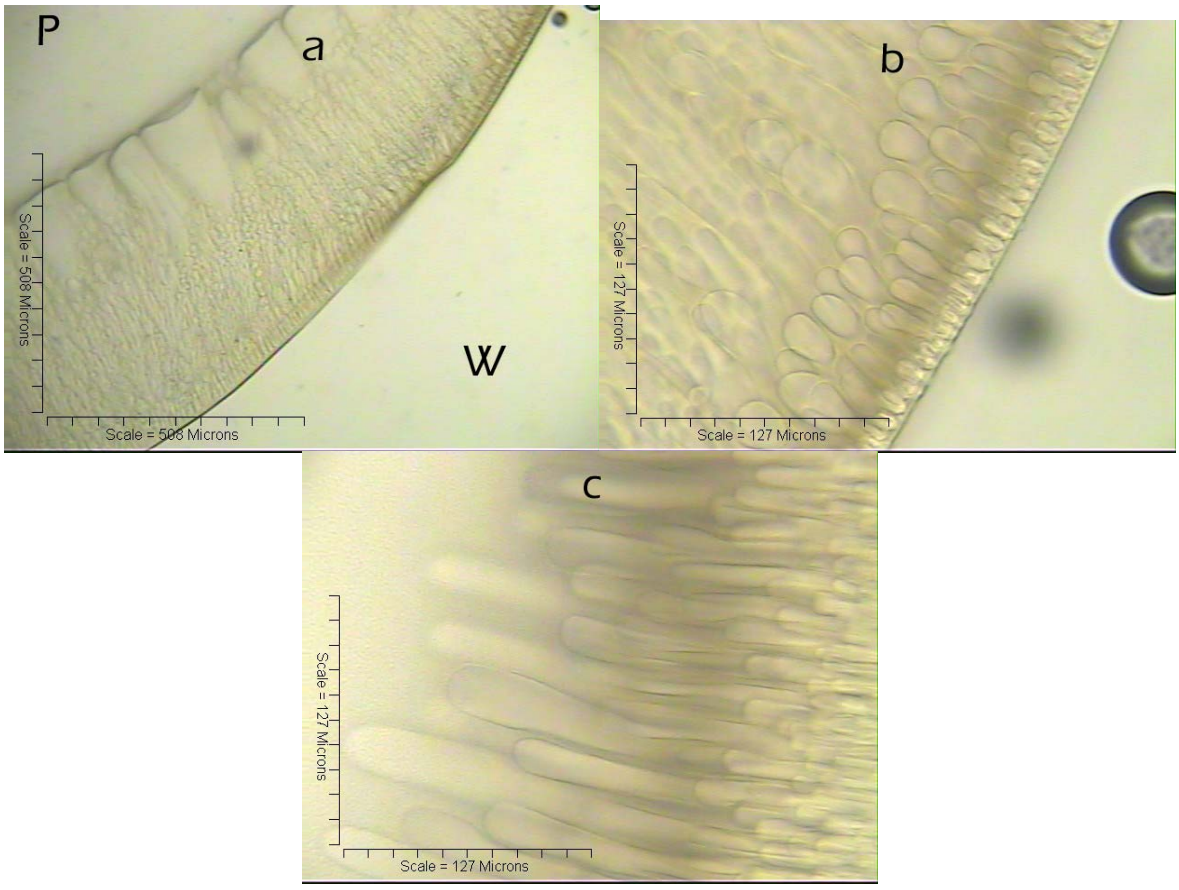


Figure 2.6: Light microscope images of solution 506-25%PLGA contacting water. Magnification is 10x for (a) and 40x for (b) and (c). For image (a), the polymer solution is marked P while the water is marked W. The interface between the polymer solution and water is shown in (a) and (b) while (c) shows the polymer phase change further back in the polymer solution.

2.5.2.2. 7.0% Levofloxacin

When solution 506-Levo is contacted to water, crystalline drug is observed (Figure 2.7). The drug crystals are not visible at or near the interface and get progressively larger further into the polymer solution. Large drug precipitates are observed beyond the PLGA coagulation front in the polymer solution.

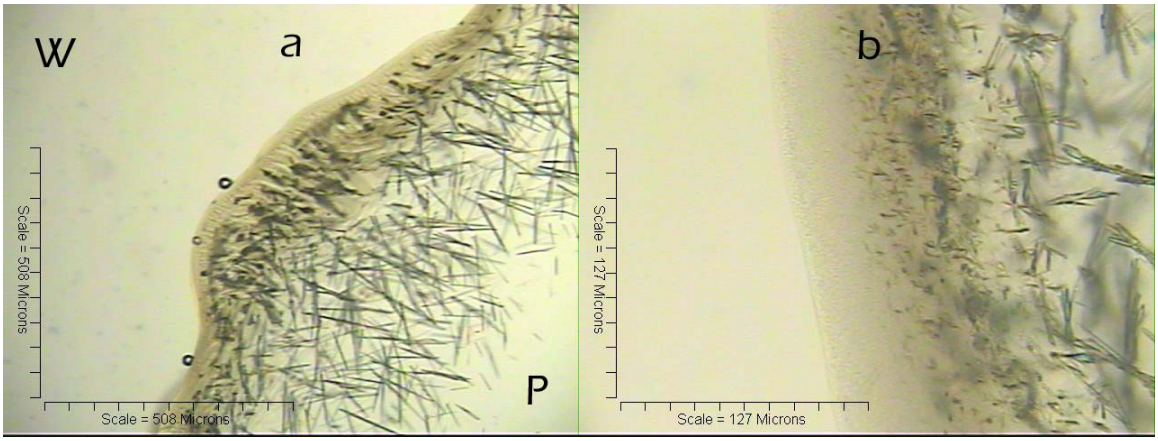


Figure 2.7: Light microscope images of solution 506-7%Levo contacting water. Magnification is 10x for (a) and 40x for (b). For image (a), the polymer solution is marked P while the water is marked W.

2.5.2.3. 0.7% Levofloxacin

When solution 506-0.7%Levo comes into contact with water (Figure 2.8), PLGA coagulation is observed, as in Figure 2.6. No levofloxacin crystals can be seen, either near the polymer/water interface or further into the solution.

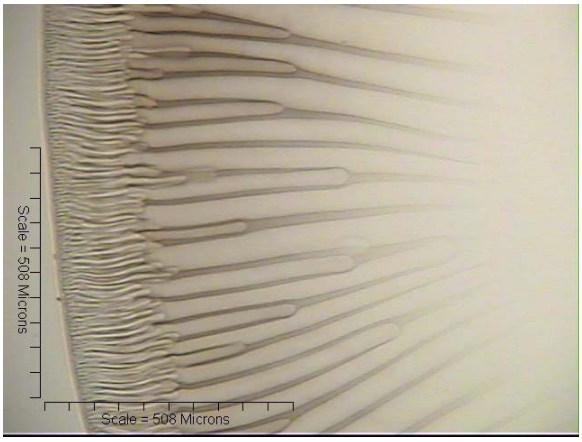


Figure 2.8 Light microscope images of solution 506-0.7%Levo contacting water. Magnification is 10x.

2.5.2.4. 7.0% Dexamethasone

For solution 506-Dex, drug crystals are visible near the polymer/water interface and in the water-rich outer solution (Figure 2.9). Visible dexamethasone crystals are not present in the finger-like region or the polymer solution beyond the polymer coagulation front.

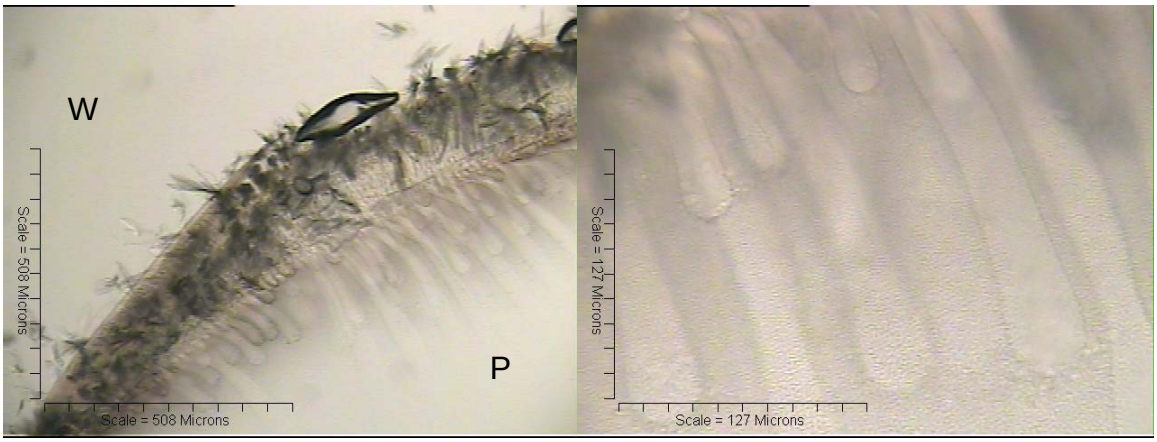


Figure 2.9 Light microscope images of solution 506-7%Dex contacting water. Magnification is 10x for (a) and shows the interface between the polymer solution and water while the magnification for (b) is 40x and shows the polymer solution away from the interface. For image (a), the polymer solution is marked P while the water is marked W.

2.5.3 Solid-State Solubility

X-ray diffractograms of films made with PLGA and drug are shown in Figure 2.10. Varying the ratio of drug to polymer allows the semi-quantitative measurement of solid-state solubility (since PLGA is an amorphous polymer). When precipitated from evaporating chloroform, levofloxacin shows a single characteristic peak at $2\theta = 5.0°$. This peak dissipates as levofloxacin content decreases until it disappears entirely between 4.4% and 5.5% levofloxacin content. Dexamethasone has several characteristic peaks, with the strongest at 16.4º. These peaks are present at 14.5% drug content but not at 12.3%.

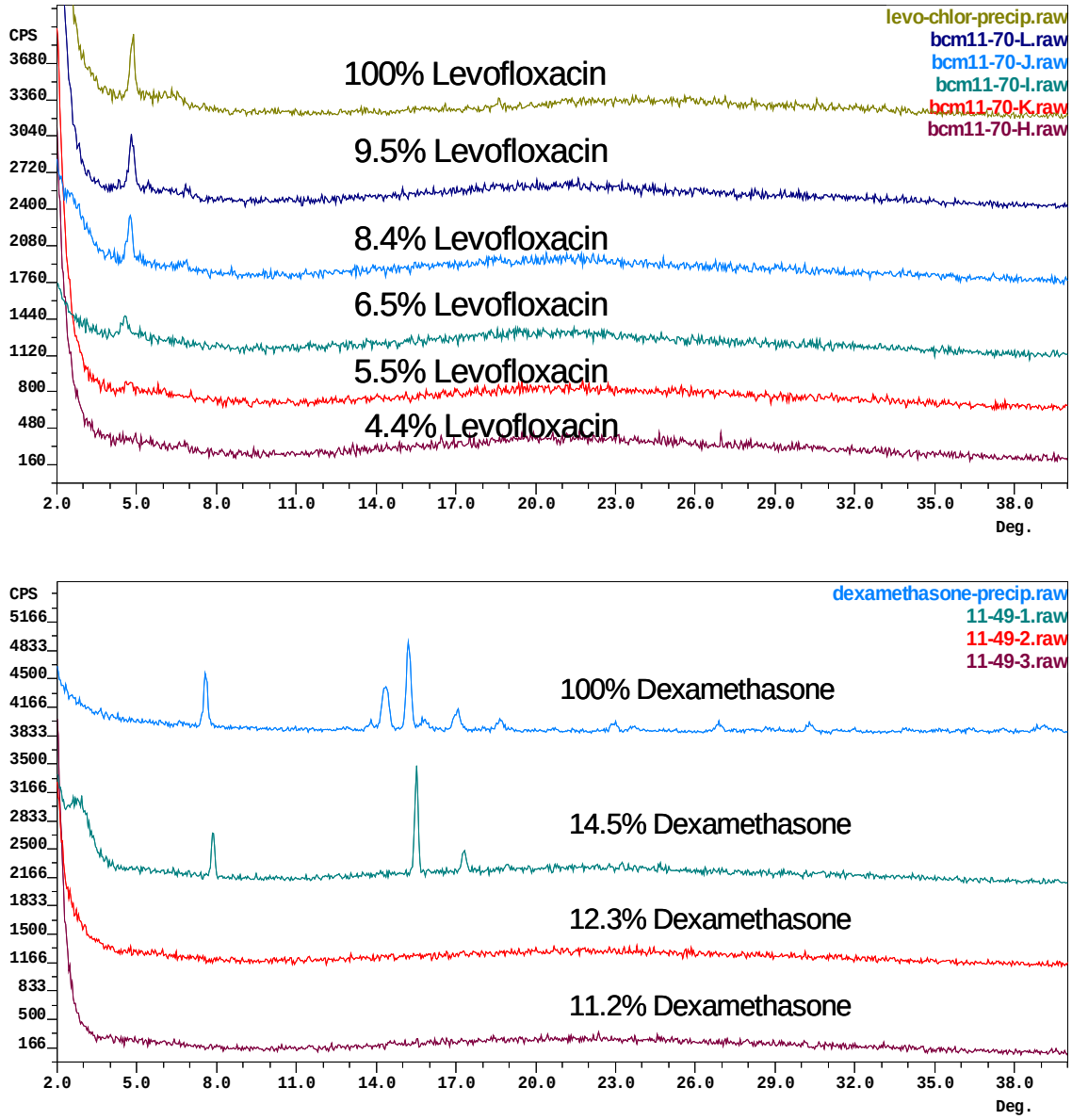


Figure 2.10: X-ray diffractograms of PLGA films containing (a) levofloxacin and (b) dexamethasone.

2.5.4 Polymer Retention

The polymer content of 506-Levo and 506-Dex was measured after a 45 s extrusion to verify full retention of PLGA. Table 2.2 shows the calculated M_{pin} and the experimentally determined

linear mass of each filament. Agreement between the two values is within the error of experimentation, proving that PLGA is retained during the extrusion.

Table 2.2: Measurement of PLGA content for filaments coagulated for 45 s

	PLGA Linear Mass (μg/cm)		
Sample	**Theoretical**	**Experimental**	**% Predicted**
506-Levo	**304**	**306 ± 24**	**101 ± 8**
506-Dex	**304**	**300 ± 11**	**99 ± 4**

2.5.5 Drug Retention

The drugs are small molecule species and may diffuse from the protofilament into the water bath. Figure 2.11 shows drug elimination as a function of bath residence time for all drug loaded filaments. For 506-7%Levo, levofloxacin diminishes steadily, but elimination slows as coagulation proceeds. During the first 19 s of coagulation, 32% of the levofloxacin is lost. Between 40 and 60 s, only 15% is eliminated, but by 60 s, 32% of the original drug remains in the filament. When only 0.7% of the formulation is levofloxacin (506-0.7%Levo), most of the drug is retained in the filament after an initial loss. For 506-7%Dex, 20% of drug is lost in the first 19 s, but drug levels remain essentially constant after the initial drop.

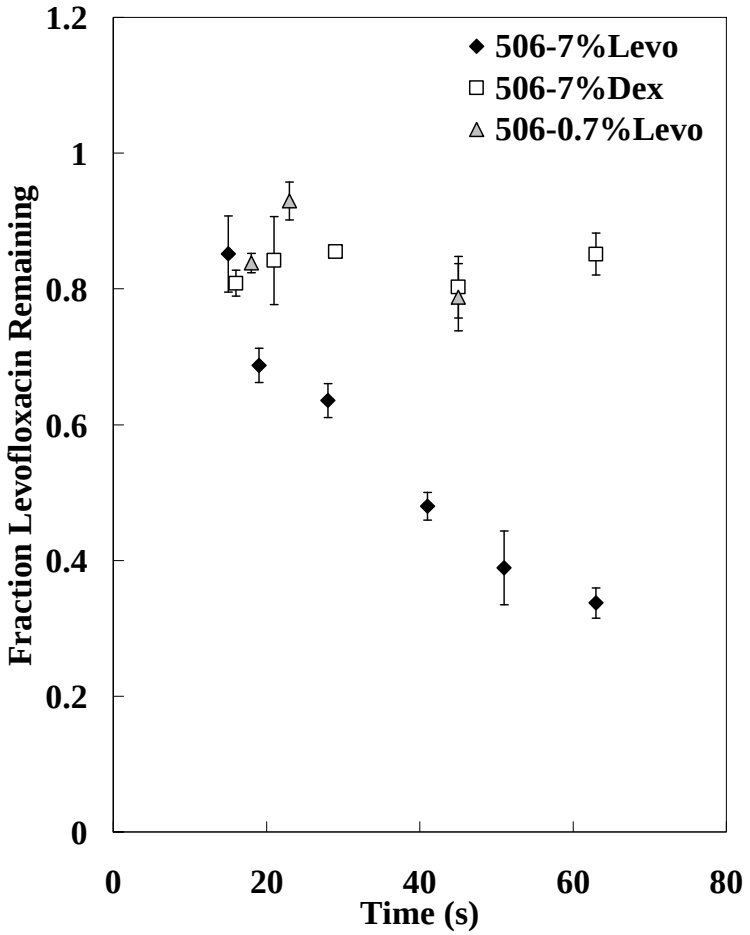


Figure 2.11 Drug retention during filament coagulation.
Error bars indicate standard deviation with n=3.

2.5.6 DMSO Retention

DMSO is rapidly eliminated from the protofilament and generally unaffected by formulation (Figure 2.12). After 20 s, all filaments contain only 25-35% of initial DMSO, with levels further decreasing as bath residence time increases. The only significantly different formulation is 506-33%PLGA, which retains 18% of initial DMSO after 45 s. Drug type does not appear to effect DMSO elimination.

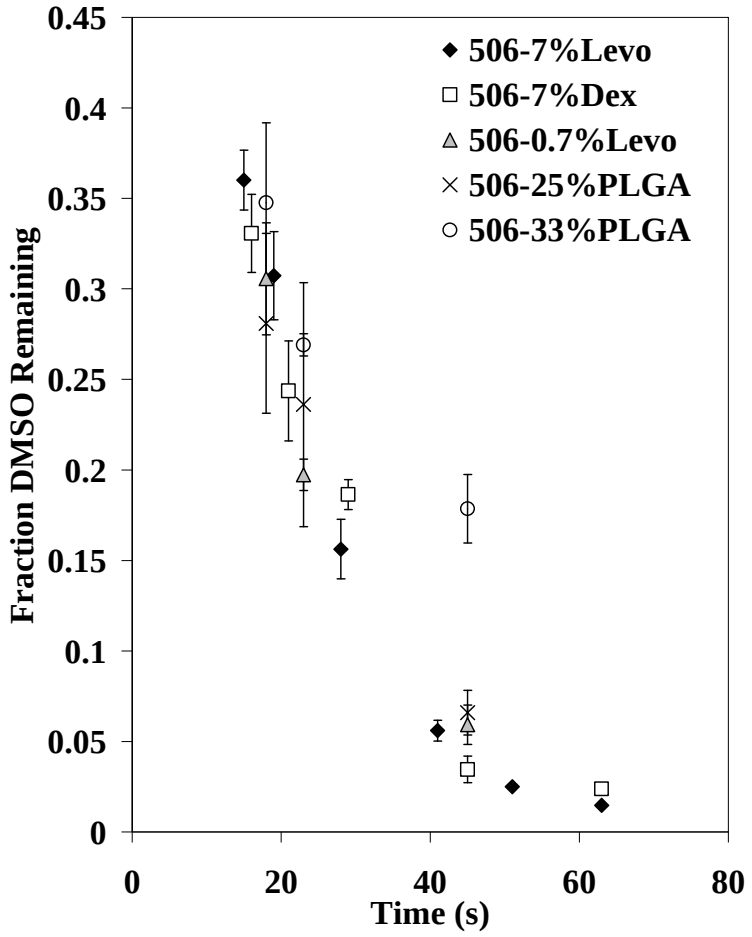


Figure 2.12 DMSO elimination during filament coagulation.
Error bars indicate standard deviation with n=3.

2.5.7 Water Uptake

The polymer solutions are formulated without water and extruded into a coagulating water bath. Figure 2.13 shows the mass/length water uptake as a function of coagulation time. Water rapidly enters the filament immediately after extrusion but is then eliminated during the remainder of the coagulation.

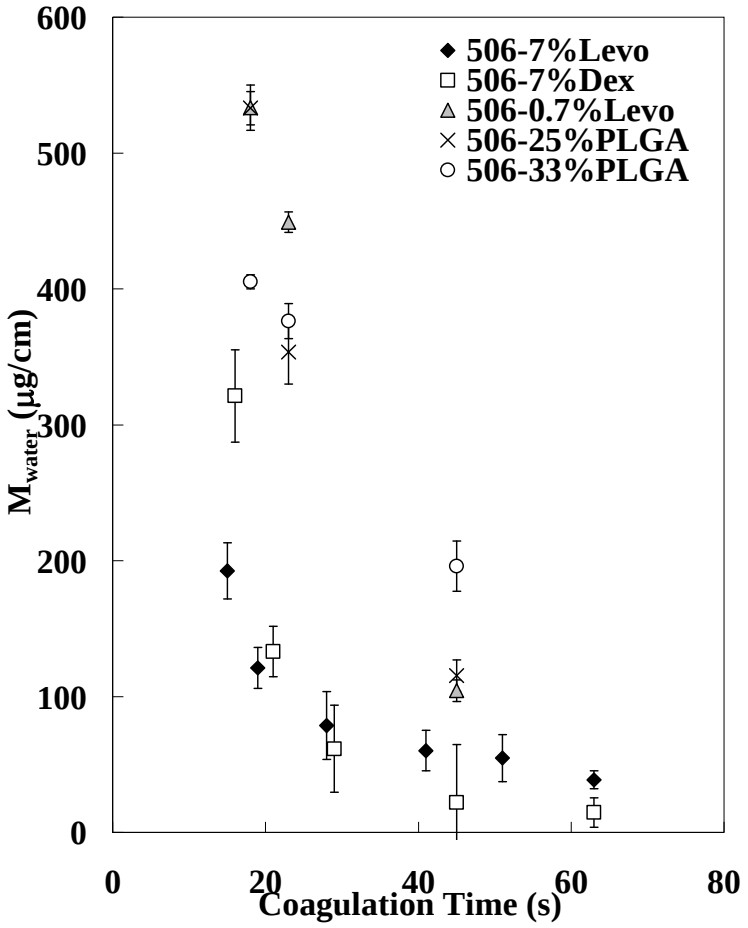
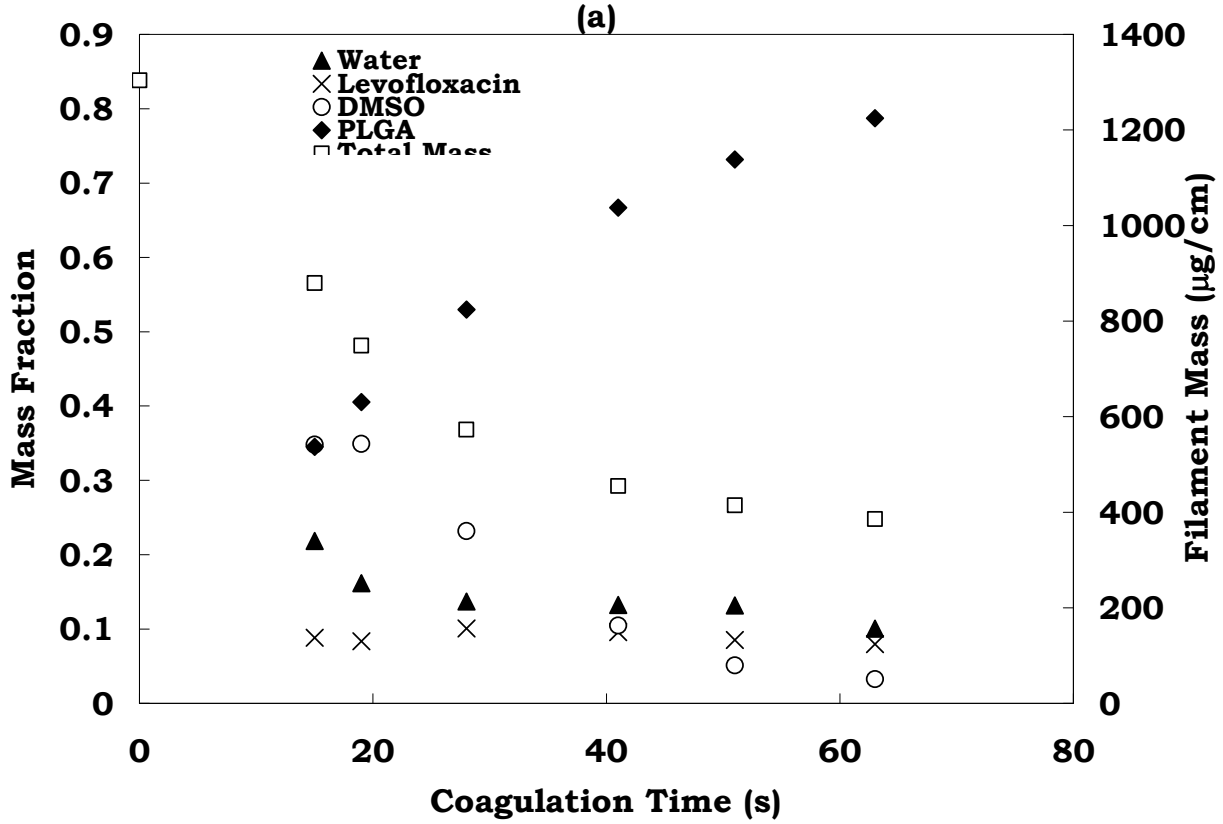


Figure 2.13: Water content of filaments vs. bath residence time.
Filaments formulations do not contain water. Error bars indicate standard deviation with n=3.

2.5.8 Total Filament Composition

Component mass fractions (w_i) are shown vs. bath residence time in Figure 2.14. Originally, DMSO makes up the largest fraction, but it is rapidly eliminated. The total mass of the protofilament decreases during coagulation. Since PLGA does not diffuse out, w_{plga} increases with extrusion time. For dexamethasone, w_{dex} also increases with coagulation time, as not much dexamethasone is eliminated past 20 s. Since levofloxacin is eliminated over time, but at a rate

slower than DMSO, w_{levo} increases to a maximum at 0.1 after 40 s of coagulation before falling slightly.



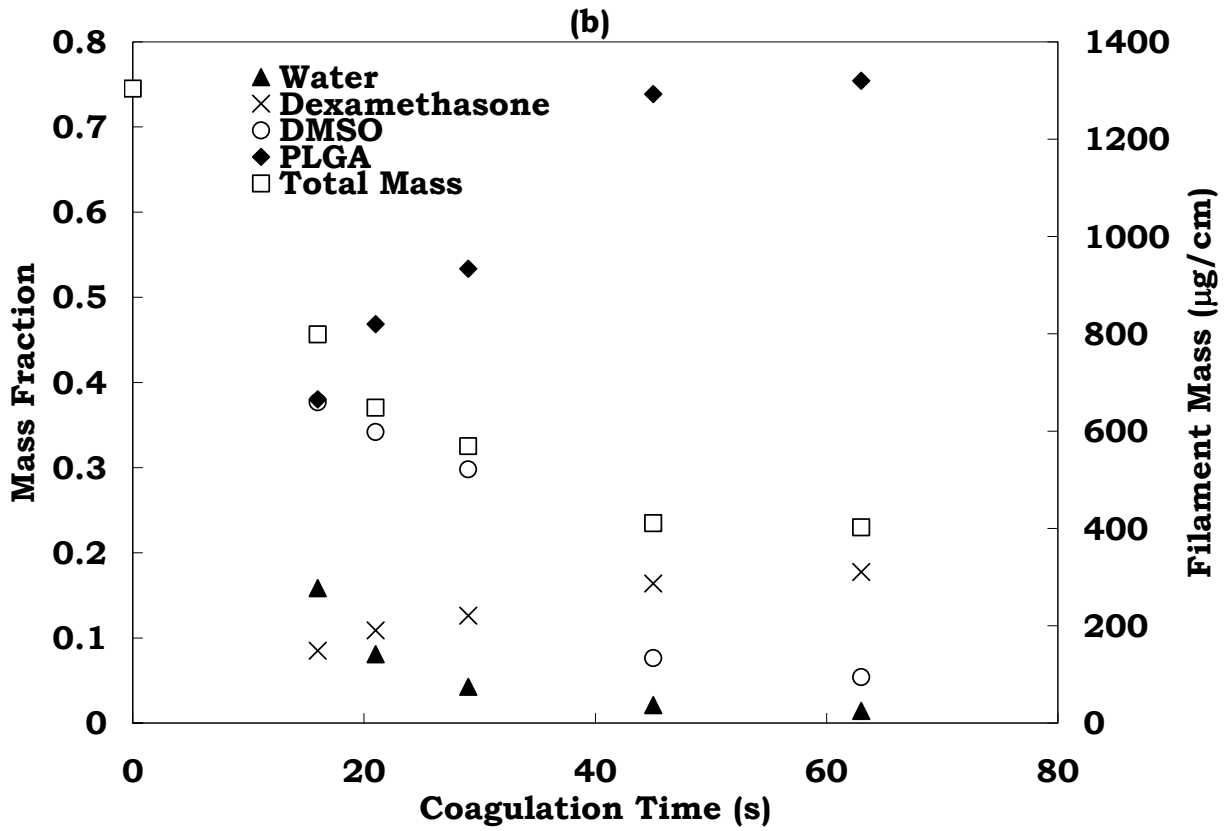


Figure 2.14. Total mass and mass fraction of each component for filaments (a) 506-7%Levo and (b) 506-7%Dex. Total mass is given in linear values and mass fractions reflect the instantaneous composition of a filament after a particular coagulation time.

2.6. *Discussion*

Wet-processing of drug loaded filaments made from suspensions[4], emulsions[1], and homogenous solutions[5] have been described in the literature, but there has been no experimental study of the mass transfer processes during filament fabrication. Mass flux during filament formation is unique to solution based processes; as devices formed by solvent-less processes (melt, compression, etc.) have no mass flux during formation. Drug delivery devices can be formed from polymer solutions by evaporation of solvent or by antisolvent precipitation. Mass transport is especially critical to filaments made by antisolvent precipitation, as solvent, antisolvent, and entrapped therapeutic can all diffuse. Devices formed by evaporation retain all non-solvent components, so analysis of the formation process follows only solvent loss over time. Monofilament wet-spinning is, in many ways, uniquely well suited to compositional investigation during processing, as bath residence times can be precisely controlled. This is in contrast to techniques such as antisolvent-aided electrospinning, where the final product is usually a nonwoven mat. Even if an antisolvent bath is involved during the electrospinning process (required when using a non-volatile solvent), different regions of the mat may have different residence times as the final product is usually left in the bath and not withdrawn in a steady-state fashion. Here, we have studied the formation of drug loaded filaments by wet-spinning. The full retention of polymer was verified and the flux of drug, DMSO, and water in the protofilament was investigated.

In order to successfully process a drug loaded filament by wet-spinning, polymer coagulation must be faster than drug elimination from the protofilament. Otherwise, drug retention would be poor and the method would be of limited use for controlled drug release applications. In the examples shown here, drug retention is moderate to high (30-90%), demonstrating the utility of wet-spinning as a drug encapsulation technique. Several formulations, containing various amounts of drug and polymer, were coagulated to examine the formation process. In formulating 506-7%Levo, levofloxacin is dissolved in DMSO at levels close to solution saturation. In contrast, 506-0.7%Levo has only a small amount of levofloxacin dissolved, and 0.7% solutions of levofloxacin should be stable in any water/DMSO mixture (as demonstrated in Figure 2.5). The solution 506-7%Dex has the same amount of drug as 506-7%Levo, but dexamethasone is much more stable in DMSO and low water content DMSO/water mixtures (see Figure 2.3). Two other formulations contain no drug. Formulation 506-25%PLGA has the same PLGA to DMSO ratio as formulations 506-7%Levo and 506-7%Dex, while 506-33%PLGA has the same total solid to DMSO ratio as the two 7% drug solutions.

The transport of solvent and nonsolvent during the coagulation process is critical, since this diffusion will determine how drug and polymer precipitate[9, 10]. Formulations 506-7%Levo, 506-7%Dex, 506-0.7%Levo, and 506-25%PLGA have essentially indistinguishable DMSO elimination kinetics. Drug loading and drug type seem to have no effect on DMSO diffusion from the protofilament. The only outlier among the filaments was 506-33%PLGA, which is indistinguishable from the other filaments at 18 and 23 s, but has much higher DMSO retention at 45 s. The reason for this high DMSO retention is not completely understood but could be due to lower DMSO diffusivity through a thicker polymer network.

At first, water is rapidly taken up into the protofilament, but then water content declines. Because water does not replace DMSO, the overall mass of the protofilament decreases with coagulation time. The early water influx seems to be influenced by drug content, as water content is greater at early time points for formulations with no drug and 506-0.7%Levo. Linear water mass is higher for 506-7%Dex than 506-7%Levo, suggesting that drug type may also influence water uptake. However, these conclusions must be made tentatively, since water flux is very fast during early sampling and the values for most of these filaments converge to a similar value later in the coagulation process. Early time points, where water is actively entering the protofilament, are inaccessible to our sampling technique. Samples at times less than 15 s are not fully coagulated and handling these samples distorts the resulting data (by squeezing or stretching them). At later coagulation times, the only outlier is again 506-33%PLGA, which shows higher levels of water at 45 s than any of the other formulations.

Both drugs and PLGA can be dissolved in DMSO to make homogenous solutions. Once water enters the solution, precipitation of drug and coagulation of the polymer will occur. The cloud point curve of DMSO solutions of PLGA (Resomer RG 506), at concentrations relevant to filament spinning, shows that phase separation occurs at 6-9% water. It is generally assumed that polymer is fully retained during wet-spinning, since the polymer is not soluble in the antisolvent and the formation of a skin prevents polymer diffusion from the protofilament. To check this assumption, and verify the mass balance over the extrusion, PLGA content was measured by a titration technique. PLGA is a polyester and ester bonds react with NaOH in a 1:1 ratio. Ignoring end effects (possible since there are an estimated 1000-3000 repeat units in

RG 506), measurement of the number of ester bonds was possible by reacting a section of filament with NaOH and back titrating with HCl to pH 7. The results confirm that there is full retention of PLGA. Measurements of the total mass of the filaments were in good agreement with full retention as well.

Solid-state solubility has been shown to be a good predictor of drug encapsulation and controlled release potential in other polymer-based systems formed by methods other than wet-spinning. Here, a semi-quantitative method of determining solid-state solubility was used to get an idea of the solubility of levofloxacin and dexamethasone in PLGA. Often, differential scanning calorimeter (DSC) measurements are used to quantify the solid-state solubility of drugs in polymers[16]. These tests measure the heat of melting for a particular drug without the polymer present and then again for the drug/matrix controlled release device. If drug is dissolved in the polymer, the heat of melting should decrease and it is possible to determine what fraction of the drug is in a crystalline state. DSC measurements were not particularly applicable to the system described here, as both dexamethasone and levofloxacin experience degradation with their melt transitions (at around 220ºC) and PLGA degrades in the same temperature range. This makes the melt transitions and degradations difficult to separate on a DSC trace. Another drawback to DSC for this system is that PLGA has a glass transition temperature in the range of 50-60ºC, meaning that measurements of solubility may reflect the amount of drug that can be dissolved in PLGA at the melt temperature of the drugs rather than at room temperature. Using a semi-quantitative method of determining drug solubility in PLGA, levofloxacin has an approximate solid-state solubility between 4.5% and 5.5% while dexamethasone has a solid-state solubility between 12.3% and 14.5%. These numbers can be compared to the percentage of drug in the

solid material (drug and polymer) during filament formation to determine what role solid-state solubility is playing in drug loading during formation. For 506-7%Levo, 9.2% of solid material is levofloxacin after 63 s, while for 506-7%Dex, 19.0% of the solid material is drug. These numbers significantly exceed the encapsulation predicted from solid-state solubility. Moreover, with filament 506-7%Levo, drug that may subsequently leave the filament can still be trapped (as for the 40 s time point) in a solidified product.

Solution state solubility may provide a better understanding of drug entrapment than solid-state solubility, since filament formation occurs in the presence of DMSO/water mixtures that are causing the co-precipitation of polymer and drug. Levofloxacin is 10% soluble in DMSO but only ~4% soluble with 6% water in the mixture. The result is that levofloxacin precipitates at lower water content than PLGA. This phenomenon is directly observed by light microscopy, where levofloxacin crystals are clearly visible beyond the coagulation front for 506-7%levofloxacin. At regions close to the coagulation interface, where phase inversion of the polymer happens rapidly, levofloxacin crystals are small or not visible. Dexamethasone is more soluble than levofloxacin in DMSO and DMSO/water mixtures. Dexamethasone stays dissolved before PGLA phase inversion, and no precipitates are visible beyond the coagulation front. Crystals are instead visible in the water-rich solution. For the levofloxacin formulation with low drug content, 506-0.7%Levo, there are no crystals observed by light microscopy. These differences in solubility and where/if drug precipitation occur could explain the difference in drug encapsulation during coagulation. Levofloxacin precipitates before phase inversion in 506-7%Levo, leading to a high initial retention since precipitates will not pass through the skin. The precipitated drug is not well incorporated into the polymer matrix and is thus available for

diffusion from the protofilament at longer coagulation times. Initially, dexamethasone is released rapidly (~20% before the first time point at 18 s), since it stays dissolved in DMSO/water mixtures and is free to diffuse through the skin. Once demixing of the polymer occurs, the polymer-rich phase thickens around the disperse drug and prevents further elimination over the course of coagulation. Supporting this hypothesis, formulation 506-0.7%Levo has drug encapsulation kinetics similar to 506-7%Dex. This indicates that it is not an intrinsic difference between drugs that dictate therapeutic encapsulation, but the kinetics of precipitation in the filament. The solution thermodynamics of the polymer and drug and kinetics of solvent/antisolvent movement, rather than the solid-state solubility of drug in polymer, are what determine drug encapsulation in a filament.

While wet-spinning is well described in the literature, there is little data on the coagulation process of wet-spun, drug containing filaments. There are some data available for other devices formed by phase inversion that might be relevant to the discussion of coagulation processes for these filaments. In-situ forming depots are the best characterized class of devices formed by phase inversion and provided some insight into the mechanism of coagulation in drug loaded filaments[17-19]. Notably, a body of work by A.J. McHugh and colleagues has investigated many aspects of phase inversion and release for injectable depots[20-22]. Much of this work stems from the formation and characterization of asymmetric membranes formed by coagulation[23-26].

Injectable depots can be categorized as either fast forming or slow forming, and the dynamics of polymer coagulation have been shown to influence drug release[20, 21, 27]. Fast forming depots

are formulated with water miscible solvents such as DMSO, NMP, or DMF. These depots rapidly take up water, expel solvent, and coagulate around the contained therapeutic. Slow forming depots use water immiscible solvents, such as benzyl benzoate or acetyltriethoxycitrate to control the uptake of water and slow coagulation. This creates a gel-like depot, where the water soluble therapeutic can not release until the water front has penetrated to the dispersed drug particles. Slow forming depots have a honeycomb like morphology after solidification, while fast forming depots have finger-like pore morphology. Based on our observations of morphology, the coagulating filaments described here behave more like fast forming depots.

Various kinetic, thermodynamic, and release studies have been performed on fast forming injectable depots. The morphology of phase inversion has been found to be the same as wet-spinning, with a thin skin forming on contact and acting as a membrane across which further mass transport occurs[20]. The drug in these systems has been found to partition partly into the polymer rich phase and partly into the water rich macrovoids. Once phase inversion has occurred, drug in the voids has an effective diffusivity that is orders of magnitude higher than therapeutic trapped in the polymer rich phase[20]. For wet-spinning, this mechanism explains why only some drug is available to diffuse out of the protofilament at short time scales and why the physical state of the drug at the moment of phase inversion is critical.

While some aspects of fast forming depots solutions are relevant to wet-spinning of drug loaded filaments, the analogy is not perfect. Firstly, the depot solutions need to control release from the point of contact with a fluid. A high, long term drug entrapment is not usually desired, since this will limit drug that is immediately able to release. The bath side conditions are determined by

physiology and can not be manipulated. The formation of an injectable depot will often be controlled by bath side considerations, as the space outside of the depot is not well mixed and will eventually contain much of the solvent. Overall, the internal formation of depots is similar to wet-processed filaments, but the objectives of the devices are different, so a direct comparison is not possible.

Based on this study, and drawing on relevant literature concerning wet-spinning[9-11] and drug encapsulation by phase inversion, we propose the coagulation/encapsulation mechanism shown schematically in Figure 2.15. Immediately after extrusion, a thin skin of solidified polymer forms on the surface of the protofilament. This skin is observed in our microscopy experiments. The skin acts as a semi-permeable membrane, allowing DMSO, water, and drug to pass through but retaining all of the PLGA in the dope. As antisolvent enters the protofilament, the precipitation of drug can occur, as in the case of levofloxacin, if the precipitation concentration for the drug is less than the cloud point of the PLGA. These drug crystals are physically trapped in the protofilament and exchange drug molecules with the surrounding solution. The polymer solution undergoes demixing when enough DMSO-water exchange has occurred. Polymer-lean nucleation sites appear and expand, creating finger-like voids in the polymer structure. The polymer-rich phase thickens around any drug remaining in solution, effectively trapping it. The phases further solidify as DMSO leaves and water is drawn out of the filament as the presence of water is no longer thermodynamically favorable without the presence of DMSO. The egress of DMSO, drug, and water from the protofilament leads to an overall decrease in mass. Drug that is present in the voids, or drug crystals that bridge to voids, can diffuse out of the protofilament.

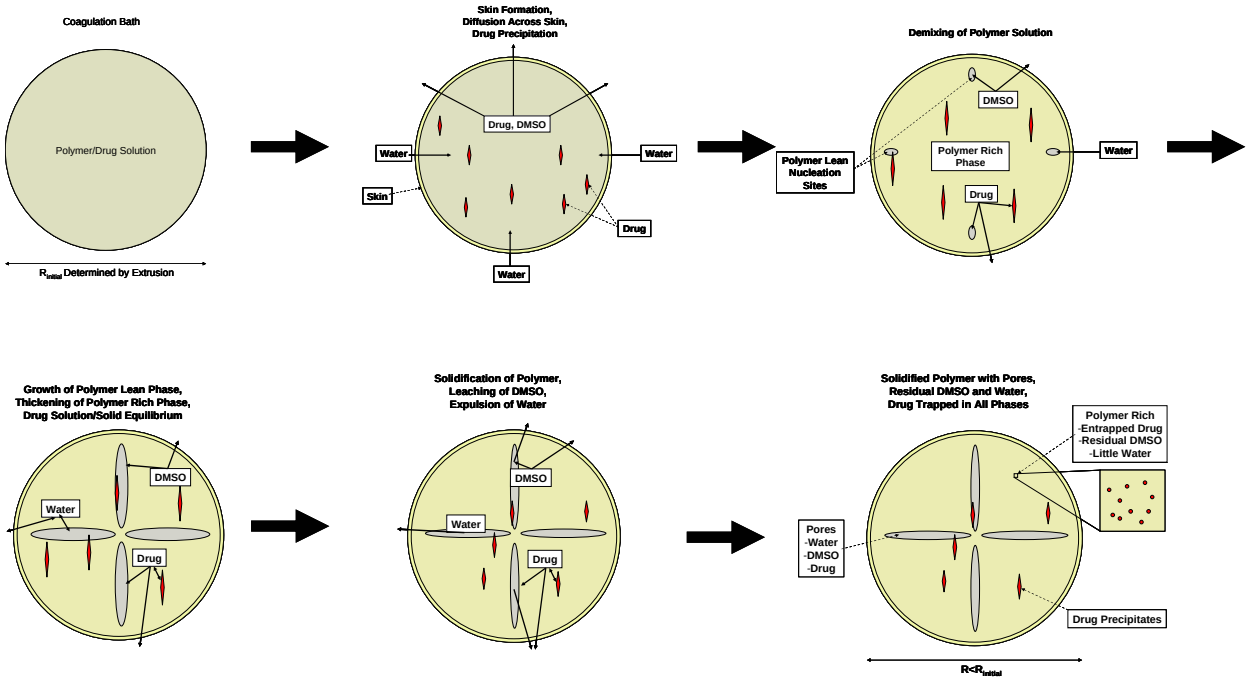


Figure 2.15. Schematic of hypothesized filament formation and drug encapsulation mechanism.

The hypothesized mechanism of filament formation draws on previous literature and explains the drug encapsulation kinetics observed in our work. Future work can be directed at clarifying and validating this mechanism. Further exploration using a variety of drugs, solvents, and antisolvents may provide more information and clarify this mechanism. This work provides guidance for choosing a proper polymer/solvent/antisolvent to encapsulate a specific drug by wet spinning and may be used in the future for determining formulation and processing conditions for drug loaded filaments produced by wet-spinning.

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Chapter 3: In-Vitro Characterization

3.1. *Abstract*

Biodegradable filaments, with diameters of 250-300 μm, for the controlled delivery of dexamethasone or levofloxacin are described in this chapter. Filaments are prepared by wet-spinning solutions of poly(lactide-co-glycolide) (PLGA) and drug dissolved in dimethyl sulfoxide (DMSO) that are coagulated in water. PLGAs of three different lactide to glycolide ratios (50:50, 75:25, and 100:0 lactide:glycolide) are extruded at numerous conditions, and the filaments obtained analyzed by a variety of methods. Compositional analyses of the filaments by independent measurements of drug, DMSO, water, and polymer give drug loadings up to 40% of filament mass and drug retention (drug in filament per drug in solution) greater than 40%. Drug release kinetics, and thermal and mechanical properties, of the filaments are reported. It is found that residual DMSO is present in the filaments, diminished with increased coagulation time, and is mostly undetectable after one day of incubation in phosphate buffered saline. Filaments prepared from homogenous solutions have low initial drug release followed by polymer-dependent, degradation controlled release, while filaments made from solutions containing suspended drug particles display rapid release regardless of polymer type.

3.2. *Introduction*

Biodegradable polymer-drug composites can be made into different morphologies[1] including microspheres[2-4], films[5], and injectable depots[6]. Fibrous morphologies are intriguing because they allow for easy implantation/removal, anchorability in tissue, and mechanical stability. Fibrous devices can also provide mechanical stability or be woven with other fibrous components that are more mechanically stable. Common methods for making fibrous devices for drug delivery include electrospinning[7-9]and wet spinning[10-13]. The synthetic method is usually chosen based on design criteria for the particular application and nature of the polymer. Electrospinning can be used to make fibers of micron to submicron diameters[14, 15], but is unable to produce large diameter monofilaments. Recently, wet spinning has emerged as a method for preparing drug-loaded filaments for a wide range of applications[10-12, 16]. Benefits of a wet-processed filament include a porous microstructure, room temperature processing conditions, and diameters of suture-like scale. Filaments made from two phase solutions, such as emulsion or solid suspensions, have been shown to be effective in controlling therapeutic release and have been used primarily for tissue engineering and cancer therapy.

The thermal properties of a controlled release device, like melting and glass transition temperatures (T_g), are linked to the release properties[17]. It is hypothesized that drug can diffuse more easily through amorphous polymers in the rubber state, due to the increased motion of polymer chains above the T_g[18]. In many reports, glass transition temperatures of the pure polymers and drug release matrices are reported as a matter of routine characterization, but some studies focus on how various aspects of polymer processing and drug encapsulation affect T_g. Plasticizers are polymer additives, usually high boiling point solvents or low melting point

solids, which make amorphous polymers, or amorphous sections of semi-crystalline polymers, more flexible[19]. It has been shown that plasticizers can affect the rate of release of a drug from a polymer matrix[17, 18, 20-22].

Here, we focus on monofilaments made from three types of amorphous PLGA, one with a 50:50 lactide to glycolide ratio, one with a 75:25 ratio, and one made entirely of d,l-lactide. Using dimethyl sulfoxide (DMSO) as a solvent and water as an antisolvent, levofloxacin or dexamethasone containing filaments are prepared. Most of the filaments are made from a solution where the drug and polymer are both completely dissolved, but some of the filaments are formulated from solutions with suspended drug particles. Here, we demonstrate the flexibility of wet-processing for the production of drug loaded filaments and explore the interplay of composition with drug release, thermal properties, and mechanical properties. In Chapter 4, we demonstrate how these filaments can be implanted with a standard surgical needle to control the delivery of a therapeutic to the eye.

3.3. *Materials and Methods:*

3.3.1 Materials

Levofloxacin, dexamethasone, and poly(d,l-lactide) (PDLLA, MW 75,000-120,000) were purchased from Sigma-Aldrich. Poly(d,l-lactide-co-glycolide)'s were obtained from Boehringer Ingleheim. The PLGA's used for this study were Resomer RG 506 (50:50 lactide:glycolide, intrinsic viscosity of 0.82) and Resomer RG 756 (75:25 lactide:glycolide, intrinsic viscosity of 0.80). DMSO, 0.10 N HCl, and 0.10N NaOH were obtained from VWR.

3.3.2 Filament Formation

Filaments containing levofloxacin or dexamethasone were processed by a wet-spinning procedure. An accurately measured amount of drug was placed in a glass vial to which dimethyl sulfoxide (DMSO) was added. This solution was sonicated for 1 minute to facilitate dissolution of the drug. After sonication, PLGA was added to each vial and the vial vortexed to mix all components. The solution was then allowed to equilibrate for 6 hours, vortexing occasionally to mix, at a given temperature (Table 3.1), to eliminate all trapped bubbles. Table 3.1 shows the processing conditions for all of the filaments used for these experiments (naming of the filaments follows the pattern of Polymer-Drug#). Most of the solutions were homogenous (with drug and polymer both dissolved to make a translucent solution) with the exceptions for 506-L2 and 756-L2, which were suspensions of solid drug particles formulated above the solubility of levofloxacin in DMSO. The samples were chosen to allow comparisons between polymer type (506-L1, 756-L1, and PDLLA-L1), drug type (506-L1 and 506-D1), coagulation time (506-L1, -L5, and -L6), amount of drug (506-L1, -L2, -L4, -D1 and -D2 and 756-L1 and -L2), and formulation temperature (506-L1 and L3).

Table 3.1. Processing conditions for filaments

Sample	Polymer	Drug Type	Solution Composition (%wt)			Solution Temperature (°C)	Coagulation Time (s)
			Polymer	DMSO	Drug		
506-L1	RG 506	Levofloxacin	23.3	69.8	7.0	25	45
506-L2	RG 506	Levofloxacin	22.2	66.7	11.1	25	45
506-L3	RG 506	Levofloxacin	23.3	69.8	7.0	60	45
506-L4	RG 506	Levofloxacin	23.8	71.4	4.8	25	45
506-L5	RG 506	Levofloxacin	23.3	69.8	7.0	25	55
506-L6	RG 506	Levofloxacin	23.3	69.8	7.0	25	35
506-D1	RG 506	Dexamethasone	23.3	69.8	7.0	25	45
506-D2	RG 506	Dexamethasone	20.4	61.2	18.4	25	45
756-L1	RG 756	Levofloxacin	23.3	69.8	7.0	25	45
756-L2	RG 756	Levofloxacin	22.2	66.7	11.1	25	45
PDLLA-L1	PDLLA	Levofloxacin	23.3	69.8	7.0	25	45

Solutions were loaded into a 5 mL syringe equipped with a 22 gauge flat-tipped needle (Small Parts Inc.) and mounted on a syringe pump. The solutions were extruded into a 16 L water bath (22 ± 2°C) and taken up onto a 1 inch bobbin rotated by a DC gear motor. The pump speed and uptake rate were chosen to fix a particular coagulation time (Table 1) with a draw ratio of 1.2. Filaments were secured tautly without stretching and allowed to dry under ambient conditions for two days.

3.3.3 Determination of Levofloxacin Content

Total drug loadings of the filaments were determined by dissolving accurately measured sections of each filament with 1 M NaOH. The drug content of the resulting solution was measure by using high performance liquid chromatography (HPLC) with a C18 column (Agilent 1200 series HPLC). The mobile phase was 49.95 % water, 49.95 % acetonitrile with 0.1 % trifluoroacetic acid. Levofloxacin was determined by fluorescence detection with an excitation at 292 nm and emission at 494 nm while dexamethasone was measured by absorbance at 254 nm. Some samples were measured, placed in 1.5 mL Eppendorf tubes, and incubated in phosphate buffered

saline (PBS, pH 7.4) at 37°C on an incubating rocker for one day. Other samples were tested for drug content without incubation.

3.3.4 Determination of Water Content

Water contents of the filaments were determined by thermogravimetric analysis (TGA). Samples were cut into small pieces, placed in an Al_2O_3 crucible and heated in air to 125°C over 45 min. Mass loss was monitored and the difference in mass before and after heating recorded. Water content of incubated filaments was done after blotting the filaments with a Kimwipe to remove water on the external surface of the filament.

3.3.5 Determination of DMSO Content

To determine DMSO contents of the filaments, approximately 30 cm (accurately measured) was dissolved in deuterated chloroform with an internal standard of 1% TMS. The sample was then analyzed by proton nuclear magnetic resonance (^{1}H NMR). By comparing the integration of the TMS peak to the DMSO peak, the amount of DMSO in the sample can be determined. Some samples containing dexamethasone and incubated samples needed to be filtered through a 0.2 μm syringe filter to remove particles before NMR analysis.

3.3.6 Polymer Content

Polymer content was directly determined by digesting an accurately measured length of filament (approximately 30 cm) with 10 mL 0.1 M NaOH. The solution was back titrated to neutral with 0.1 M HCl, with the amount of HCl accurately recorded. For every ester bond that is

hydrolyzed, one hydroxide ion will be consumed, so the amount of ester bonds can be determined and converted into the mass of polymer in solution. Incubated filaments were first rinsed with water three times before digestion.

3.3.7 Filament Imaging

Bulk fiber morphology was observed by light microscopy and scanning electron microscopy (SEM). Light microscopy was performed using a Micromaster I light microscope (Fisher Scientific, Pittsburgh, PA) at 10x magnification. The size measurements were calibrated using known diameter wires (Small Parts Inc.). For SEM, samples were coated with Pt/Pd and magnifications up to 400x were possible without destroying the samples.

3.3.8 Drug Release

One inch sections of filament were placed in 1.5 mL tubes. One mL of phosphate buffered saline (pH 7.4) was added to each vial. The vials were incubated at 37°C under gentle rocking, and at various times, the buffer was replaced with fresh PBS. The removed PBS was analyzed for drug content by HPLC as described above.

3.3.9 Mechanical Testing

Load vs. strain curves for the fibers were collected on an Instron 1150 mechanical properties testing apparatus. Experiments were run under ambient conditions, where the temperature was 22 ± 2°C with relative humidity of 50 ± 10%, and under incubated conditions, with the filament submerged in PBS at 37°C. The samples were drawn at a strain rate of 1 %/s and the resulting

tensions were recorded. The loads that the filaments were bearing at the time of breakage were recorded, as were the maximum elongations of the filaments. Other samples were tested by producing a step change in the strain of the filament and measuring relaxation properties. These filaments were subjected to a 10% strain at 50%/s strain rate and the relaxation was recorded.

3.3.10 X-Ray Diffraction

Samples of filaments 506-L1 and 506-D1 were incubated in PBS at 37C. At various time points, samples were removed, dried by lyophilization, and cut into appropriate sections for analysis by x-ray diffraction. Samples were scanned from 2θ angles of 2º to 40º. Samples of levofloxacin and dexamethasone, precipitated from concentrated solutions in DMSO by water addition, were tested along with samples of PLGA Resomer RG 506.

3.4. *Results*

3.4.1 Extrusion and Composition

Filament compositions are shown in Figure 3.1, and are displayed as mass fractions. The polymer used for fabrication is the most abundant component of every filament. A small amount of DMSO is present after extrusion (this amount is only a very small fraction of the DMSO used for formulation) and varies with formulation conditions and compositions. After one day of incubation in PBS at 37°C, the amount of DMSO in several of the filaments fell below the sensitivity of detection for the assay, with only small amounts present (less than 0.25%) even when detected. While residual water is present after extrusion, the filaments all swell significantly after a day of incubation in PBS at 37°C. The degree of swelling ranges from 25% to 49%. Influx of water is often accompanied by an increase in filament diameter (Table 3.2), particularly for filaments made with Resomer RG 506. Drug is retained in each filament, with loading varying with original drug composition. Figure 3.2 shows the amount of drug retained in the filament relative to the initial formulation. Drug retention after extrusion ranged from 48% to 89% of initial drug depending on formulation conditions. After one day of incubation in PBS at 37°C, all of the fibers display some degree of fast drug release with drug retention after one day ranging from 6% to 78% of drug used in its formulation.

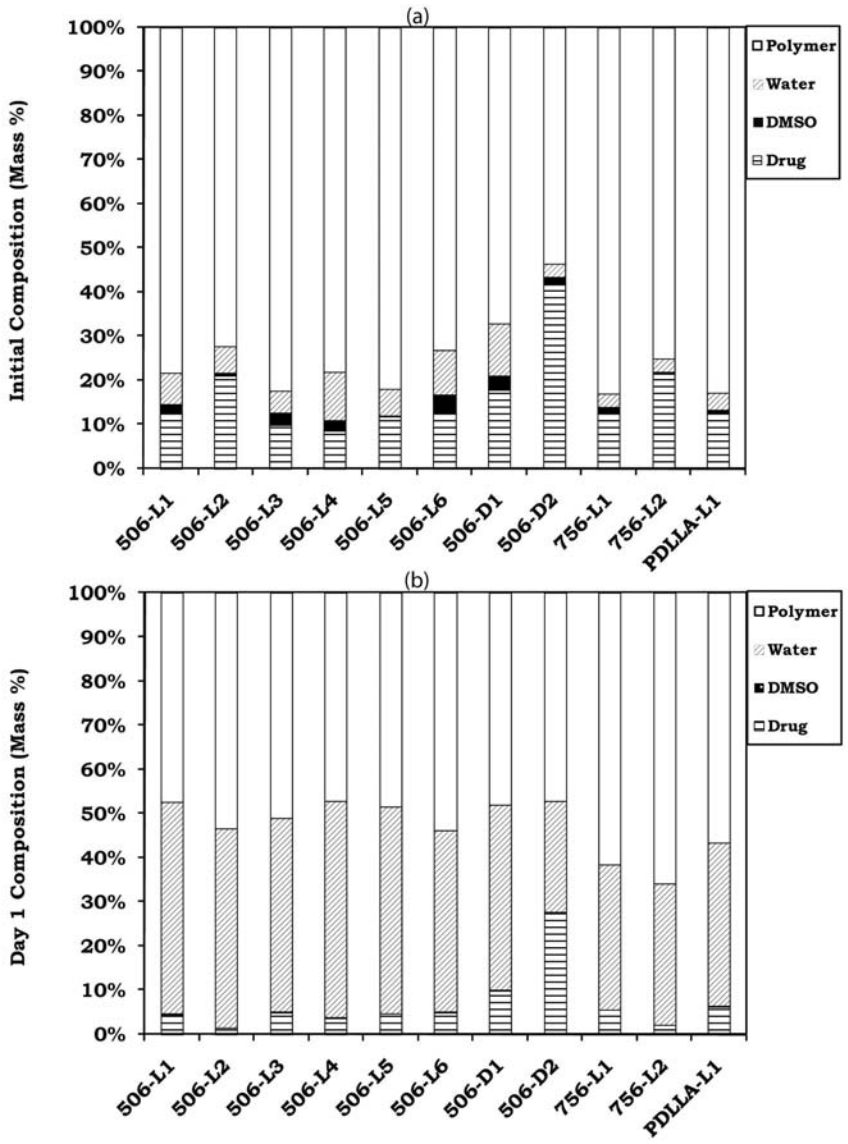

Figure 3.1. Composition of filaments after a) extrusion and b) 1 day of incubation in PBS at 37°C.

DMSO, drug, polymer, and water contents are shown. Values are the averages of three measurements for each component.

Table 3.2. Filament diameters after extrusion and one day of incubation in PBS at 37°C.

	Diameter (μm)	
Sample	Initial	Day 1
506-L1	255±2	287±5
506-L2	259±5	314±10
506-L3	266±4	283±11
506-L4	250±4	284±12
506-L5	261±5	322±13
506-L6	252±7	271±8
506-D1	270±7	269±6
506-D2	285±5	304±7
756-L1	288±15	297±3
756-L2	294±6	300±7
PDLLA-L1	299±7	311±5

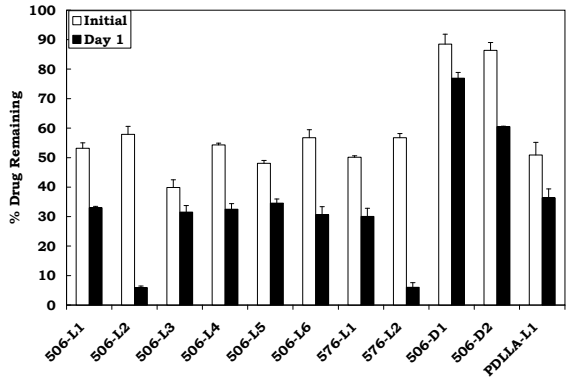

Figure 3.2. Drug remaining in the filaments after extrusion and 1 day of incubation in PBS at 37°C as a percentage of drug in formulation.
Error bars indicate one standard deviation (n=3).

The swelling kinetics of filament 506-L1 are shown in Figure 3.3. After 5 min, 25% of the filament is water and the water content of the filament increases to 47% after 45 min. The water content then levels off, and the water content at 90 min is essentially unchanged from 45 min. Figure 3.1 shows that the filament does not swell significantly more over the course of the next 22.5 hrs.

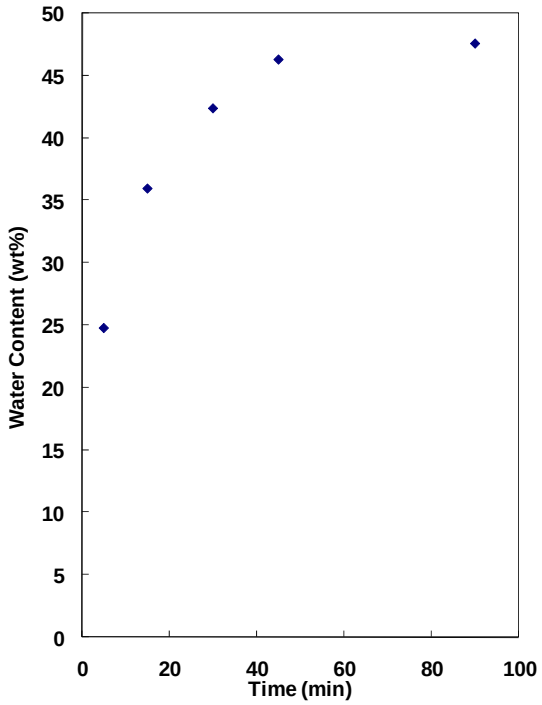

Figure 3.3. Swelling kinetics of filament 506-L1.

3.4.2 Morphology

Light microscopy allowed for the determination of filament diameter. Table 3.2 lists the diameters of each filament after extrusion and one day of incubation in PBS at 37°C. Representative SEM's of filaments 506-L1, -L2, and -L3 are shown in Figure 3.3. A cross-section of 506-L1 shows the internal porosity of the polymeric filaments. The other filaments reveal essentially the same cross sectional morphology. The exterior surfaces of the filaments are different. Filaments 506-L1 and -L3 have a rough surface on the sub-micron length scale while larger surface protuberances are apparent for 506-L2.

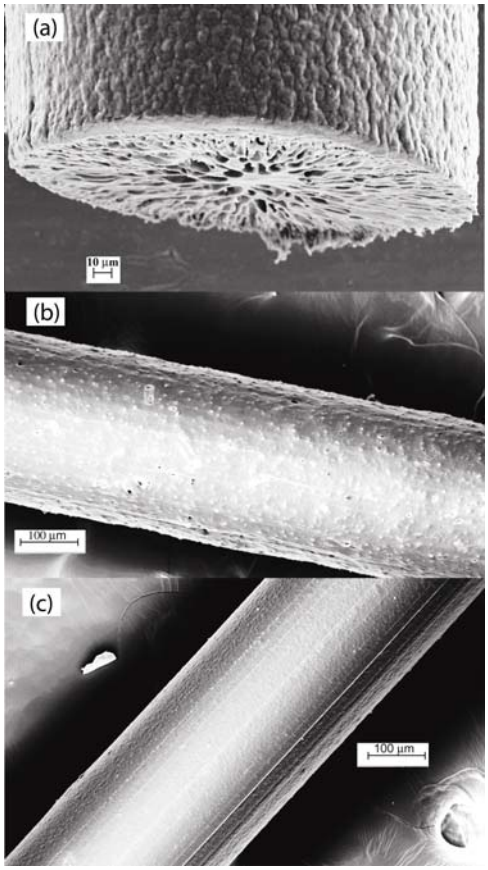

Figure 3.4. SEM images for filaments (a) 506-L1, (b) 506-L2, and (c) 506-L3.

3.4.3 Drug Release

Figure 3.5 shows release profiles for each of the filaments separated into groups, with 506-L1 present in each panel for comparison. Figure 3.5(a) shows the release of 506-L1, -L3, -L4, -L5, and -L6, all filaments made from Resomer RG506 with fully dissolved levofloxacin. All filaments gave similar release profiles. Figure 3.5(b) illustrates the release profiles of filaments made from fully dissolved solutions of levofloxacin (506-L1 and 756-L1) and from suspensions of levofloxacin (506-L2 and 756-L2). Figure 3.5(c) shows the release profiles of dexamethsasone loaded filaments 506-D1 and 506-D2 compared to levofloxacin loaded 506-L1. The differences in release due to polymer type are illustrated in Figure 3.5(d), with 506-L1, 756-

L1, and PDLLA-L1 all made with identical ratios of polymer:DMSO:levofloxacin and identical processing conditions.

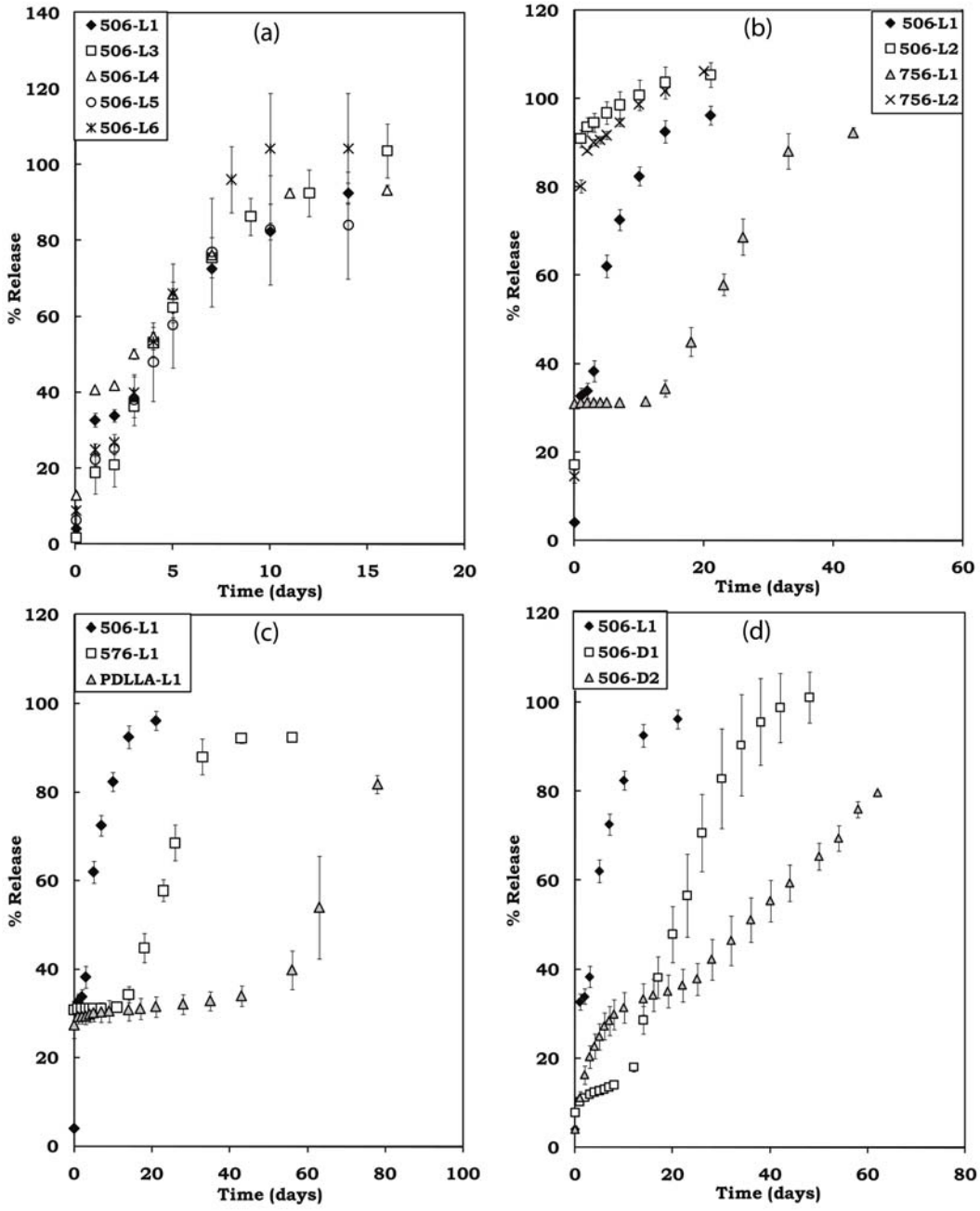

Figure 3.5. Drug release profiles for filaments.
The release profiles of (a) filaments containing levofloxacin formulated from single phase solutions, (b) filaments formulated with dissolved and suspended levofloxacin, (c) filaments made from different polymers, and (d) filaments formulated with dexamethasone (506-L1 given in each panel for comparison). Error bars represent one standard deviation (n=3).

3.4.4 Effect of Dissolved vs. Suspended Drug in Formulation

To further explore the influence of drug solubility on release profile, filaments were made from solutions at 25ºC and 60ºC with various levels of dissolved or suspended levofloxacin. The solubility of levofloxacin in DMSO increases with temperature as shown in Figure 3.6. Filaments were either formulated below the solubility point of levofloxacin (solutions) or above (suspensions). Figure 3.7 shows release from both suspensions and solutions of levofloxacin loaded in RG 506 filaments. For filaments formulated from solutions at 25ºC, levofloxacin is dissolved when it comprises 4.8% and 7.0% of the solution, but is in suspension at compositions of 9.1% and 11.1%. At 60ºC, all of these solutions are homogenous. Filaments formulated from solution all show approximately the same release, with a triphasic profile, but when there is suspended levofloxacin, the release happens much faster, with approximately 90% of the drug released in a single day. This same fast release is observed regardless of polymer, as shown in Fig. 3.4. Filaments 506-L1 and 756-L1 both have 7.0% drug in formulation, while 506-L2 and 756-L2 have 11.1% (at 25ºC). SEM images of 506-L2 show that the large particles visible in Figure 3.3 are no longer present after a day of incubation in PBS (Figure 3.8). This is in contrast to filament 506-L1, which retains a rough surface after incubation.

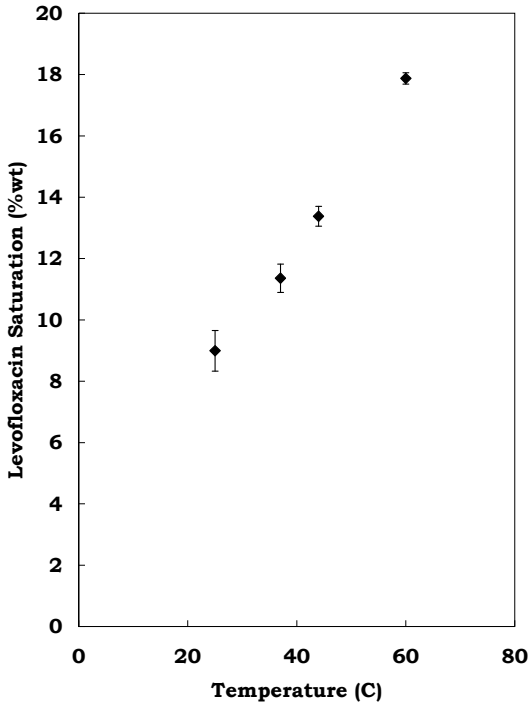

Figure 3.6. Levofloxacin saturation as a function of solution temperature.

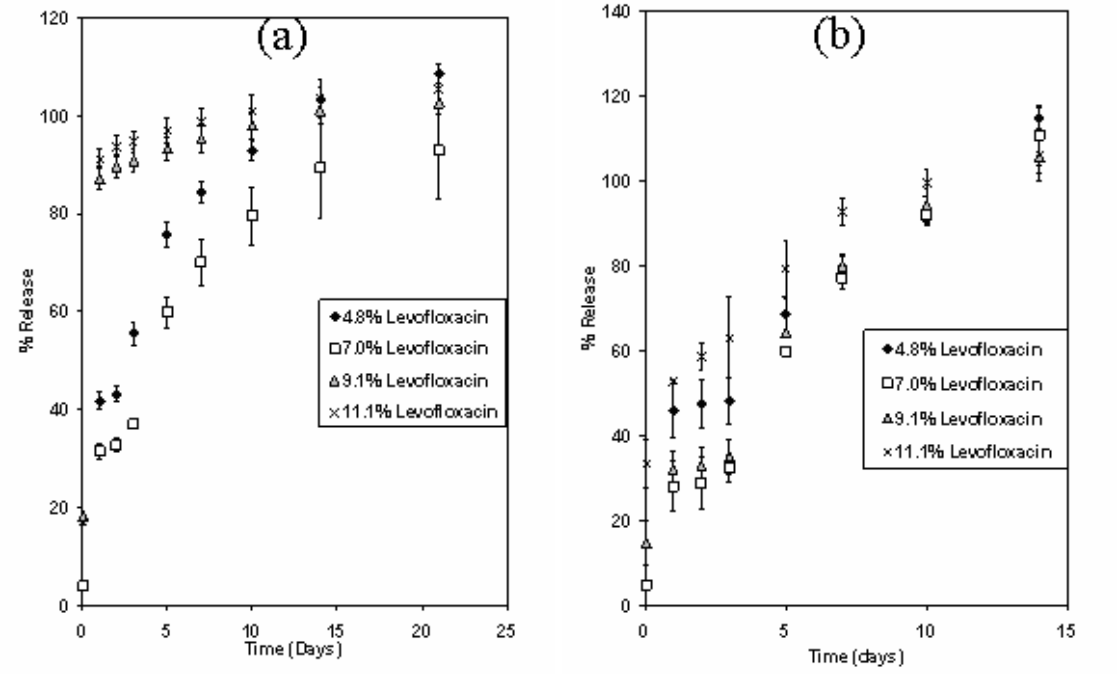

Figure 3.7. Drug release profiles for filaments formulated with various amounts of levofloxacin.

Drug release profile changes as a function of levofloxacin in formulation for (a) filaments formulated at 25ºC and (b) filaments formulated at 60ºC.

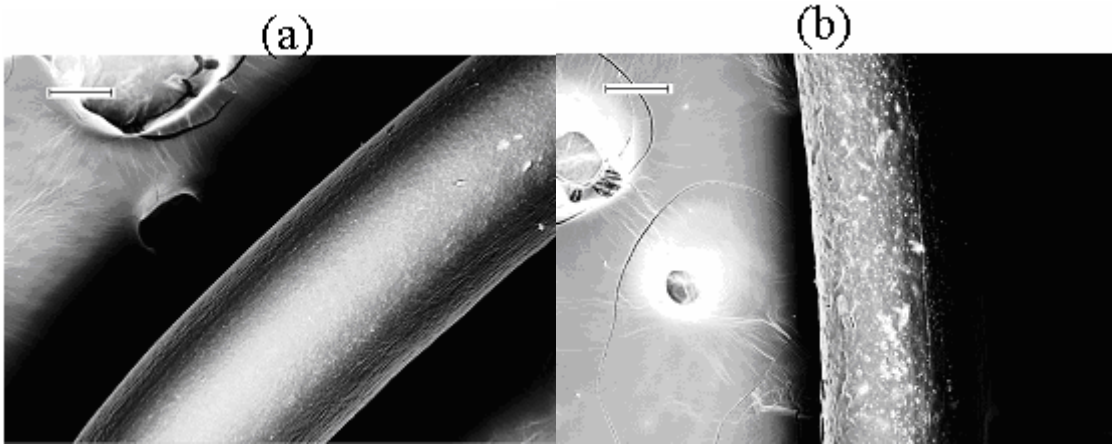

Figure 3.8. SEM images of filaments (a) 506-L1 and (b) 506-L2 after one day of incubation in PBS at 37ºC. Scale bar represents 100 μm.

3.4.5 Effect of Bulk Morphology

The effects of bulk morphology on drug release for filament 506-L1 was also studied. Samples of filament 506-L1 were subjected to one of several post-processing operations. Samples were cut into approximately equal sections averaging 0.8 mm in length, stretched to 300 % of their initial length, flattened into a strip with a hydraulic press, or annealed at 100ºC for 1 hr. Only annealing influences drug release (Figure 3.9).

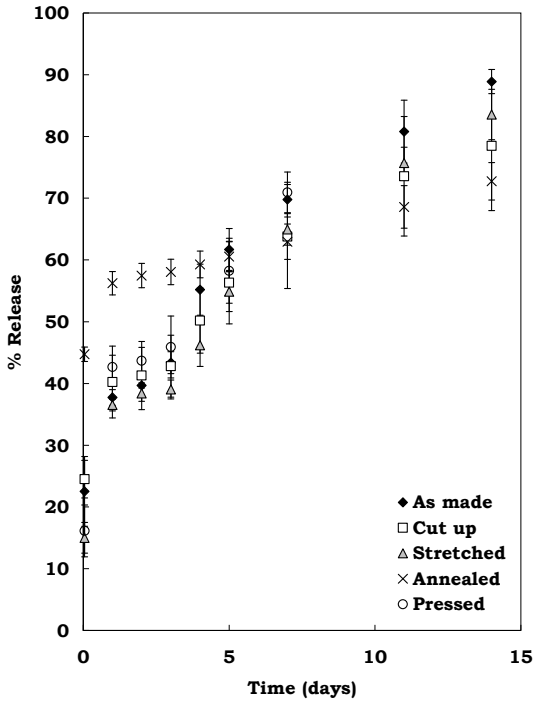

Figure 3.9. Effects of bulk morphology on drug release.

3.4.6 Early Stage Levofloxacin Release

Figure 3.10 shows early stage release from three samples of filament 506-L1. Data is plotted as amount released per unit length of filament. Samples start at 2 min and proceed at short time intervals over the next 25 hr. There is an initial burst release at 2 min, followed by a region of slow release for the following 1-2 hr. There is a transition to a faster release rate at approximately 2 hr and release slows to a plateau after the release of approximately 18 μg/cm of levofloxacin.

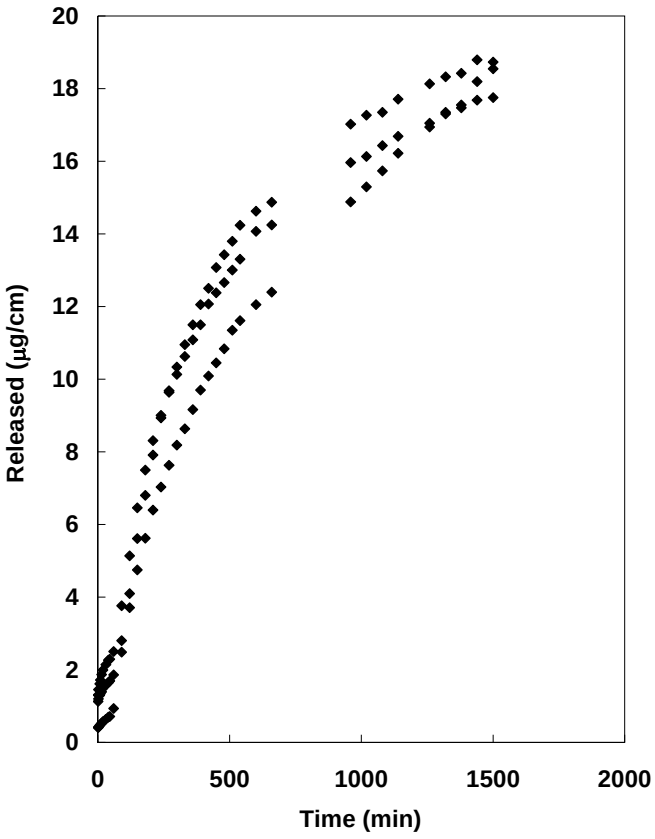

Figure 3.10. Early drug release from filament 506-L1.

From the shape of the curve, release after 90 min was hypothesized to be diffusion controlled. The Higuchi model of diffusion controlled release was applied to this area of drug release. The

Higuchi model is a one dimensional release model that assumes a higher drug loading (A) than drug solubility (C_s) in the release media and has the form:

$$Q = \sqrt{Dt(2A - C_S)C_S} \qquad 3.1$$

Where Q is the amount of drug released, D is the effective diffusion coefficient, and t is time. Figure 3.11 shows Q as a function of $t^{1/2}$ for the three samples of 506-L1. The data in Figure 3.11 is normalized to 90 min and only cover up to 14 μg/cm released, as the assumptions behind the Higuchi model start to break down as drug is depleted from the matrix. The average linear fitting parameter is 0.56 ± 0.08 μg/cm/min$^{1/2}$ with an average R^2 value of 0.998 ± 0.001.

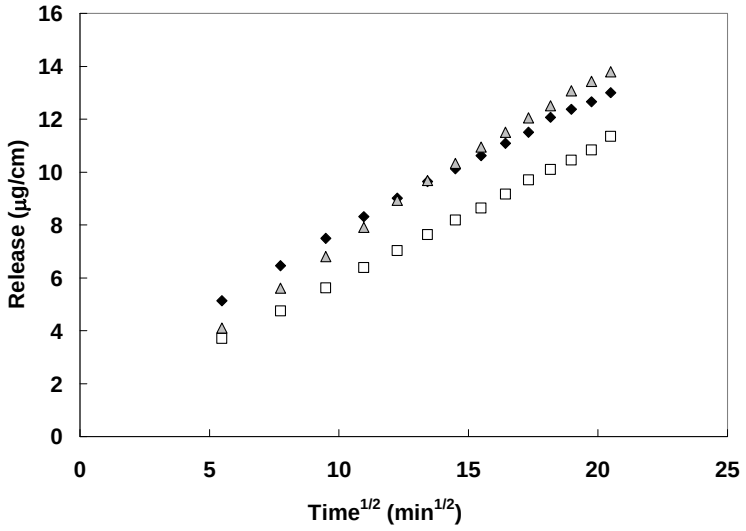

Figure 3.11. Release from three filaments of 506-L1 plotted against time½.

3.4.7 X-Ray Diffraction

To determine the physical state of each drug before and during release from the filaments, x-ray diffraction experiments were run. Figure 3.12 shows the x-ray diffraction patterns for PLGA (RG 506), levofloxacin and dexamethasone. PLGA is an amorphous polymer, so only a small, broad peak is visible.

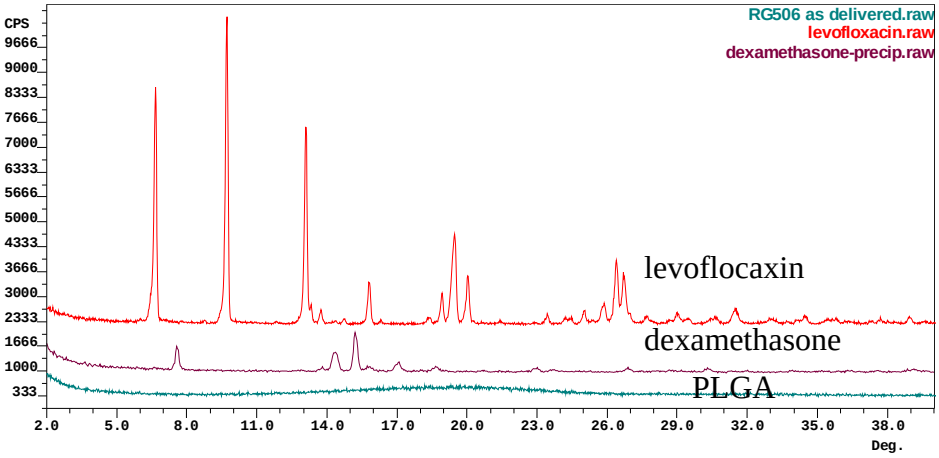

Figure 3.12. X-ray diffractograms of PLGA, levofloxacin, and dexamethasone.

When levofloxacin is encapsulated in a filament, characteristic peaks at 6.9º, 10.0º, and 26.8º can be seen after extrusion (Figure 3.13). This indicates that at least some of the levofloxacin is present in the crystalline form. After a single day of incubation in PBS at 37ºC, all of the characteristic peaks have vanished, with only a peak at 32º present due to residual salt from the PBS.

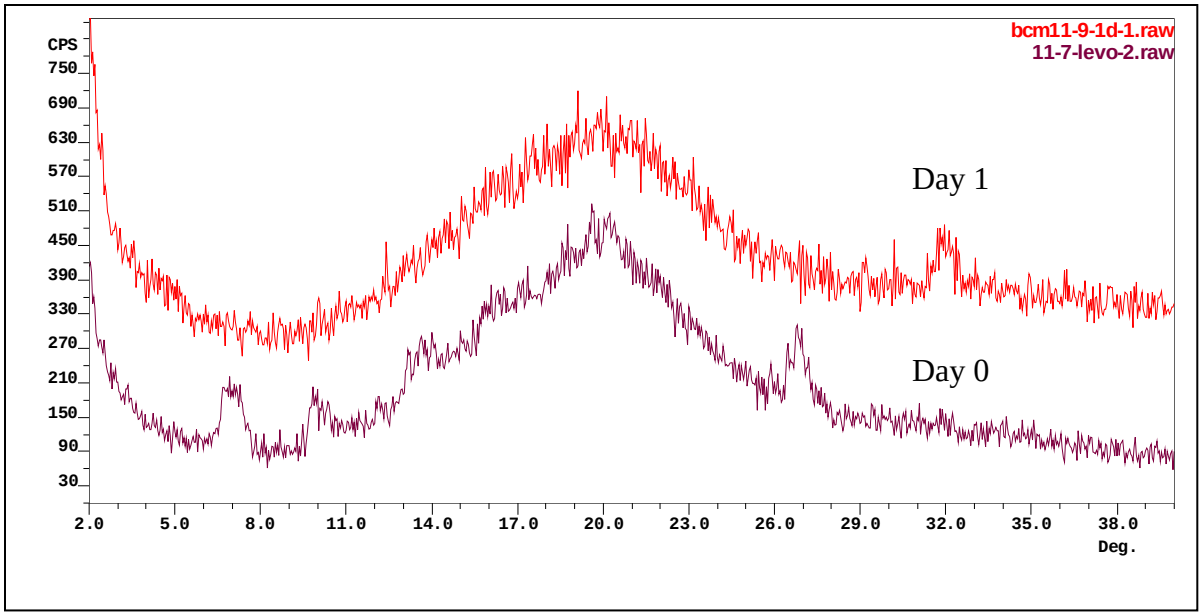

Figure 3.13. X-ray diffractograms of levofloxacin loaded filaments after extrusion and one day of incubation in PBS at 37°C.

Dexamethasone loaded filaments display the characteristic peaks at 14.5º, 17º, and 18.7º (Figure 3.14). These peaks do not fade with incubation time and are present through 25 days of testing, indicating that crystalline dexamethasone is present throughout most of the release phase. The peak at 18.7º does dissipate over time, but the other two peaks remain visible. Again, a peak at 32º is visible after incubation in PBS.

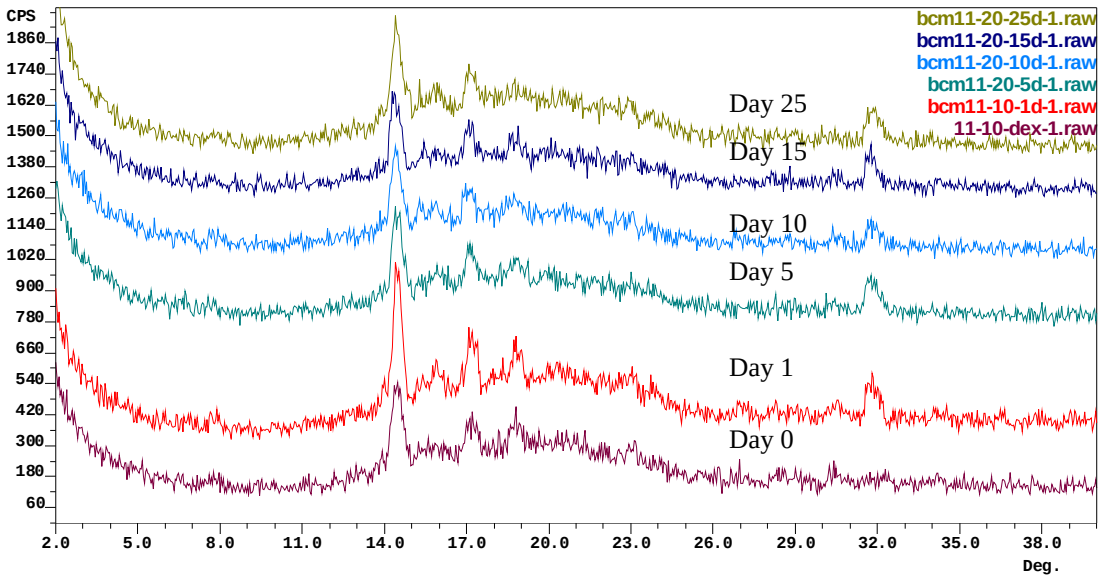

Figure 3.14. X-ray diffractograms for dexamethasone loaded filaments after incubation times in PBS at 37°C from 0 to 25 days.

3.4.8 Glass Transition Temperature

The glass transitions temperatures (T_g) for each filament under wet and dry conditions are shown in Table 3.3. After extrusion, glass transition temperatures are depressed below the raw polymer values. This depression is more pronounced for filaments made of RG 506 and is barely noticeable for PDLLA-L1. In each case, water significantly decreases the glass transition temperature of the filaments and the pure polymers. All the glass transition temperatures are essentially the same when the filaments are saturated with water, while they vary greatly for the initial filaments when dry. The variation in T_g is almost entirely eliminated after the filaments are incubated for one day.

Table 3.3. Glass transition temperatures for filaments and polymers

	T_g Dry (°C)		T_g Wet (°C)	
Sample	Initial	Day 1	Initial	Day 1
RG 506	54	-	39	-
506-L1	32	51	37	39
506-L2	37	54	39	39
506-L3	30	52	38	39
506-L4	42	54	39	39
506-L5	23	51	39	38
506-D1	32	52	39	39
506-D2	34	54	40	38
RG 756	58	-	44	-
756-L1	56	58	44	44
756-L2	54	57	44	44
PDLLA	57	-	48	-
PDLLA-L1	56	57	48	48

3.4.9 Mechanical Properties

Mechanical properties depend greatly on the conditions of experimentation. Some of the filament samples were tested under ambient conditions (air at 22°C) and others under incubation conditions (PBS at 37°C). Figure 3.15 shows the results of both elongation at steady strain rate, and step-strain response for filament 506-L1 after one day of incubation in PBS to remove residual solvents.

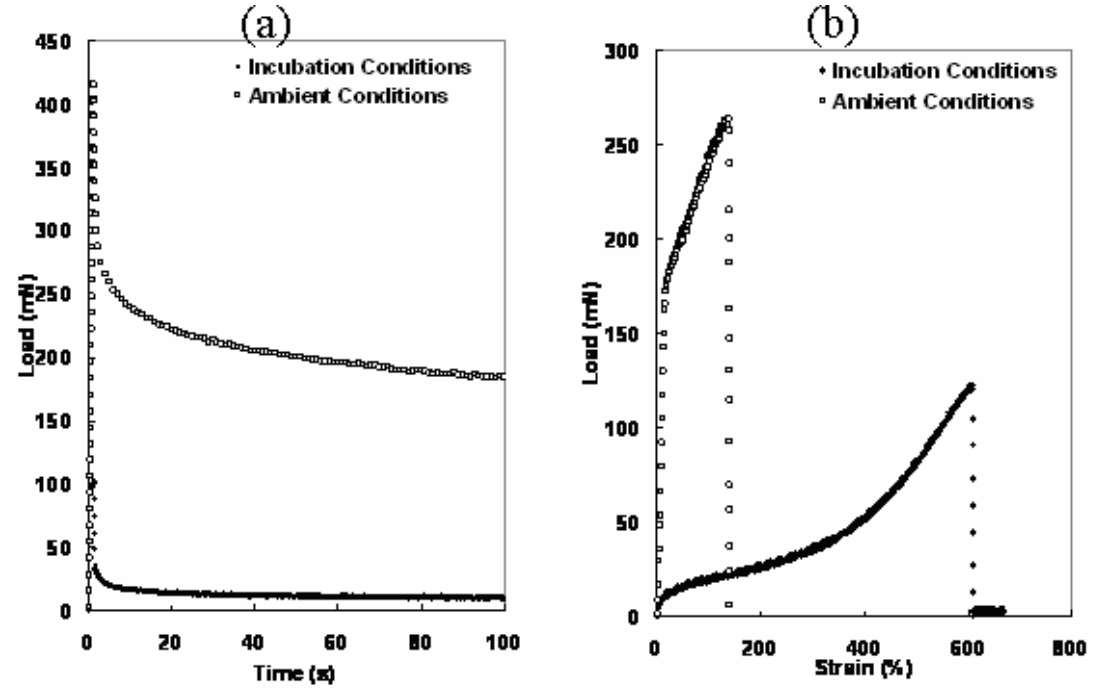

Figure 3.15. Mechanical properties of filament 506-L1 under ambient and incubation conditions.
Step-strain relaxation is shown in (a) while load/strain curves at a strain rate of 1%/s are shown in (b)

For filaments tested under ambient conditions, the as-made filaments displayed distinctly different behavior from filaments tested after incubation in PBS. Figure 3.16 displays the ambient mechanical properties filament 506-L1 as-made and after 1 day of incubation.

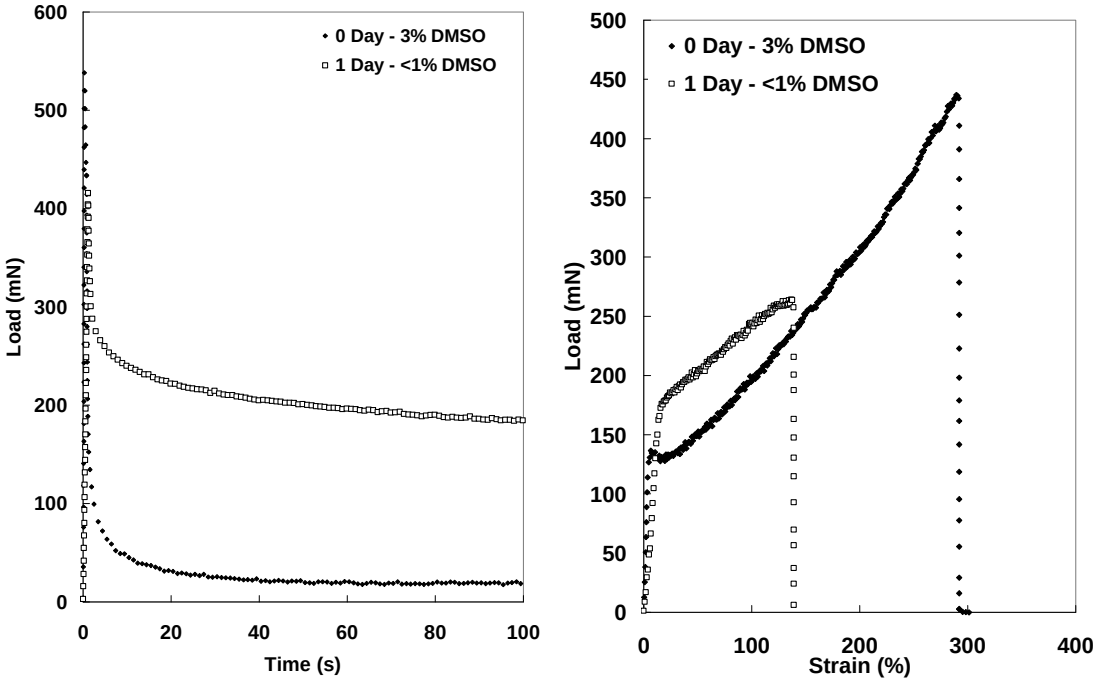

Figure 3.16 Mechanical properties of filament 506-L1 as made and after one day of incubation in PBS at 37ºC.
Load/strain curves at a strain rate of 1%/s are shown in (b) while step-strain relaxation is shown in (a)

Figure 3.17 shows an overview of the mechanical properties of all of the filaments. Figure 3.17(a) lists the load at break in both ambient and incubation conditions after processing and after 1 day of incubation in PBS at 37°C. Incubated filaments tended to elongate and break consistently, independent of whether they were previously incubated. The variation between initial properties and properties after 1 day of incubation in PBS at 37°C is noticeable under dry conditions, where loads tend to increase and maximum elongation decreases notably.

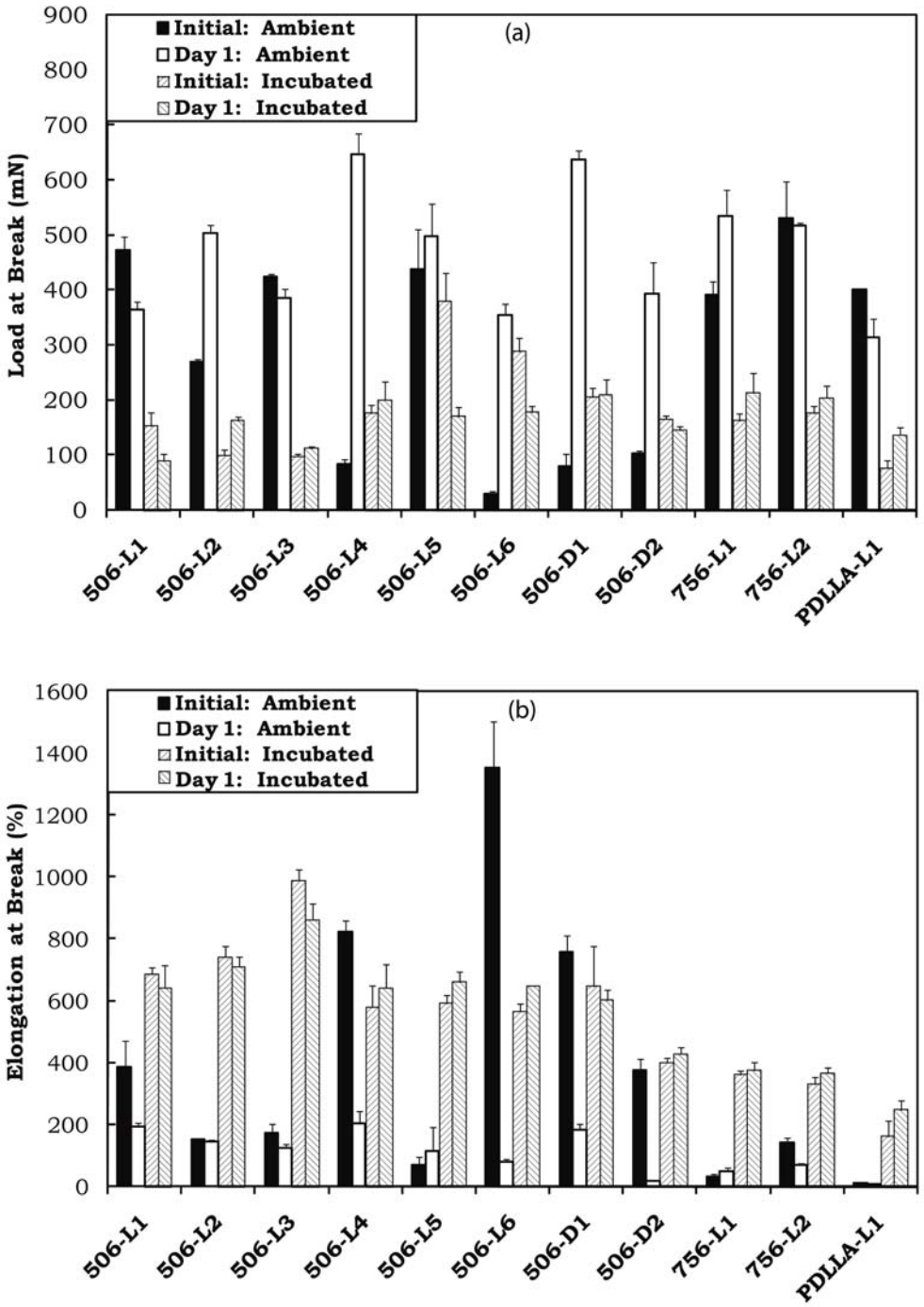

Figure 3.17. Mechanical properties of filaments:(a) load at break and (b) maximum elongation.

Each sample is tested after extrusion and after 1 day of incubation in PBS at 37°C, under ambient and incubated conditions. Error bars represent one standard deviation (n=3).

Filaments lose strength over time when tested under incubation conditions, but become more brittle when tested under ambient conditions. Figure 3.18 shows how the maximum load and strain of 506-L1 changes over time when tested under both conditions. Maximum load decreases steadily under incubation conditions while maximum strain decreases under ambient conditions.

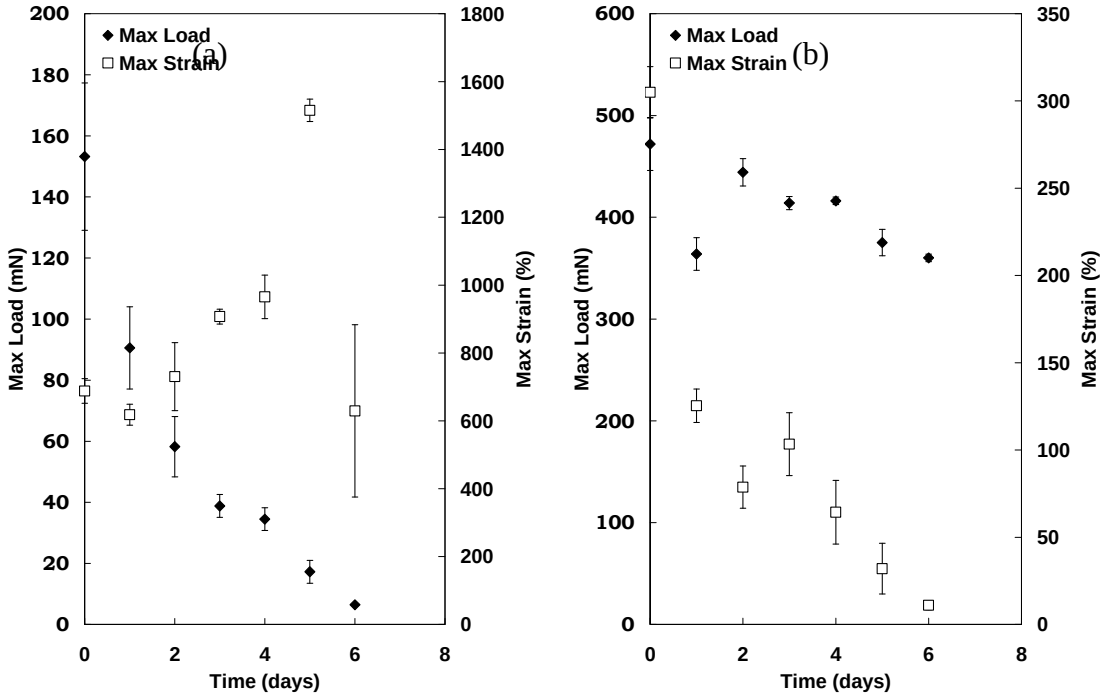

Figure 3.18. Maximum load and strain for filament 506-L1 over time when tested under ambient (a) or incubation (b) conditions.

Figure 3.19 shows how polymer type affects the rate of mechanical properties deterioration. Filament 506-L1 loses strength over one week, while 756-L1 lasts close to 20 days and PDLLA-L1 retains some strength through 45 days. Samples after this point for each filament were difficult to handle and accurate measurements were not possible.

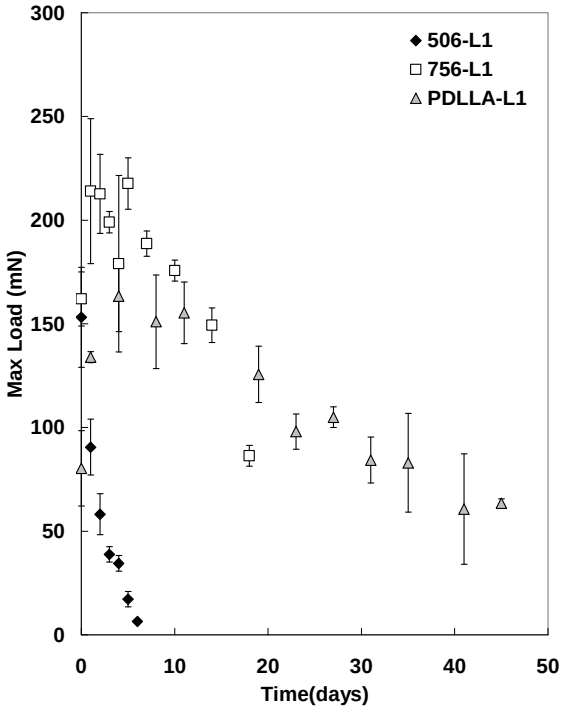

Figure 3.19. Maximum load over time for filaments 506-L1, 506-L2, and PDLLA-L3 tested under incubation conditions.

3.5. *Discussion*

Wet-spinning is a well established process for preparing polymer filaments, and has proven to be a versatile method for therapeutic encapsulation for controlled release applications. The filaments prepared here are formulated of copolymers with glycolide and d,l-lactide monomers. PLGA copolymers are a good release platform due to the ability to manipulate polymer degradation by changing the ratio of lactide to glycolide. Both drug and polymer were dissolved (or suspended) in DMSO. DMSO was chosen due to its low toxicity, miscibility with water, and ability to dissolve the drugs and polymers used in this study. Other polymers and drugs could easily be processed into similar filaments as long as care is taken with the choice of solvent and anti-solvent. All filaments formed displayed a porous, finger-like internal morphology consistent with polymers processed by wet-spinning, and were 250-300 μm in diameter.

Full compositional analysis provides insight into what chemical species affect the in-situ properties of the filaments. Here, the presence of residual solvent was carefully monitored. DMSO was chosen as a solvent due to the relatively low toxicity (the FDA considers DMSO to be a class 3, or low toxic potential, solvent) compared to organic solvents such as chloroform and dichloromethane. Residual DMSO was present in all filaments post-extrusion, but levels were often below detection after a single day of filament incubation in PBS at 37°C. Residual solvent acts as a plasticizing agent in polymeric devices[23], and affected both thermal and mechanical properties of the filaments. The difference in glass transition temperatures of dry filaments can be linked to the presence of residual DMSO (Table 3.3). Initially, the filaments have residual DMSO, which leads to a depression of T_g and makes the filaments more flexible(Figure 3.17). After a day of incubation in PBS, the T_g of the filaments is returned to a value very close to the

raw polymer value, while the filaments lose ductility as evidenced by a reduction in elongation at break. The difference in mechanical properties due to residual solvents is demonstrated by the change in tension response to a step change in strain for filament 506-L1(Figure 3.16). After extrusion, when the filament contains some residual DMSO, the tension on the filament rapidly declines to a load of 19 mN over 100 s. When that same filament is incubated in PBS for one day, eliminating residual solvent, the load on the filament stays above 185 mN over the same time. These differences can not be attributed to drug release, as in most filaments more than 60% of the initial drug is retained over a single day of incubation in PBS at 37°C. The clearest demonstration of the effect of residual DMSO can be found by examination of three filaments made with the same formulations but with different coagulation times, 506-L1, L5, and L6. Filament 506-L6 had the shortest coagulation time, followed by 506-L1 and 506-L5. Residual DMSO in 506-L6 was 16.6±3.6 μg/cm filament (4.2% by mass), while 506-L1 had 7.6±1.7 μg/cm (2.1%) and 506-L5 had 0.5±0.1 μg/cm (0.1%). The glass transition temperatures of dry filaments (without incubation) were 506-L6<506-L1<506-L5 while the elongation at break for the filaments in ambient conditions were 506-L6>506-L1>506-L5. However, after a single day of incubation, these properties converged to a single value while DMSO was eliminated from the filaments. Filaments made without drug display the same decrease in initial glass transition temperature and increase initial in elongation (data not shown), further indicating that DMSO strongly influences the thermal and mechanical properties of the filaments under ambient conditions.

Drug content does not appear to have an effect on the glass transition temperatures of the filaments. The ability for drugs to plasticize polymers, including PLGA, has been reported but is

a property specific to the particular drug and polymer. After one day of incubation, where the filaments still contain most drug in the cases of 506-L1 and 506-D1, dry glass transition temperatures are essentially unchanged from the raw polymer T_g. Drug content significantly affects the early properties of filaments only in the case of 506-D2, which has 40% drug by mass and is extremely brittle under dry conditions (maximum elongation of approximately 10 %) after incubation for one day to remove residual DMSO. Filament 506-D2 also has a decreased elongation at break under incubation conditions, stretching only 400% before breaking compared to over 600% for all other RG 506 filaments. The decrease in maximum elongation and lack of T_g depression suggest that levofloxacin and dexamethasone do not act as plasticizers for PLGA.

Filaments had very different properties when tested under ambient and incubation conditions (Table 3.3 and Figure 3.15). Water swells poly(lactide), acting as a plasticizer[24], but often mechanical and thermal properties are reported for dried samples[25-27]. The behavior observed under such conditions may not be the same under physiological conditions[28]. Filament 506-L1 displayed characteristics indicative of viscoelastic solids when under ambient conditions, similar to previous mechanical testing of PLGA devices under similar conditions[25, 27]. The stress decreased over time as a result of a step change in strain, but the load on the filament stayed high over 100 s. Under incubation conditions, the filament behaved more like a viscoelastic liquid, with the load on the filament falling to just 9 mN over 100 s. This change in properties is actually desirable for our present applications, as the filament can be implanted like a thread but will deform easily when placed under any in-situ stress. This should reduce tension on the tissue surrounding a filament implantation.

Overall, these findings suggest that the presence of water dominates all other considerations when determining in-situ mechanical and thermal properties. Drug has little influence on T_g or mechanical properties, unless extremely high drug loading is used. Residual solvent only affects samples tested outside of water. When water is present, both the effect of drug and the effect of residual DMSO are difficult to determine because under incubation conditions, the effects of water are dominant.

By examining at the initial day of release of filament 506-L1, the phenomena that govern early drug release can be elucidated (Figure 3.10). There is an immediate burst of release (around 2%), present even after only a single minute of incubation in PBS. Burst release like this is likely due to dissolution of surface drug, as water has not permeated the filament. Over the next 1-2 hours, release is very slow. This corresponds to a period of rapid water uptake (Figure 3.3). The swelling slows release, by forcing diffusion of drug against a bulk flow of PBS. After swelling, release kinetics are largely diffusion controlled for the first day with a constant diffusion coefficient, as exemplified by the close fit to the Higuchi model of release (Figure 3.11). The Higuchi model assumes a higher drug loading in the filament than the saturation concentration of drug in the release media. A shrinking core of solid drug is assumed, with the concentration of drug at the saturation concentration next to the solid and essentially zero outside of the release matrix. An effective diffusion coefficient of $6.1 \pm 1.7 \times 10^{-9}$ cm^2/s is calculated from the data in Figure 3.11 and Equation 3.1 with the C_s = 25 mg/mL and A = 40 mg/mL (calculated from drug loading and geometry)

It can be concluded that diffusion governs this early portion of drug release. This result sheds some light on a previous report by Gao et al on a filament made of poly(l-lactide) containing suspended particles of the drug 5-fluoricil[12]. From our results, it can be concluded that that Gao et al showed only diffusion controlled release from the filament, as release times (1-5 days) were far shorter than the degradation time of the polymer. Their report showed that by thickening the polymer structure, and decreasing the size of particles trapped in the filament, release could be extended from a single day to several days for wet-processed filaments.

Filaments formulated from solution of drug (not suspensions) displayed triphasic release profiles consistent with release from PLGA devices. There are two populations of entrapped drug in the filaments. Some of the drug is accessible to initial diffusion, while other drug is trapped in the polymer until later times. The drug with longer residence time does not appear to be trapped beneath the skin, but rather is tapped by the polymer itself. This is demonstrated by the morphological changes not affecting drug release (Figure 3.9). If the drug was simply trapped in the pores under the outer polymer skin, then reducing the length scale of the filaments to the order of diameter should allow for a greater amount of initial release. This is not observed, which indicates that the length scale relevant to the remaining drug is much smaller than the diameter. This is consistent with previous reports, which show the necessity of drug dispersion throughout the polymer matrix in order for the control of release past an initial burst[29, 30]. Previous reports indicate that phase inversion will trap part of the drug in a crystalline state and part in an amorphous state. The crystalline drug easily accesses pores in the polymer structure and rapidly diffuses out of the device. Amorphous drug is dispersed throughout the polymer

system, and is effectively trapped. The drug trapped in the polymer matrix has a much lower diffusivity than drug in the pores[31].

A recent report by Ma et al on non-degradable, drug containing films produced by phase inversion suggests that crystalline drug will diffuse out of the system relatively quickly, leaving behind amorphously trapped drug[29]. This leads to the conclusion that drug solubility in the polymer is the main factor in determining when the drug will release. The XRD studies above throw some doubt on the generality of this claim. Levofloxacin behaves much as expected from the previous report. Crystalline levofloxacin is present after extrusion for 506-L1, but there is no detectable crystalline drug after a single day of release. However, for 506-D1, dexamethasone characteristic peaks were observed well into the second release phase of the filament (25 days). This means that for dexamethasone, crystalline drug is trapped in the polymer matrix in a way that prohibits the fast release of drug. Further exploration of the physical state of the drugs inside of the filaments was found to be difficult. Typically, DSC would be used to find what fraction of drug is in a crystalline state. In this case, both drugs do not melt below 200°C and melting is only accomplished with drug degradation. Furthermore, heating filaments to temperatures in excess of 100°C leads to polymer degradation and liquefying. From annealing filaments at 100°C, we observed a significant change in release properties, indicating that drug interaction with the polymer is not the same after significant heating. It is likely that, even if melting transitions can be adequately observed, such measurements will only record the solubility of the drugs in PLGA at their melting temperature. We found that the competing phenomena at the melting temperatures of the drugs did not allow for easy analysis of crystallinity.

The encapsulation of levofloxacin in copolymers of lactide and glycolide having monomer ratios of 50:50 (RG 506), 75:25 (RG 756), and 100:0 (PDLLA) lactide:glycolide allowed the manipulation of release times for the drugs (Figure 3.5). Higher lactide content should lead to slower polymer degradation and drug release. As degradation causes polymer cleavage and erosion, the diffusivity of trapped drug increases, leading to degradation controlled drug release. To compare the effects of PLGA type to final fiber properties, 506-L1, 756-L1 and PDLLA-L1 filaments were made with formulations that were the same except for polymer type. Filaments prepared from each polymer displayed a typical release curve for such devices, with an initial burst followed by a plateau of slow release that accelerates as the polymer structure degrades. As expected from previous studies using similar polymers[32, 33], 506-L1 releases over the shortest time period of 15 days, while 756-L1 releases over 40 days, and PDLLA-L1 over 80 days.

Thermal and mechanical properties are different depending on polymer type. Filament 506-L1 retains more DMSO than the other two filaments, leading to differences in thermal and mechanical properties. Even without residual DMSO, glass transition temperatures are different for each polymer, with RG 506 more susceptible to T_g depression by water. Mechanical properties are also different between polymers, with 506-L1 elongating more than 756-L1 or PDLLA-L1. After 1 day of incubation, 506-L1 elongates more than 756-L1 or PDLLA-L1, under ambient or incubation conditions.

Mechanical deterioration is a sign of polymer degradation in biodegradable devices. This deterioration manifests differently depending on the environment under which filaments are tested (Figure 3.18). For filaments under incubation conditions, this corresponds to a decrease in the maximum stress before breakage. Under ambient conditions, filaments get more brittle, as demonstrated by the steady decline in maximum elongation for filament 506-L1. For different polymers, the mechanical deterioration of the filaments happened on the same time scale as drug release, with 506-L1 losing strength over a week, 756-L1 over 20 days, and PDLLA-L1 over 50 days (Figure 3.19). This results in logistical problem for these devices, as mechanical deterioration and release are linked due to the dependence of both on polymer degradation.

The physical state of drug in the formulation can influence various aspects of the processed filament[9]. Filaments 506-L2 and 756-L2 were formulated from solutions containing levels of levofloxacin above the solubility point and contained suspended particles of drug. In contrast, filaments 506-L1 and 756-L1 were formulated with less drug (under the same conditions otherwise) with the levofloxacin fully dissolved with the polymer in DMSO. SEM images reveal rough surfaces for 506-L1 and -L3, which were processed from homogenous solutions, while 506-L2 has discrete protuberances that are not visible on the other filaments (Figure 3.4). These images likely indicate that drug particles exist in the matrix of 506-L2 in discrete clumps. Filaments formulated from suspensions exhibited fast release, with most of the levofloxacin releasing over the course of one day. This burst was so extreme, that only 6% of drug in the formulation was retained after a day of incubation in PBS at 37°C. Even though 506-L2 and 756-L2 had more drug before and immediately after extrusion than 506-L1 and 756-L1, they lost so much levofloxacin during burst release that they were no longer the more highly loaded

filaments. The clumps visible by SEM are no longer observed after this fast stage of release, leaving a pocked surface morphology. These “overloaded” filaments also retained less DMSO than other filaments, which had a commensurate influence on mechanical and thermal properties. Filament 506-L4 had even less drug and retained slightly more DMSO, but was otherwise not distinguished from 506-L1. The release profiles from filaments formulated from homogenous levofloxacin solutions were remarkably similar (Figure 3.5 and Figure 3.7).

To further investigate the differences between drug solutions and suspensions, several filaments were formulated at different levofloxacin contents and temperatures (Figure 3.7). The solubility of levofloxacin in DMSO is highly temperature dependent. Solutions containing levofloxacin at 25ºC below the solubility point displayed a sustained release profile, while suspensions of levofloxacin showed fast release. When heated to 60ºC before extrusion all of the levofloxacin solutions were completely dissolved. This led to lower drug loading, but all of the release was sustained and all of the profiles were similar. The initial period of release for the filaments appears to be diffusion controlled, with only part of the drug accessible to this initial burst. It is reasonable, based on the work described in the introduction, that having large particles of drug present makes a pathway by which the drug can diffuse out. These particles create disruption in the polymer matrix, dissolve upon polymer swelling, and simply diffuse out like other available drug. The particles make the burst release even worse by acting as nucleation sites for drug precipitation during extrusion. This behavior is consistent with previously reported filaments made from wet-spinning of suspensions. Gao et al demonstrated that by making the particles small enough, diffusion can be controlled. One drawback to this method is the requirement that the drug is relatively insoluble in the polymer solution relative to the coagulation media, or the

nanoparticles may act as nucleation sites and grow. This method also limits the release profile to a diffusion-based profile, but still is effective for releasing drugs over several days.

The type of drug can influence the properties of the filament. Dexamethasone is more soluble in DMSO, and filament 506-D1 was able to be processed from a homogenous solution even though dexamethasone comprised 18.4% of the initial formulation. Dexamethasone is retained in the filament better than levofloxacin. Filament 506-D1 retained 89% of formulated drug after extrusion and 78% after a day of incubation in PBS at 37°C, compared to 53% and 33%, respectively, for 506-L1. Filament 506-D2 was an outlier in several of the above studies; it was more brittle than other 506 filaments, it released drug over a longer period, it swelled less (only 25% compared to 40% + for other RG 506 filaments) and it was the only filament to have a wet T_g above the native polymer. The unique properties of 506-D2 may be due to the extremely high drug loading, with dexamethasone comprising 42% of the initial filament mass. This surprisingly high filament loading, while retaining mechanical integrity and control of release, was probably due to the extreme solubility of dexamethasone in DMSO and the thermodynamics of coagulation in this system. The phase diagrams of PLGA (RG 506), levofloxacin, and dexamethasone in water/DMSO (see Chapter 2) may provide insight into why dexamethasone can be captured so efficiently relative to levofloxacin. At PLGA concentrations relevant to this study (20-30% by mass), phase separation occurs when water comprises approximately 6% of the solution. Levofloxacin, which can dissolve up to about 10% in pure DMSO, only makes up 4% of a solution comprising 6% water. Dexamethasone is more soluble in pure DMSO (approximately 50%) and is less sensitive to water addition, still comprising 36% of a saturated solution with 6% water. These results show that more dexamethasone can be initially dissolved

in DMSO, and that more dexamethasone will stay in solution during coagulation of PLGA. This solvent/anti-solvent compatibility seems to be a critical parameter of making drug loaded filaments by wet processing. Highly loaded, mechanically stable filaments are possible to prepare by wet-spinning if these process is carefully designed based on solubility properties of the particular solvent/anti-solvent/drug/polymer system.

Here, we have described a methodology to prepare a wide variety of drug loaded filaments and have studied their compositional, release, thermal, and mechanical properties. These characterizations provide insight into how these filaments may perform in medical applications, but in-vivo corroboration of in-vitro data is a necessary next step. In Chapter 4 we examine how several of these filaments perform in-vivo by testing their usefulness to control the delivery of levofloxacin to the eye.

3.6. *References*

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Chapter 4: In-vivo implantation of levofloxacin containing filaments

4.1. *Abstract*

Biodegradable filaments for the controlled delivery of levofloxacin to the eye are described. The filaments are synthesized from poly(dl-lactide-co-glycolide) (PLGA) and implanted in the conjunctiva using a standard surgical needle. Three filaments with levofloxacin contents of 46 ± 2, 85 ± 4, and 36 ± 2 μg/cm (denoted 506-L1, 506-L2, and 506-L3, respectively) prepared by a wet-spinning process are investigated. The strength of all the filaments decreases over 5-7 days in PBS at 37°C. Filaments were implanted using 3 different techniques. Filaments were either (1) pulled under the conjuntiva with only a small length of filament exposed, (2) looped on the surface of the tissue with two needle passes with unsecured ends, or (3) looped on the surface with knots securing the filament ends. It was found that filaments had to be exposed to the tear film and knotted to release drug into the tear film for as long as physically possible. The time dependent, in-vivo tear concentrations of levofloxacin from filament implants in New Zealand white rabbit eyes are in general agreement with the results from the in-vitro release profiles, with one of the filaments (506-L1) showing effective levels of levofloxacin in the tears for 6 days. The filaments are generally well tolerated with none of the rabbits displaying any overt signs of irritation or discomfort after recovery from the initial surgery. Histology from tissue taken directly adjacent to degrading filament revealed inflammation and immune response consistent with previously described PLGA devices. Filament failure occurs at 6-8 days within the rabbit eyes and this time to failure is essentially the same observed from in-vitro mechanical properties testing results.

4.2. *Introduction*

Eye drops are a standard way of delivering drugs to the eye. However, efficacy suffers from numerous factors that include poor patient compliance, physical difficulties in administering drops, variable drop size, and low residence time in the eye[1-3]. Winfield et al found that 25% of patients do not comply well with drop instructions, with 57% reporting some difficulty with drop installation. Dosing problems can be exacerbated with children due to the necessity of adults administering drops to uncooperative adolescents[1]. Eye drops are rapidly cleared by blinking and reflex lacrimation, so that only a very small portion of the dose remains available for efficacy[4-6]. The eye has the ability to hold approximately 30 ml of solution, but drops approach 50 ml. This means that a significant portion of a drop will not fit into the eye. Additionally, drug concentrations fall rapidly due to a 16% turn over in the tear film every 2 min, and effective levels are not maintained for extended periods of time. The rapid turn over of drug instilled from eye drops means that multiple dosing is necessary for almost every application. Forcing patients to take multiple drops each day highlights the necessity of good patient compliance.

Previous attempts to control the release of drugs to the eye include the fabrication of both biodegradable and non-degradable[7] devices. The morphologies of these systems include microspheres[8, 9], tablets[10-14], rods[15, 16], and contact lenses[17, 18]. Problems with these systems include irritation, short effective lives, the necessity for device removal (for non-degrading systems and for systems that would cause an adverse reaction in a patient), and premature elimination from the eye. Devices can also be implanted subconjunctival[19-21] or by

other periocular routes[22], but the delivery of drugs to the tear film from these positions is unknown.

In Chapter 3, we demonstrated the ability of a biodegradable filament to encapsulate and control the release of levofloxacin and dexamethasone. The goal of this investigation is to use several of the previously described filaments to control the release of levofloxacin to the eye, that for certain indications could replace or complement eye drops. The suture-like morphology of the device allows for easy implantation with a surgical needle. Here, we illustrate the concept by focusing on a device that could provide an alternative to post-operative antibiotic eye drops, and facilitate the treatment of severe external bacterial infections such as bacterial corneal ulcers. The target performance of the device is that it should be easily implanted after surgery, and maintain an effective concentration of the antibiotic in the tear film for 5-10 days. The filament is anchored in the eye by passing it through the conjunctival tissue and removal surgery is unnecessary by the controlled biodegradibility. Levofloxacin is used for this model system due to its potency against a broad spectrum of bacteria and its natural fluorescence, which makes detection at low concentrations possible. PLGA has been used extensively in implantable devices, including devices implanted in the eye. Levofloxacin not only inhibits bacteria, but is also bacteriocidal, in many cases at concentrations just above the MIC90. Since MIC90 values fall below or are close to 1 μg/mL for most bacteria, this value was utilized as the minimum levofloxacin tear concentration that would be acceptable for sustained release[23].

4.3. *Materials and Methods*

4.3.1 Filament Preparation

Filaments containing levofloxacin or dexamethasone were processed by a wet-spinning procedure. An accurately measured amount of drug was placed in a glass vial to which dimethyl sulfoxide (DMSO) was added. This solution was sonicated for 1 minute to facilitate dissolution of the drug. After sonication, PLGA was added to each vial and the vial vortexed to mix all components. The solution was then allowed to equilibrate for 6 hours, vortexing occasionally to mix, at a given temperature (Table 3.1), to eliminate all trapped bubbles. Filaments 506-L1, 506-L2, and 506-L3 from Chapter 3 were synthesized for these experiments.

Solutions were loaded into a 5 mL syringe equipped with a 22 gauge flat-tipped needle (Small Parts Inc.) and mounted on a syringe pump. The solutions were extruded into a 16 L water bath (22 ± 2°C) and taken up onto a 1 inch bobbin rotated by a DC gear motor. The pump speed and uptake rate were chosen to fix a particular coagulation time (Table 1) with a draw ratio of 1.2. Filaments were secured tautly without stretching and allowed to dry under ambient conditions for two days.

4.3.2 Drug Loading

Total drug loadings of the filaments were determined by dissolving accurately measured sections of each filament with 1 M NaOH. The drug content of the resulting solution was measure by using high performance liquid chromatography (HPLC) with a C18 column (Agilent 1200 series HPLC). The mobile phase was 49.95 % water, 49.95 % acetonitrile with 0.1 % trifluoroacetic

acid. Levofloxacin was determined by fluorescence detection with an excitation at 292 nm and emission at 494 nm.

4.3.3 In-Vitro Release

One inch sections of filament were placed in 1.5 mL tubes. One mL of phosphate buffered saline (pH 7.4) was added to each vial. The vials were incubated at 37°C under gentle rocking, and at various times, the buffer was replaced with fresh PBS. The removed PBS was analyzed for drug content by HPLC as described above.

4.3.4 In-Vitro Mechanical Testing

Load vs. strain curves for the fibers were collected on an Instron 1150 mechanical properties testing apparatus. Experiments were run under incubation conditions, with the filament submerged in PBS at 37°C. The samples were drawn at a strain rate of 1 %/s and the resulting tensions were recorded. The loads that the filaments were bearing at the time of breakage were recorded.

4.3.5 In-Vivo Implantation

All use of animals in these experiments was approved by the California Institute of Technology Institutional Animal Care and Use Committee.

Filaments were prepared and dried for two days as described. The day of implantation, they were sterilized by dipping in isopropanol followed by dipping in sterile saline. New Zealand white rabbits were anesthetized with isoflurane (5% originally, falling to 2.5% typically) and their eyes were treated with 0.5% proparicaine drops. A speculum was used to hold open the

eyelids while implantation was accomplished. Filament was threaded through a standard surgical needle creating a double-stranded device.

Filaments were implanted by three techniques. In the first technique, the filament was passed under the conjunctiva in the inferior fornix and the ends of the filament were clipped close to the tissue surface. This resulted in a single band of double-stranded filament residing, primarily, under the tissue surface. In some cases, small tags of filament were present outside of the tissue.

The second implantation technique allowed for an exposed strip of filament outside of the tissue. Filament was passed under the conjunctiva, as with the first technique, but the needle was looped back to the starting position and a second pass through the tissue was completed. The ends of the filament were cut, allowing for ~ 0.5 cm exposed end tags, but were not secured further.

In the third implantation technique, the free end of the filament was knotted and the needle was passed under the conjunctiva until the knot rested against the tissue. The needle was returned close to the initial position and passed a second time under the tissue, leaving an exposed stand of about 1.5 cm above the tissue and in contact with the tear film. The filament was knotted again at the exit point of the tissue so as to secure the device at both ends with knots. The length of the filament before implantation and the remainder after implantation were accurately measured to give the length of filament implanted. Six implantations of each type of filament were performed.

Tear samples were collected by Schrimer's test strip. The mass of a strip before and after 15 s contact with the tear film was recorded. The strip was allowed to dry and then the drug on the strip was dissolved in 200 μL PBS. The sample was filtered and levofloxacin concentration was determined by HPLC. The tear concentration measurements were normalized to the average length of filament implanted. For each tear sample, the presence of filament was verified by visual observation. The failure point of the filament was recorded at the first day where no filament was visible on the surface of the tissue.

To further correlate in-vitro models with in-vivo performance, samples of 506-L3 that had been incubated for two days in-vitro were implanted by technique #3 in three rabbits. Filament 506-L3 was made as above and three lengths of approximately 30 cm (accurately measured) were cut and each was placed in 10 mL of PBS at 37°C. For two days, these filaments were incubated at 37°C like for the in-vitro experiments described above. After two days, the filaments were removed from the PBS, sterilized with isopropanol, and implanted in rabbits.

After 9 days, the rabbits were anesthetized and the area around the implantation was examined for filament remnants. In most cases, no remnants could be found, since filament failure and elimination usually involved the entire filament coming out of the tissue. In some cases, when a filament remnant could be positively identified, a biopsy of the site was taken for analysis. These samples were sent for blinded, professional histological analysis and a report detailing inflammation, foreign body reaction, and other histology features was generated.

Some filaments of 506-L1 were implanted using technique 3 and removed periodically for drug loading analysis. These filaments were not allowed to extrude from the eye, but instead were located and collected while still residing in the eye. Small sections of these filaments were cut, measured, and analyzed for levofloxacin content to track filament drug release rather than tear concentration.

4.4. *Results*

4.4.1 In-Vitro Properties

Table 4.1 lists the linear concentrations of levofloxacin in each of the three filaments. Filament 506-L2 was formulated with 1.67 times as much levofloxacin as 506-L1 and -L3 and has a significantly higher drug loading (ca. 2X) than the other two filaments. Each filament has a very similar diameter (Table 4.1), which is expected due to their processing under identical extrusion conditions

Table 4.1 Filament Properties

	Levofloxacin Loading (μg/cm)	μg levofloxacin/g tear at 3 hr	Average In-vivo Failure Time (Days)
506-L1	**46 ± 2**	**48 ± 33**	**7.3 ± 0.5**
506-L2	**85 ± 4**	**171 ± 140**	**8.3 ± 0.8**
506-L3	**36 ± 2**	**30 ± 17**	**6.8 ± 0.7**

Figure 4.1 shows the in-vitro levofloxacin release for each of the filaments. Filament 506-L2 reveals a quick release, while 506-L1 and -L3 show fast release for the first day, followed by a plateau from days 1-3, and then ends with a faster release until completion.

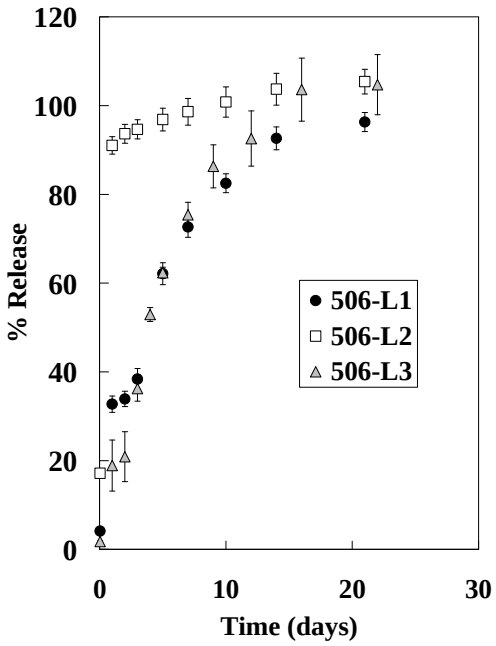

Figure 4.1. In-vitro levofloxacin release.
Error bars represent one standard deviation for n = 3.

The in-vitro strength of the filaments decreases with incubation time when tested in PBS at 37°C (Figure 4.2). Filament 506-L2 maintains strength the longest, followed by 506-L1 and 506-L3. All of the filaments last at least 5 days before they are no longer strong enough to handle without breaking.

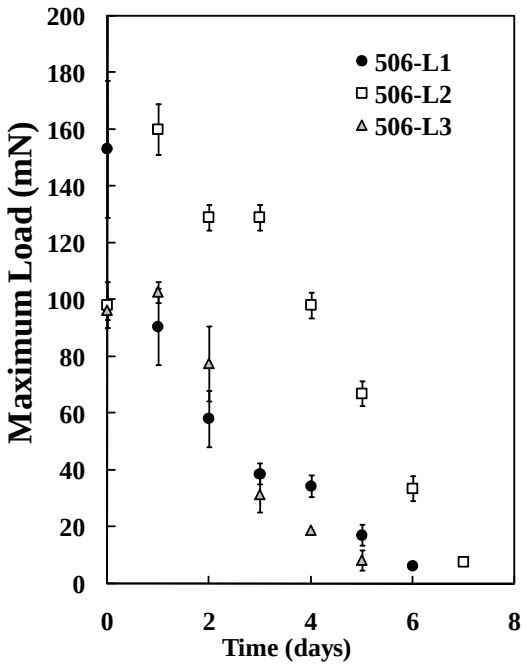

Figure 4.2. **In-vitro ultimate strength of filaments under physiological conditions over incubation time.**

4.4.2 Implantation Technique #1: Under Conjunctiva

The first implantation technique attempted consisted of a double-stranded filament pulled under the conjunctiva with the ends cut off close to the tissue surface as shown in Figure 4.3(a). This configuration was used for 506-L1 in three rabbits and drug concentrations in the tear film are variable (Figure 4.4). In some samples, drug is present up to 7 μg/g tear (one sample after 3 hours, and one on day 4), but in most samples, drug content is below 1 μg/g, with many samples below detection limits (about 0.05 μg/g) for levofloxacin. After 3 days, none of the samples showed levofloxacin and the filament was fully healed over in the eye (Figure 4.3(b)).

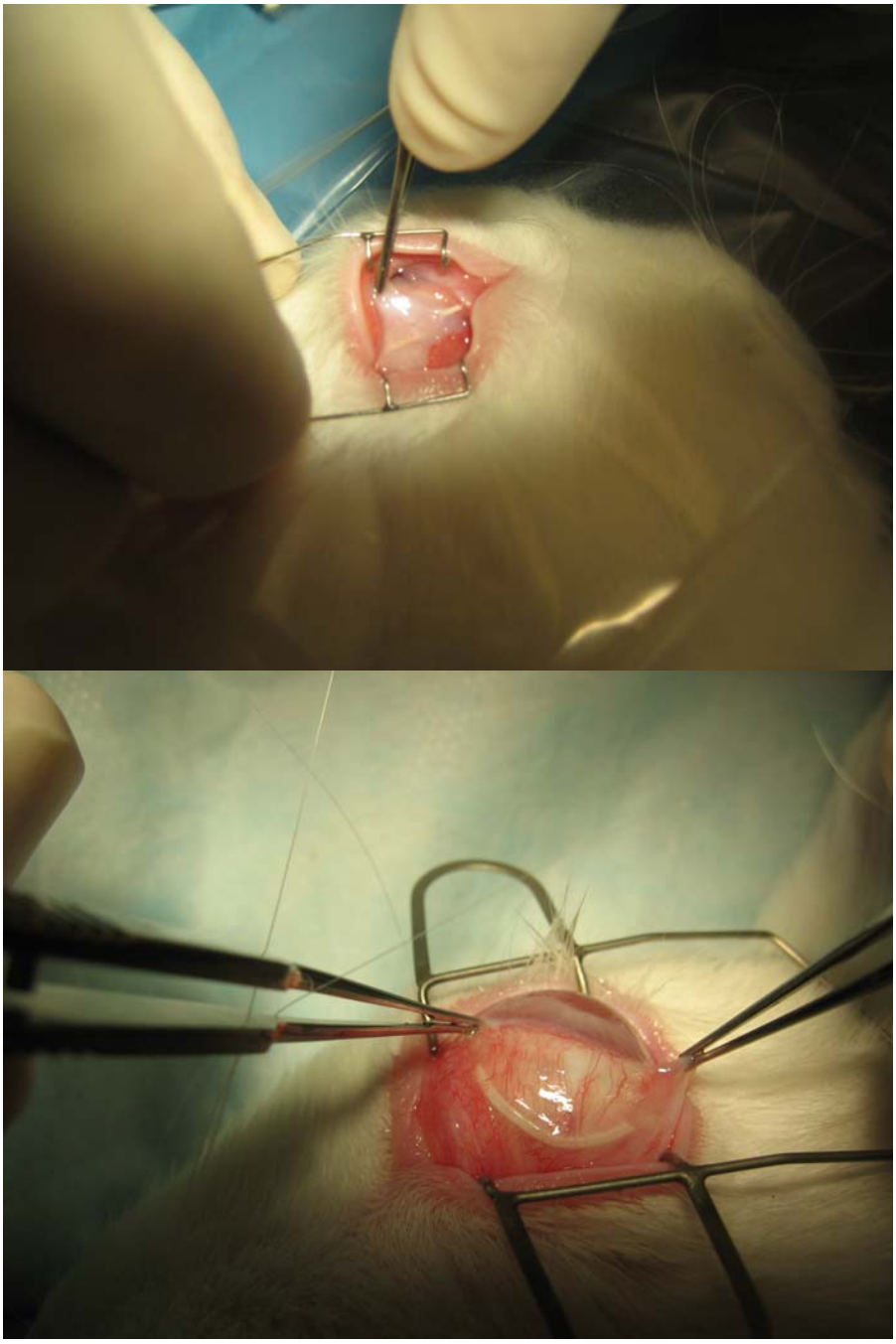

Figure 4.3. Filaments implanted by technique #1.
Technique #1 implants (a) are passed under the conjunctiva with small external tags and (b) show total tissue encapsulation after 5 days.

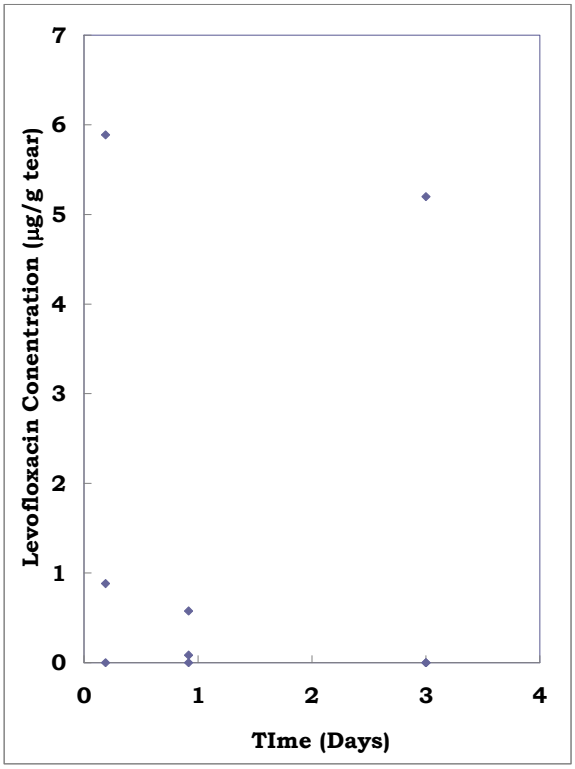

Figure 4.4. Levofloxacin levels in tears following filament implantation by technique #1.

4.4.3 Implantation Technique #2: Looped

Three 506-L1 filaments were implanted with an exposed loop on the surface of the tissue, but without securing the filament ends in any fashion (Figure 4.5). The average length of filament implanted was 7.3 cm. Figure 4.6 shows tear concentrations over time. After 3 hours, drug levels are uniformly high. As the trial progressed, 2/3 of the total samples fell above the 1 µg/g target. Two of the samples lasted until day 5 and one lasted until day 6. It was observed that they day 5 samples were recovered intact, without any breakage of the filament.

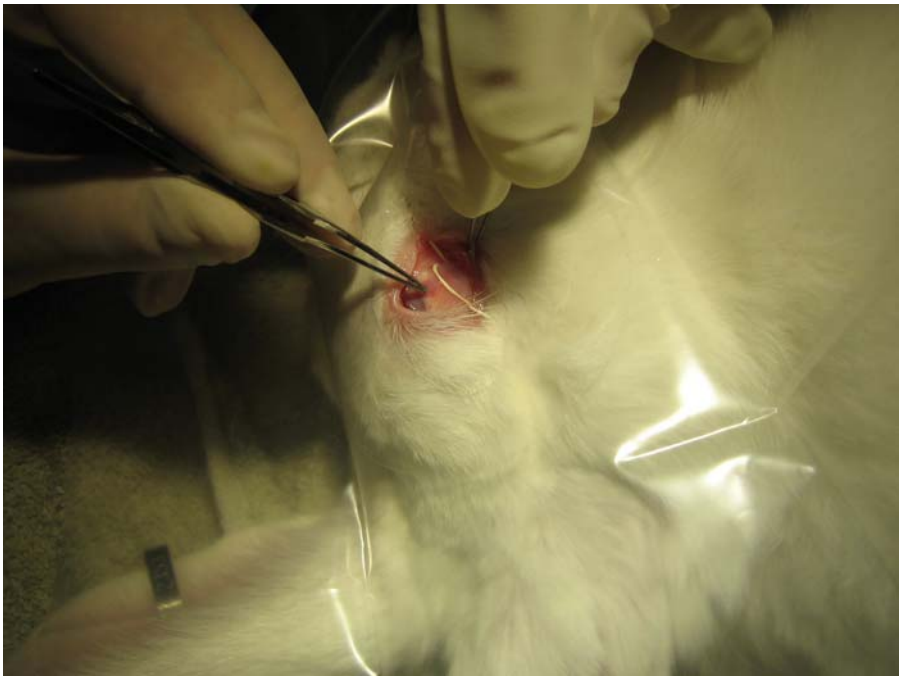

Figure 4.5. Filament implanted by technique #2.

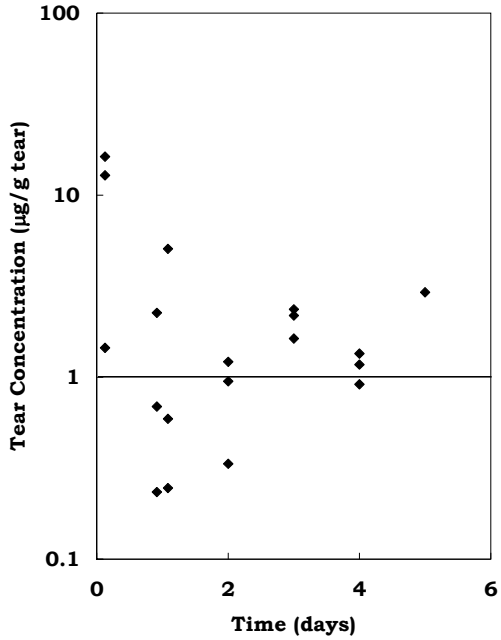

Figure 4.6. Levofloxacin tear concentrations for filaments implanted by technique #2.

4.4.4 Implantation Technique 3: Secured Ends

Six samples of each of the filaments were implanted into the eyes of New Zealand white rabbits as illustrated in Figure 4.7 with a tear-exposed strip and knots at the ends. The average length of implantation was 9.9 ± 1.8 cm. Levels of levofloxacin in the tears were tested 3 hours after implantation, and then every day until the end of the trial. The filaments were allowed to fall out on their own and every day, when tears were sampled, the eyes were examined to determine exposed filament presence. With the ends secure, failure occurs when the polymer degrades to a point where the filament breaks and falls out of the eye and can no longer be seen by simple observation. Table 4.1 listed the average time to device failure for each of the filaments. Filament 506-L2 lasts longer than 506-L1 and 506-L3 failing the earliest. Some filaments would extrude out of the tissue and fall out in entirely, while others left small pieces of filament embedded in the tissue.

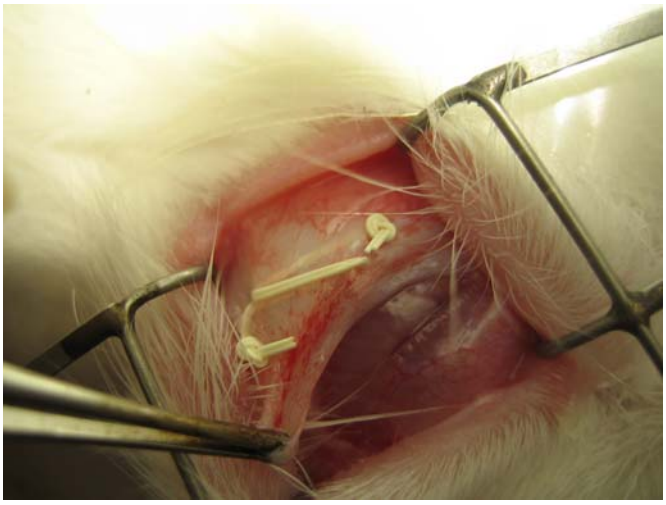

Figure 4.7. A filament implanted by technique #3.

After 3 hours, there were high levels of levofloxacin in the tears of each animal for all the filaments implanted, with the highest levels for filament 506-L2 (Table 4.1). The time

dependent levofloxacin tear concentrations are shown in Figure 4.8 where the concentrations are normalized to the average implantation length to eliminate bias from variations in the amounts implanted. The plots are shown on the semi-log scale to accentuate trends in the tear concentrations. Data points after device failure are omitted, though tear concentrations typically fell below detection levels when no filament was visibly present. Filament 506-L1 exhibits stable release, with most values falling between 1 and 10 μg levofloxacin/g tear. Filament 506-L2 shows significantly lower concentrations for the majority of samples. Filament 506-L3 has a release profile that varies with time, showing a dip at 1 day, rising concentrations through day 3, then a gradual decline until device failure.

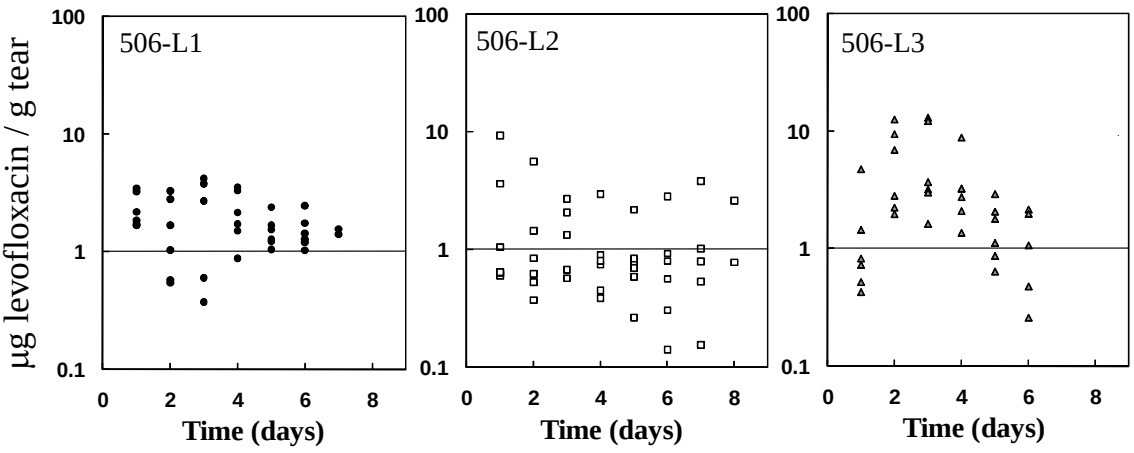

Figure 4.8. In-vivo levofloxacin tear concentrations for each filament implanted by technique #3.
Concentrations are not reported past the time when filament mechanical failure occurs. The line at 1 μg/g tear denotes the target tear concentration.

Filaments of 506-L1 were removed at various time points and drug content was analyzed (Figure 4.9). The levofloxacin loading decreases as a function of implantation time. As shown in Table 4.1, the original levofloxacin loading of 506-L1 is 46 μg/cm but this level drops to 26 μg/cm after one day of implantation and by day 7 is down to 5 μg/cm. These samples were taken from

filaments that were removed after days of implantation and may have been stretched or compromised in other ways during the implanted time.

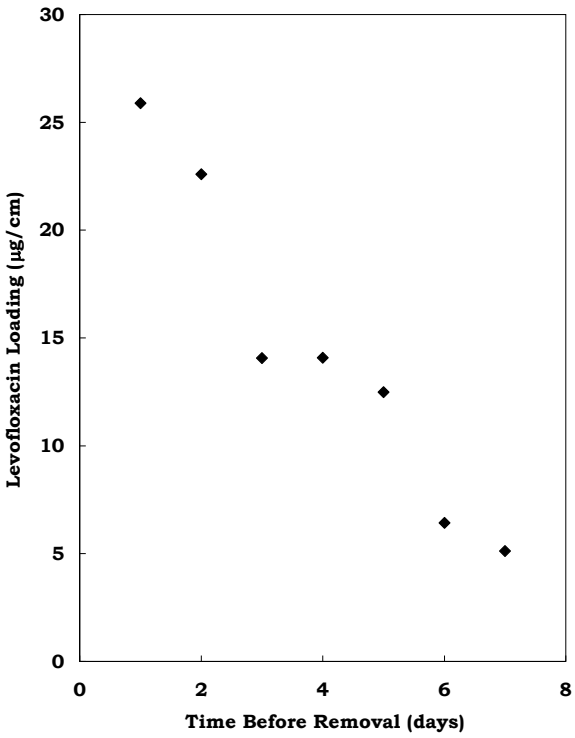

Figure 4.9. Drug loading of filament 506-L1 vs. in-vivo implantation time.

The transient release profile of filament 506-L3 allowed further investigation into the relationships between the in-vitro and in vivo results by incubating samples of 506-L3 for two days in PBS at 37°C before implantation. Figure 4.10shows the results of this study, with the samples for the pre-incubated filament shifted to match the timeframe of the filament implanted without incubation, so time 0 on the graph corresponds to when un-incubated 506-L3 was implanted in the rabbits and when pre-incubated 506-L3 was placed in PBS. Pre-incubated 506-L3 was then implanted on day 2. The pre-incubated filaments show levofloxacin concentrations that match well to the original filaments, and the release profiles correlate with the same gradual

decline until failure. The pre-degraded filaments also failed after an average of 4 days, matching the average lifespan of original filaments minus the two day incubation period.

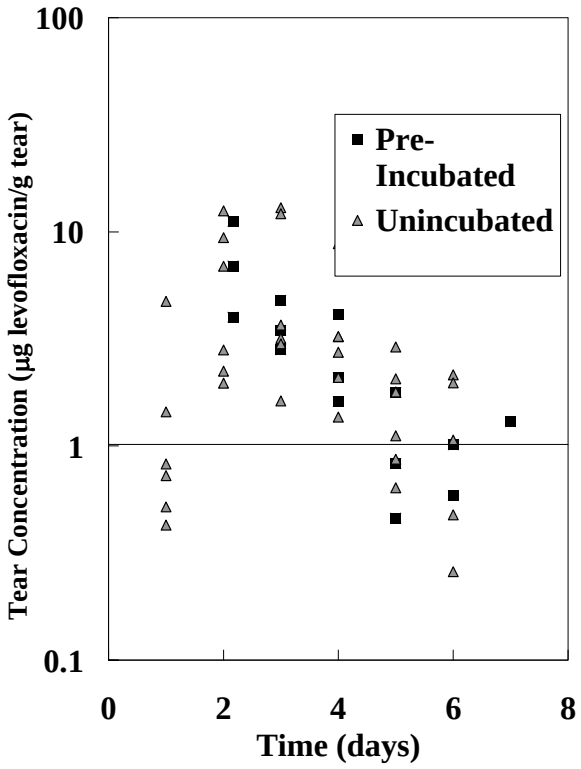

Figure 4.10. Comparison of in-vivo levofloxacin tear concentrations between filament 506-L3 that was implanted as-made and 506-L3 that was incubated in PBS at 37°C for two days before implantation.

There were visible signs of irritation to the eyes immediately after implantation, but these signs dissipated as the rabbits fully recovered from the surgery. By day 2, there were no visible signs of irritation or pain (no scratching, rubbing, or keeping the eye shut). After nine days, examination revealed the discernable remnants of only 5 of the filaments that were implanted. Histology of biopsies from these sites revealed chronic inflammation in all samples with foreign body reaction and fibrosis each present in 3 out of the 5 samples. Inflammatory cells made up of lymphocytes, plasma cells and eosinophils were present in the subepithelial stroma for all of the

samples and foreign body giant cells were seen in 3 of the samples. This type of tissue response is in agreement with previous studies on implanted PLGA and other degradable devices[24-28].

4.5. *Discussion*

Biodegradable filaments for the controlled release of levofloxacin to the eye have been prepared and characterized both in vitro and in vivo in the eyes of New Zealand white rabbits. Levofloxacin is a highly potent antibiotic against a broad spectrum of microbes (under 1 μg /mL in the vast majority of cases) and is FDA approved for topical, ophthalmic use[29]. Levofloxacin is a good model drug for this delivery system since it is easily detectable in low concentrations due to its natural fluorescence. Wet-spinning of PLGA creates a porous, easily deformable product that likely aids in the minimization of irritation upon implantation[30]. The diameters of these filaments are similar to suture diameters, with these three filaments having diameters typical for a size 2-0 or 3-0 suture. While this is larger than sutures typically used for ophthalmic applications, it is similar to the dimensions of previously described ophthalmic controlled release devices[10, 13, 15, 31], including some that have been used clinically[32].

The method of implantation is critical to the success of a filament. In order to study the release and failure properties of filaments in-vivo, a method of implantation that allowed for investigation had to be established. In this study, filaments were implanted by three different techniques: 1) placing the filament under the conjunctiva, 2) looping the filament to provide an exposed strip but not securing the filament ends, and 3) looping the filament and tying the ends. If a filament is implanted under the conjunctiva, with minimal or no direct tear exposure, than drug levels are almost uniformly low. This is corroborated by the failure to detect levofloxacin after filament breakage for the other types of implantation, despite filament remnants present under the conjunctiva. The conjunctiva heals over quickly and after a few days, the entire sample is encapsulated under tissue. The barrier to drug diffusion through the tissue is too great

to have a fully implanted filament provide adequate drug release to the tears, and that having part of a device exposed to the tear film is necessary.

Two implantation techniques with exposed filament were performed. It was found that a filament could provide adequate drug release if a strip of filament was exposed to the tear film. When the filament's ends were not secured (technique 2), the filament tended to extrude intact from the implantation site. These filaments were collected whole, indicating that failure to properly anchor the filament, rather than mechanical failure, was the cause of filament loss. Before these filaments were lost, most of the tear concentrations were above 1 μg/g tear, indicating that filaments exposed to the tear film could release adequate amounts of drug. The final implantation technique used knots to secure the ends of a looped filament. A secured filament provided a stable system for investigating filament drug release and mechanical failure properties.

The three filaments used here were characterized more fully in Chapter 3 and limited data are provided here for comparison to in-vivo results. The differences in filament formulation are reflected in release profiles and drug loading. Filament 506-L2 was fashioned from a solution containing drug particulates, while 506-L2 and -L3 were processed from homogenous solutions. This led to much higher drug loading for 506-L2, while 506-L1 had only slightly higher loading than 506-L3. For a typical implantation, the total amount of levofloxacin in the implanted length of filament ranged from approximately 300 μg (506-L3) to 750 μg (506-L2).

The time dependent release properties from in-vitro studies correlated well with in vivo results. The release profiles of filaments 506-L1 and -L3 involved an initial burst followed by a small plateau and a second phase of release. These results are similar to many previous studies of devices fashioned from PLGA or related polymers[33]. The initial burst of antibiotic release is clinically desirable as high antibiotic concentration is most important during the time immediately after an operation. In Chapter 3, it was shown that filaments made from solutions containing particles of levofloxacin released the drug rapidly, and this is the case for 506-L2. The differences in the release behavior in the in-vitro release kinetics are also observed in the in-vivo tear concentrations. Filaments 506-L1 and -L3 provide relatively high levels of drug over the entire time course of the study while 506-L2 shows mostly low levels of drug after one day. The in-vivo verification of in-vitro release profiles is important, since far more filaments were made in Chapter 3 than were tested here. It can now be reasonably assumed that the in-vitro release of drugs from the filaments previously described is a good indicator of in-vivo release.

Tears were sampled by contacting the eye with Shirmer's test strips (mass determinations are more accurate than volume and thus were used here). This method may be affected by reflex lacrimation diluting the tear samples as they are collected, resulting in levofloxacin concentration readings that are lower than actual levels in the tear film. The Schirmer's strips were used since alternatives, such as capillary tubes and sterile sponges, also suffered from similar problems and were more difficult to use for collection. Topical anesthesia, that would prevent reflex lacrimation, can not be used for tear collection, since any infusion of drops into the eye would wash out the levofloxacin.

There were also significant rabbit to rabbit differences in tear concentration, with the most obvious outlier being a sample of 506-L2. For one rabbit, 506-L2 gave very high tear concentrations throughout the entire course of release. In figure 4.8, all of the highest drug measurements for 506-L2 come from a single implantation. This significantly skews the results of the trial and blurs the destinctions between 506-L2 and the other filaments. With the outlier included, it appears that 33% of the tear concentrations for 506-L2 are over 1 μg/g tear but without the outlier, this falls to 17%. This compares to 87% for 506-L1 and 70% for 506-L3. When the highest single rabbit is eliminated, 84% of the measurements for 506-L1 are still over 1 μg/g tear while 65% are for 506-L3.

One of the primary goals of a local drug delivery system is to minimize the amount of drug used in the dose while maximizing the effectiveness of the dose. For filaments of the type used here, these criteria translate into maintaining an effective level of levofloxacin while minimizing the total exposure. Any side effects or tissue toxicity due to levofloxacin exposure should be minimized while effectiveness is retained. For filament 506-L1, the ability to maintain effective levels of levofloxacin has been demonstrated, while the total amount of levofloxacin present in a particular eye averages only 455 μg. Eye drops, which maintain effective levels of drug in the eye for only a short time, require a far larger total amount of drug. For example, a typical drop of 0.5% levofloxacin contains approximately 250 μg of drug (assuming a standard drop of 0.05 ml). Thus, the total amount of drug in a standard implantation of 506-L1 is equivalent to the total drug content of only two drops of levofloxacin ophthalmic solution.

Overall, the filaments were easy to implant with the procedure taking only a couple of minutes for each eye. The rabbits showed some irritation in the time immediately after implantation, but they recovered from the implantation and eyes with filaments implanted were not immediately discernable from eyes without filaments. With few exceptions, the eyes with filaments did not display excessive tearing and none of the rabbits were observed trying to scratch or rub the implantation site. The 50:50 PLGA that was used for these studies in not often fashioned into filaments, but other reported devices made by wet-spinning have been described. These filaments, made by wet spinning, easily yield under wet conditions as shown by the low maximum stress obtained during mechanical properties testing, as shown in Chapter 3. Moreover, these maximum stresses are often achieved at strains of over 500% which demonstrates that the filaments will deform in response to stress rather than recoil. The low irritation could be the result of the easy yielding of the filaments to stress, thus preventing any pinching or tightening that could result from a device retracting against any forces acting on the devices in the eye. The irritation may also be lessened by the porosity of the filaments, which makes the devices able to deform in the radial direction. While the rabbit results are promising in that the filaments are well tolerated, humans may respond differently to such a device. Implantation technique and size may need to be further tuned. For example, the practice of knotting the ends of the filaments may need to be re-evaluated based on patient concerns and another method of securing the ends of the filaments may need to be devised.

After the filament has been eliminated, histology reveals that the part of the filament embedded in the conjunctiva does cause an inflammation and foreign body response. This inflammation is of a moderate intensity and is similar to the types of inflammation seen in response to degrading

sutures and other degradable implants[24-28]. PLGA devices have been approved for human implantation by the FDA despite the inflammation caused by degradation products of the filament[25]. Since this inflammation is moderate, localized only to the tissue in contact with the embedded part of the filament, and is only for devices that are not fully eliminated after filament failure, it may be that most of the reaction can be eliminated by changing the implantation technique. If future implants only have a small length embedded in the tissue, with most of the filament on the tissue surface, then there is less area for inflammation and not much embedded filament to prevent complete device elimination.

Previous attempts at sustained drug delivery to the tear film have mostly focused on shorter release times and devices that extend drug residence times when the drug is administered as a drop. Diffusion based drug delivery systems, such as contact lenses[17, 18], unanchored devices[12, 13, 15], and self-forming gels[6], are often used to stall the release of drug for several hours, but rarely work for multiple days. There have been reports of molecularly imprinted contact lenses that release drug for several days, but they must reside on the cornea, and this may limit their use for immediately after surgery on the cornea. Other systems that are not anchored may be placed in the conjunctival sac, but can come out due to mechanical disturbances. These devices show promise for multiple-day release, but may ultimately have to be anchored in-vivo.

Devices developed for drug delivery to the tear film that are unanchored will be cleared from the eye due to blinking or other types of mechanical disturbance[15]. The filaments are self-anchoring, so they must maintain mechanical strength over the course of drug release. Eyes that

had no visible filament also had no detectable levels of levofloxacin, so the ability of the filament devices shown here to reside exposed to the tear film was of critical importance. At the same time, a device that resides in the eye long past the end of drug release is undesirable. The filaments described here have mechanical properties that are time dependent due to the biodegradation of the PLGA. In-vitro studies of mechanical properties closely predict the in-vivo lifespan of the filaments. The order of survival (506-L2>-L1>-L3) is especially important, but the days to failure are even closer in agreement for the in-vitro and in-vivo studies. In-vivo failure tends to happen about a day later than predicted in-vitro, but this is probably due to the method of recording failure in-vivo (only looking at tear sampling times) and to filaments remaining trapped in the eye past their mechanical failure point. A critical consideration in the design of the filaments is that the mechanisms for drug release and mechanical failure are not independent. This leads to a filament releasing over 70% of its total drug load before failure in-vitro.

It is important that in-vivo results follow in-vitro trends, as the relative difficulty, time, and expense are much higher for in-vivo testing. Two experiments, the serial removal of implanted filaments and the implantation of a pre-incubated filament, were run with the goal of correlating in-vitro release and mechanical data to in-vivo results. Samples of 506-L1 were implanted and removed after various time intervals. Direct measurements of filaments loadings may provide a better comparison to in-vitro data, since tear sampling provides an instantaneous measurement of release rather than information on the total amount released at any time. Drug retention in the filament was close to expected from in-vitro experiments. The filament starts at a loading of 46 mg/cm, but by one day the loading has fallen 39%. The filament loading then decreases steadily

so that only 13% of the initial drug remains by day 7. The average drug release in a given day (past day 1) is 3.5 μg/cm. This data correlates to the in-vitro data but does not provide perfect verification. First, drug levels remaining in the filament are uniformly lower than predicted by in-vitro experiments. Usually, the data points are off by 7-15%, but the loading at 3 days is a significant outlier beyond the normal deviation. These results may be skewed by the filament deforming during implantation and subsequent conditions in the eye. If the filaments are stretched, the linear drug loading may decrease. Assuming as little at 10-20% deformation brings most of the numbers in line with in-vitro experiments. A better method may be to mass the filaments and determine drug loading by mass fraction of drug, but with such small sections of filament implanted and recovered, the measurement of mass was not reliable. Overall, the filament drug loadings reinformced the overall trend of drug release and verified the amount of release/day from the filament.

The implantation of a filament that was pre-incubated in-vitro further shows the validity of the in-vitro models used for this system. The transient in-vivo tear concentrations for 506-L3 had the largest variation and thus made this filament the most appropriate one to investigate variation in the time dependent release properties. The pre-incubated filament has the same release profile as filament 506-L3 implanted without any incubation when adjusted two days to account for the overall incubation time. Moreover, the pre-incubated filament fails two days earlier than the un-incubated one.

Another significant benefit of the fibrous morphology of this system is the ease of removal after implantation. Since filaments maintain mechanical strength for several days, they could be

easily cut and removed should the patient experience adverse affects due to interactions with either the filament material or delivered drug. This ease of removal is a property of the bulk morphology of the device and the type of implantation. Since the ends of the filament, and a loop in the center, are exposed on the tissue surface, a physician would be able to simply cut the central loop and pull the entire device out by the ends. This procedure takes less than a minute to complete based on informal testing performed during these experiments. The ease of removal is in contrast to other systems, since fully implanted devices would require a second surgery to remove. Microparticulate devices are not easily removed due to the difficulty in finding and eliminating all particles when the devices are small and able to migrate.

4.6. *Conclusions*

We have developed biodegradeable filaments (PLGA) for controlled delivery of levofloxacin to the eye. Our initial goals of creating a device that will release effective levels of levofloxacin over 5-10 days have been achieved, with tear concentrations above 1 μg levofloxacin/g tear from the eyes of New Zealand white rabbits obtained for up to 7 days. The filament material is fully absorbable, and the total residence time of the device in the eye varies from 5-9 days, depending on formulation. We believe that this process for drug delivery can be applied to other drugs with different target release spans and that biodegradable filaments are an effective way of delivering therapeutics to the eye.

4.7. *References*

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Chapter 5: Wet-spinning of side-by-side multicomponent, drug containing filaments

5.1. *Abstract*

Side-by-side, multicomponent, polymer filaments containing either levofloxacin or dexamethasone are synthesized by wet-spinning and characterized to determine physical and drug release properties. Polymer and drug are dissolved in a water miscible organic solvent (DMSO) and side-by-side filaments produced by co-extrusion through a hypodermic Y connector into a water bath. The biodegradable polymers used here included three poly(dl-lactide-co-glycolide) (PLGA) copolymers (with 50:50, 75:25, or 100:0 ratios of lactide:glycolide) and polycaprolactone (PCL). Scanning electron microscopy and differential scanning calorimetry revealed that individual filament morphology and thermal properties are maintained in the bicomponent filaments. Filaments prepared from PCL and slower degrading 75:25 PLGA can mechanically stabilize a 50:50 PLGA component that would normally lose all strength over 7 days in PBS at 37ºC. Drug release from a bicomponent filament is an additive composite of the drug release profiles obtained from single component filaments, and multicomponent filaments can control the release of drug over much longer times than a monocomponent filament. Drug release from a tetracomponent filament is calculated based on the individual component drug release profiles and shown to be a good predictor of experimentally determined drug release.

5.2. *Introduction:*

Wet spinning has emerged as an attractive method for processing controlled release devices[1-5]. Wet-processed filaments have been used to control the release of both proteins and small molecule therapeutics. Previously, we demonstrated that a range of poly(d,l-lactide-co-glycolide) (PLGA) filaments could be prepared with encapsulated antibiotics and steroids, and these filaments were tested for ophthalmic applications via a rabbit eye model[4]. In-vivo sustained release of levofloxacin was demonstrated over a 7 day period, a time frame appropriate for post-surgical antibiotic delivery. In-vitro release and mechanical failure experiments correlated well to in-vivo results. While single component filaments have been demonstrated to show in-vivo function, their use as a versatile controlled release platform is limited by several drawbacks. These features include polymer dependent, triphasic release profiles (with an initial fast release, a plateau of slow release, and a second phase of degradation controlled release) and mechanical properties failure during the course of release. Since degradation controls long-term release in PLGA devices, faster degrading polymers release drug over a short period of time, while slower degrading polymers can exhibit long induction periods before the onset of release. The degradation also causes mechanical properties failure, so drug release and filament strength are strongly linked. To extend the utility of these filaments beyond the previously demonstrated applications and to prepare a more versatile controlled release filament-based platform, a method of processing filaments with multiple side-by-side layers was developed.

Multicomponent filaments have long been produced for commercial applications where one polymer type does not have all the necessary properties. These filaments have mostly been bicomponent, but systems containing more than two components have been reported[6]. The

production of bicomponent filaments for drug delivery has primarily focused on core-shell configurations. These filaments usually have drug contained in only one component. Some shell-core devices for drug release have a drug containing matrix surrounded by a structural shell, and are useful for applications like guided nerve growth[7-9]. Alternately, coatings have been made on structural cores[10-12]. A drawback to core-shell devices is that the rate of release from internal layers can be influenced by diffusion through external layers. Additionally, processing conditions for multiple concentric components become increasingly complex, and spinerette design can become limiting.

Side-by-side bicomponent configurations for controlled release are less represented in the literature, but several recent publicationa of side-by-side multicomponent spinning indicate that interest in making multicomponent fibers is increasing. Side-by-side examples mostly come from the field of electrospinning, where techniques originally described for the electrospraying of two compartment particles has been adapted to make fibers[6, 13-15]. Under certain conditions, bicomponent electrospun fibers can separate during formation[15] with the resulting fiber mat being a mixture of the two fiber types rather than a single filament having a split composition. At least one previous example of a drug-loaded bicomponent fiber has not shown a release profile that was a composite of the single components, but instead had properties dominated by the most abundant component[14]. Recently, Lahann and coworkers have electrospun multicomponent polymer fibers where the chemical compositions of the different compartments are distinct[6]. These fibers were made from polymers relevant to drug delivery with encapsulated fluorophores, but release profiles for these devices were not reported. These

filaments were based on earlier work by the same group on electrospraying of multicomponent particles[16, 17].

Here, we describe a simple method of producing wet-spun, multicomponent filaments. The described filaments are mostly bicomponent, but it is shown also how this method can be extended to accommodate more than two components. We show that the release, thermal, and mechanical properties of each individual component are retained and decoupled from other component properties. Using a side-by-side spinning method, a filament can be produced that has properties that can be tailored to a particular application.

5.3. *Materials and Methods*

5.3.1 Materials

Levofloxacin, dexamethasone, polycaprolactone (MW 80,000) and poly(d,l-lactide) (PDLLA, MW 75,000-120,000) were purchased from Sigma-Aldrich. Poly(d,l-lactide-co-glycolide)'s were purchased from Boehringer Ingleheim. The PLGA's in this study were Resomer RG 506 (50:50 lactide:glycolide, i.v. of 0.82 dl/g) and Resomer RG 756 (75:25 lactide:glycolide, i.v. of 0.80 dl/g). DMSO, acetone, HCl, and NaOH were obtained from VWR.

5.3.2 Multicomponent filament extrusion

Single component filaments were prepared as previously reported[4], while multi-component filaments were made by a modification of this method. Polymer and drug were dissolved in a common solvent, with solution compositions shown in

Table 5.1. Solutions were loaded into glass syringes fitted with 22 gauge flat-tipped needles. For muticomponent filaments, syringes were connected to 22 gauge hypodermic Y-connectors (Small Parts Inc.) by Teflon tubing. Component solutions were extruded in equal amounts. Filaments were extruded into a coagulation bath of water at 20°C and taken up on a bobbin with a draw ratio of 1.2. The flow rate out of the spinneret was 70 ml/min for all filaments and coagulation time was 45 s except where noted. Schematics for the combination of polymer streams for bicomponent and tetracomponent extrusions are shown in Figure 5.1.

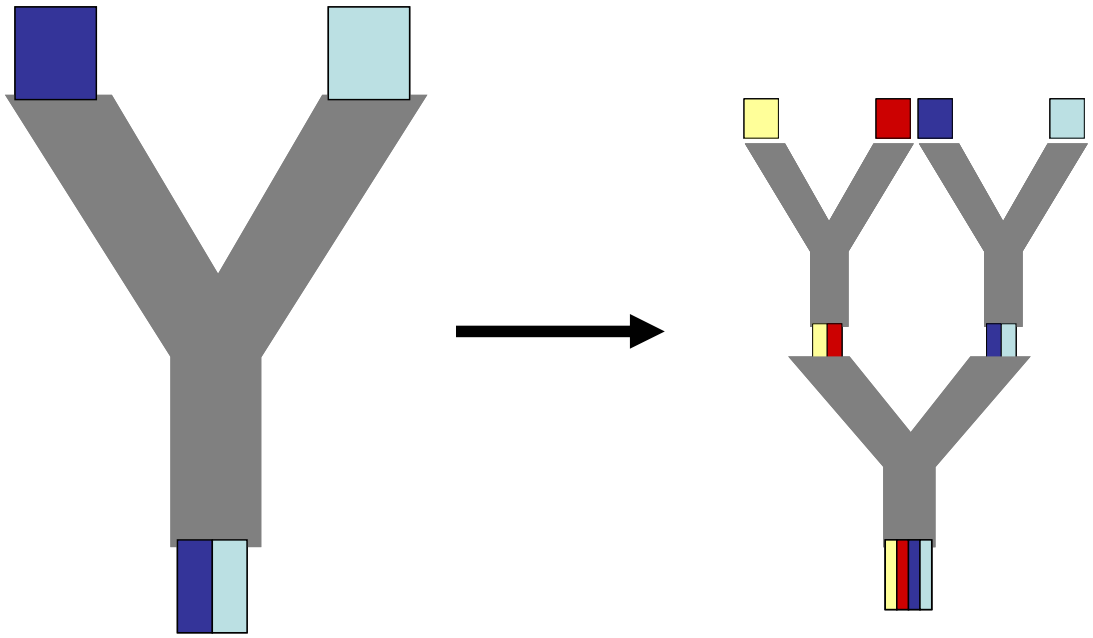

Figure 5.1. Schematic of combining streams for a multicomponent filament.

Table 5.1. Solution contents and compositions.

				Solution Composition (%wt)		
Solution	Polymer	Drug	Solvent	Polymer	Solvent	Drug
A	RG 506	Levofloxacin	DMSO	23.3	69.8	7.0
B	RG 506	Levofloxacin	DMSO	22.2	66.7	11.1
C	RG 506	Dexamethasone	DMSO	23.3	69.8	7.0
D	RG 756	Levofloxacin	DMSO	23.3	69.8	7.0
E	PDLLA	Levofloxacin	DMSO	23.3	69.8	7.0
F	PCL	-	Acetone	30.0	70.0	0.0
G	RG 756	Dexamethasone	DMSO	23.3	69.8	7.0

5.3.3 Drug Loading

Drug loading was determined by dissolving a measured length of filament in 1 M NaOH. The solution was neutralized with 1 M HCl and diluted to an appropriate concentration for analysis in PBS. Drug concentration in the resulting solution was determined by HPLC using a C18 column with a mobile phase of 49.95% water, 49.95% acetonitrile, and 0.1% trifluoroacetic acid.

Levofloxacin was detected by fluorescence, with an excitation of 292 nm and emission of 494 nm. Dexamethasone was determined by UV absorbance at 254 nm.

5.3.4 DMSO content

DMSO content was determined by NMR using an internal standard. An accurately measured section of filament (approximately 10 cm) was dissolved in d-chloroform (approximately 1 g, accurately measured) with an internal standard of 1% trimethoxysilane. The proton peak for DMSO was compared to the internal standard to give the concentration of DMSO in the sample.

5.3.5 Drug Release

Release studies were performed in PBS at 37°C on an incubating rocker. Sections of filament measuring 2.5 cm were placed in 1.5 mL Eppendorf tubes, to which was added warmed PBS. At each time point, the PBS was removed for analysis and replaced with fresh PBS. Drug concentration was measured using the above HPLC method.

5.3.6 Differential Scanning Calorimetry

Differential scanning calorimetry was performed, with selected samples cycled three times from -50 to 80°C at a heating rate of 5K/min. Samples were saturated with water, as previously described[4], to allow the separation of transition temperatures for multicomponent filaments.

5.3.7 Filament Imaging

Bulk fiber morphology was observed by light microscopy and scanning electron microscopy (SEM). Light microscopy was performed using a Micromaster I light microscope (Fisher

Scientific, Pittsburgh, PA) at 10x magnification. The size measurements were calibrated using known diameter wires (Small Parts Inc.). For SEM, samples were coated with Pt/Pd and magnifications up to 400x were possible without destroying the samples.

5.3.8 Mechanical Testing

Load vs. strain curves for the filaments were collected on an Instron 1150 mechanical properties testing apparatus. Experiments were run under ambient conditions, where the temperature was 22 ± 2°C with relative humidity of 50 ± 10%, and under incubated conditions, with the filament submerged in PBS at 37°C. The samples were drawn at a strain rate of 1 %/s and the resulting tensions were recorded. The loads that the filaments were bearing at the time of breakage were recorded, as were the maximum elongations of the filaments.

5.4. *Results*

5.4.1 Filament Morphology

In Figure 5.2, filaments prepared from solutions A and B (denoted as filaments A and B, respectively) are shown with a bicomponent filament of AB. Discrete drug particles are present in filament B, while filament A has a rough surface without large protuberances. Filament AB (cross section in Figure 5.2(d)) clearly shows two different morphologies, one side containing drug particles (Figure 5.2(c), right) and the other without (Figure 5.2(c), left). These images suggest that morphology of an individual component is retained during multicomponent spinning.

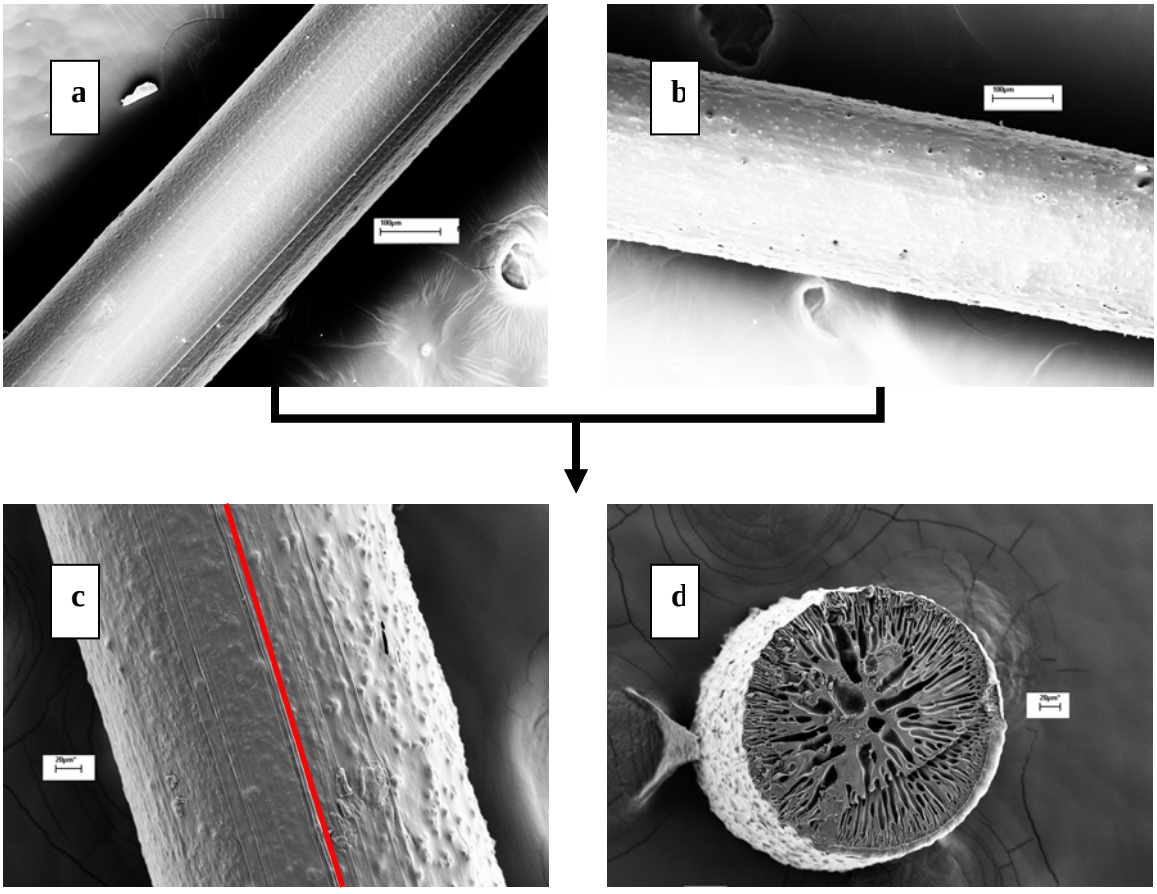

Figure 5.2. SEM images of filaments A (a) and B (b) as well as filament AB (c) and (d).

5.4.2 Filament Formation

Data for drug and DMSO retention vs. coagulation time are shown in Figure 5.3 for filament AC as well as single component filaments A and C. Filament AC is a combination of a levofloxacin containing component (A) and a dexamethasone containing component (C) with both components containing the same polymer (RG 506). Dexamethasone is highly retained while levfloxacin loading steadily decreases with coagulation time. The retention of drug during bicomponent filament extrusion is almost identical to the individual retention profiles for single component filaments (Figure 5.3(a)). DMSO is rapidly eliminateded from all filaments (Figure 5.3(b)).

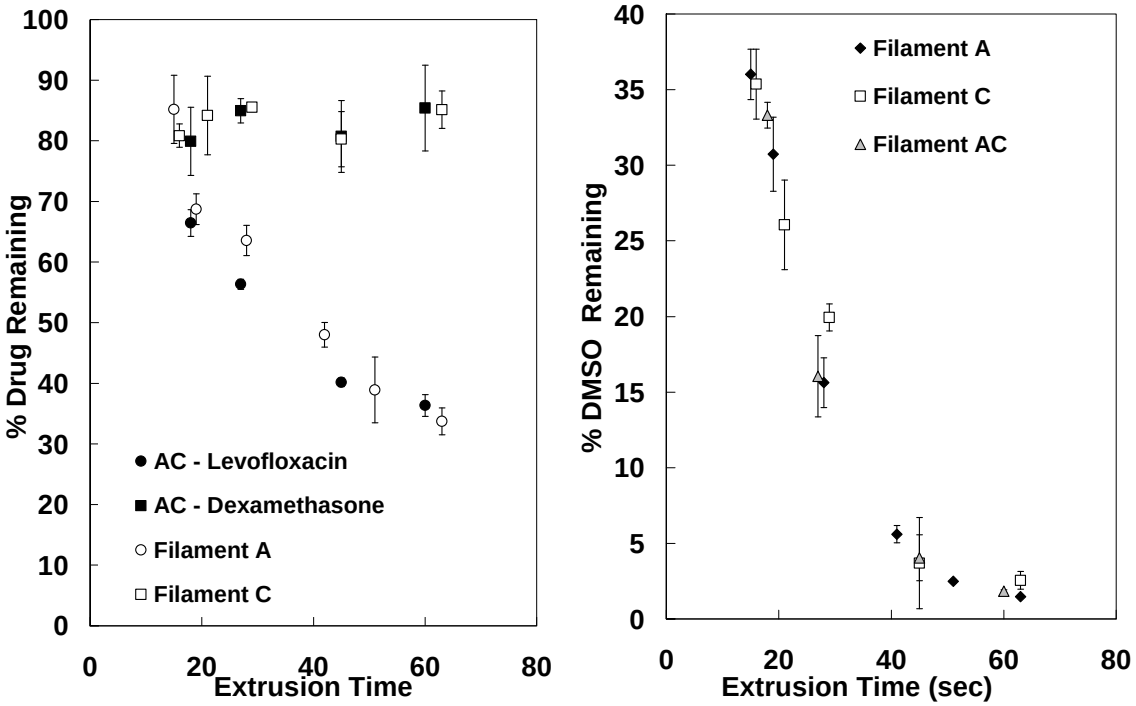

Figure 5.3. Drug (left) and DMSO (right) retention during extrusion.
Profiles for the single component filaments A and C and the bicomponent filament AC are shown.

5.4.3 Drug Release

Drug release profiles for several bicomponent filaments are illustrated in Figure 5.4. In Figure 5.4(a), a component containing fast-releasing drug particles (B) is combined with a homogenous component (A) to achieve a profile that has a large, fast initial release but still retains drug for sustained release. Figure 5.4(a) is shown with a y-axis of total drug released/cm filament to highlight the differences in drug loading. Figure 5.4(b) shows release from filaments A, C and AC. The release profiles of individual components are retained by the bicomponent filament, which releases both levofloxacin and dexamethasone according to the single component profile. This method can also be used to release a single drug over an extended period of time. Figure 5.4(c) demonstrates how a combination of a quickly degrading component (RG 506 in filament A) and a slowly degrading component (RG 756 in filament D) can be combined to achieve a composite release profile for levofloxacin.

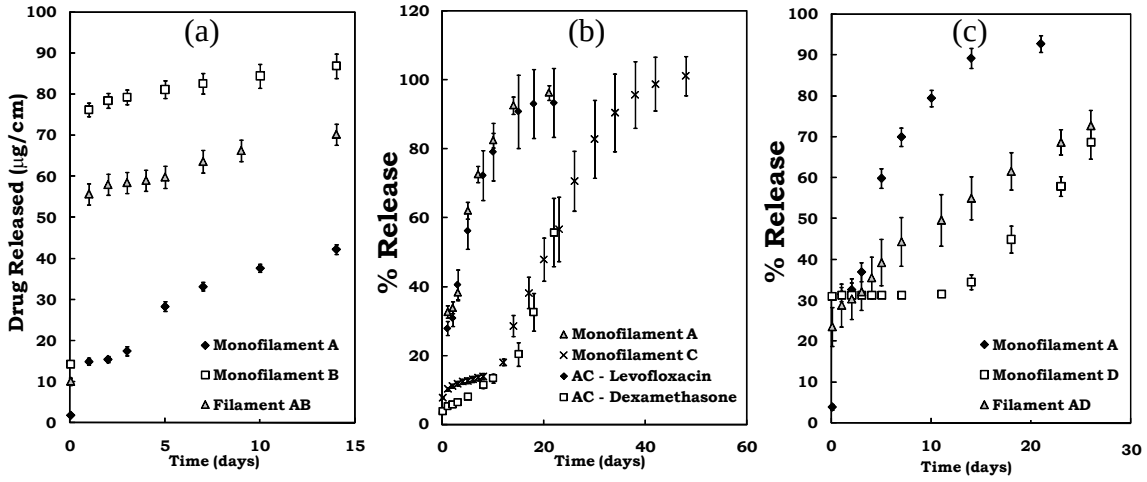

Figure 5.4. Drug release profiles for bicomponent filaments and monofilaments made of their individual components. Bicomponent filaments are shown along with filaments made from each of the individual components for different (a) drug forms, (b) drug types, and (c) polymer types.

5.4.4 Thermal Properties

DSC measurements of bicomponent filaments reveal little if the PLGA type was the same for both components. If the polymers were different in the different components, multiple transitions were observed. For filament AF, the T_m of PCL and the T_g of PLGA could be separated by the addition of H_2O to the sample crucible (Figure 5.5(a)). Samples of water saturated PCL show a T_m at 57°C, while PLGA has a T_g at 39°C. Bicomponent filament AF showns both transitions, indicating that the polymer phases are retaining their individual thermal properties. Both transitions can be seen for filament AD as well (Figure 5.5(b)), but the results are less clear since the glass transitions of the two PLGA copolymers overlap.

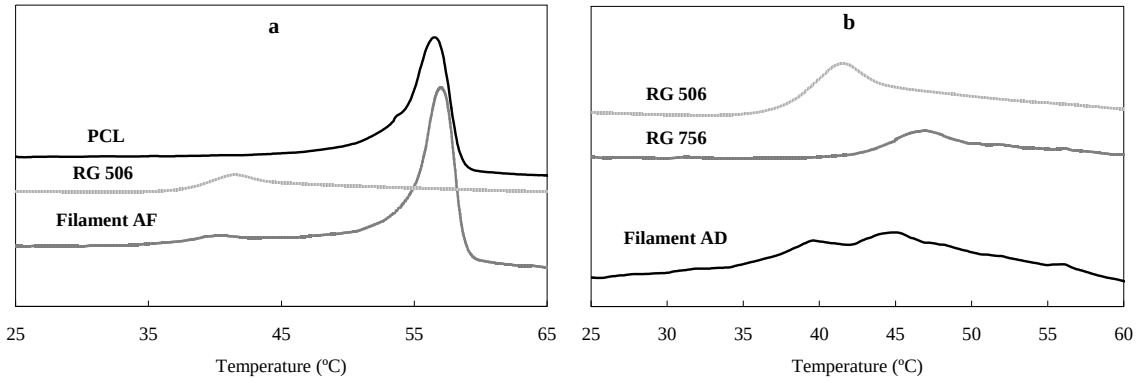

Figure 5.5 DSC traces of multicomponent filaments (a) AF and (b) AD along with their individual polymer components.

5.4.5 Mechanical Properties

Filament AF was prepared to have a structural PCL component with a PLGA component loaded with drug. Figure 5.6(a) shows the maximum load over time at a strain rate of 1%/s, when tested under PBS at 37°C. The PCL component of the filament provides mechanical stability for longer than PLGA alone. When samples of filament AF are dried and tested in air, the

individual breaking points of the components can sometimes be observed. Figure 5.6(b) shows the stress-strain curve of a sample of AF that was freeze dried after incubation in PBS at 37°C for one date to eliminate residual solvent. The PLGA phase breaks at a strain of 210% while the PCL component does not break until an elongation of 740%.

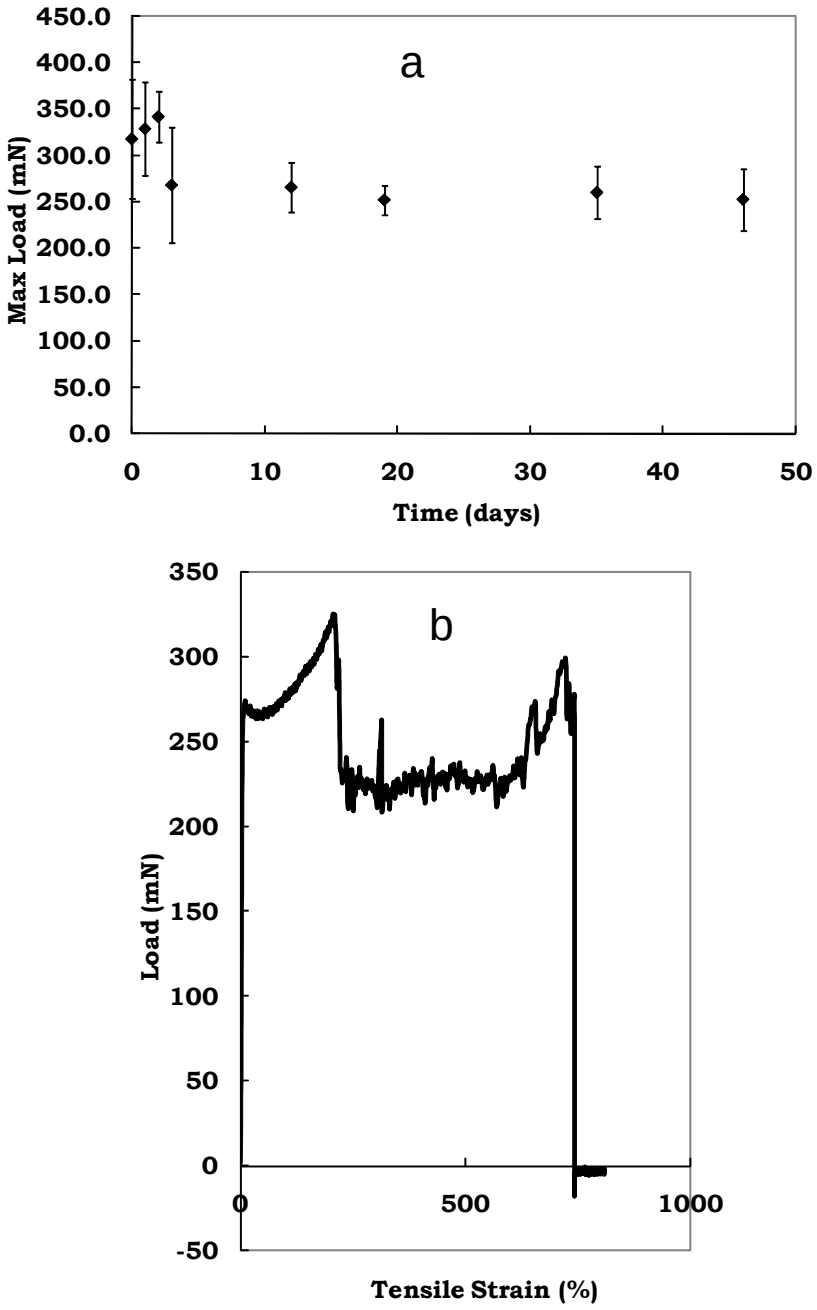

Figure 5.6: Mechanical properties of filament AF, a bicomponent PCL/PLGA filament. (a) shows that the strength of AF under incubation conditions over several weeks while (b) shows a load-strain curve of a dry filament.

Another example of mechanical decoupling could be seen from filament AD (Figure 5.7). Filament A loses strength rapidly, with total mechanical deterioration happening by day 7 but

filament D lasts for 2-3 weeks (Figure 5.7a). Filament AD has a combined profile, showing attributes of each monocomponent filament. Since filament D has a much higher maximum load after the first day, the mechanical properties of AD are dominated by the D component. Filament AD has two breakage events for the first several days of incubation, with each component breaking at different strains (Figure 5.7b). Fig. 6a shows both breakage events at day 0 and 1 for filament AD.

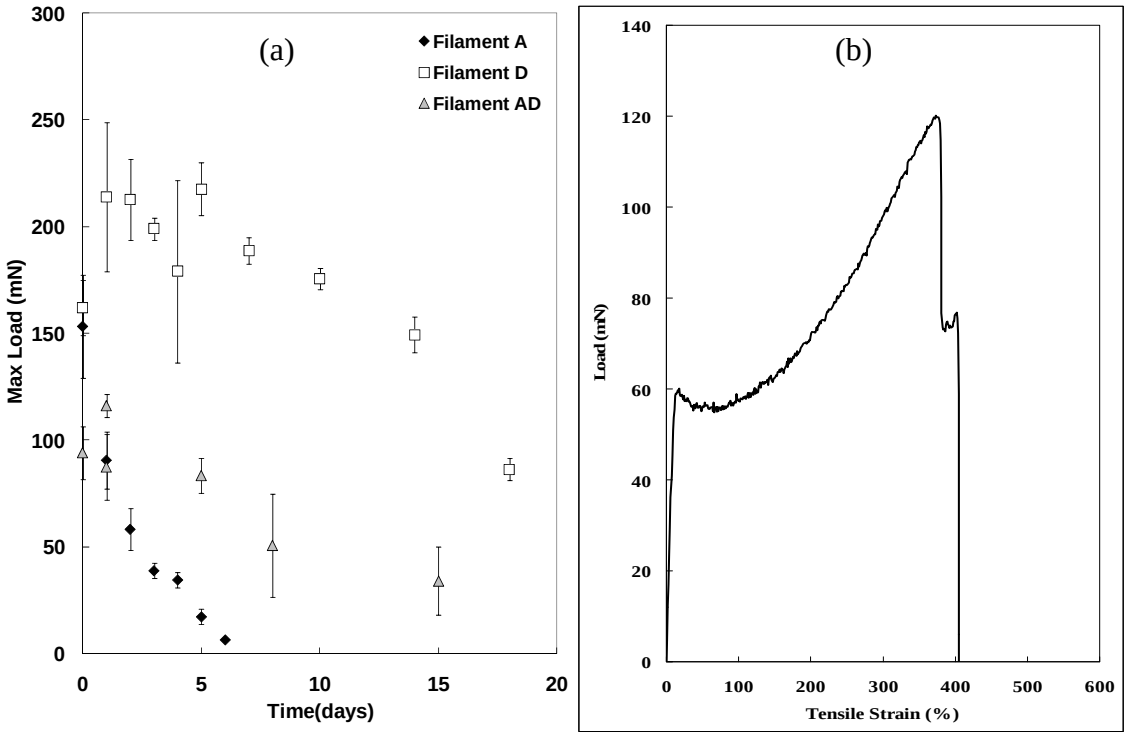

Figure 5.7. Mechanical properties of filament AD. The maximum filament load over time for filaments A, D, and AD is shown (a) as well as a load/strain curve for AD at a strain rate of 1 %/s (b).

5.4.6 Tetracomponent Filament Release

A tetracomponent filament was prepared by combining two bicomponent polymer streams. This filament is composed of components A, D, E, and G. Levofloxacin release from components A,

D, and E is shown in Figure 5.8(a) compared to the release predicted by averaging the release of the three monofilaments. This filament has three levofloxacin containing components, each formulated with a different PLGA copolymer. Predicted release values were determined by adding the amount released from each single component filament at each time point and dividing by 3. One phase is composed of 75:25 lactide:glycolide PLGA with dexamethasone (G). The release of dexamethasone is independent of the levofloxacin release, and consistent with the release profile of monocomponent filament G (Figure 5.8(b)).

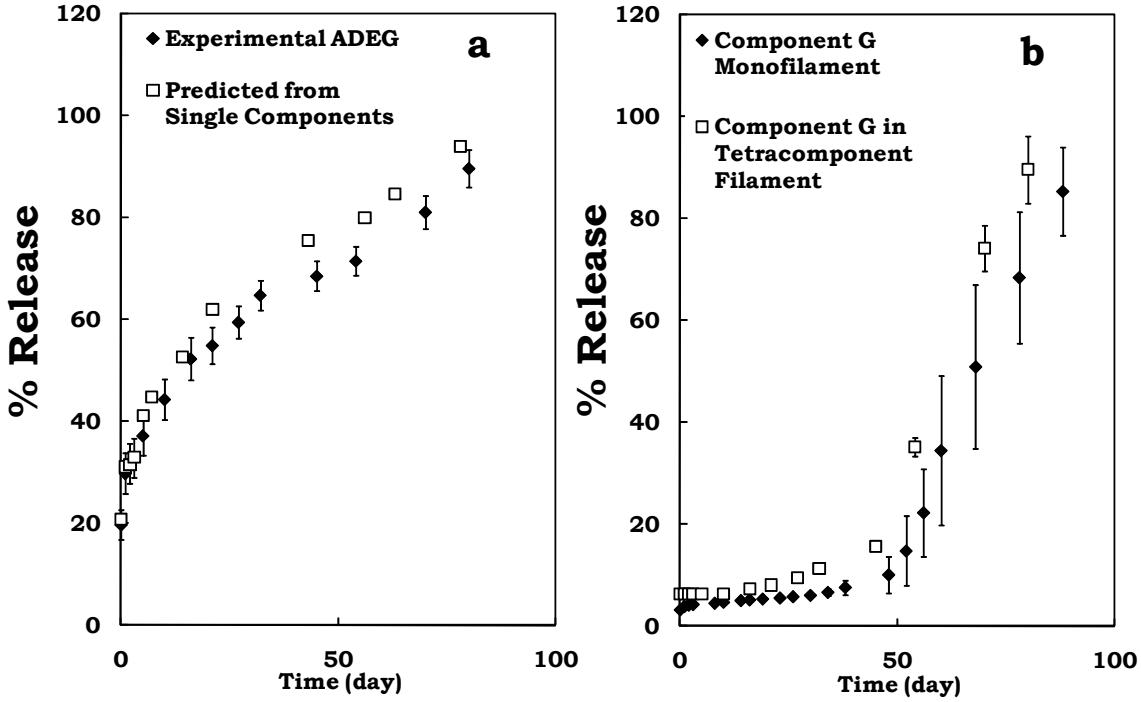

Figure 5.8. Drug release from a tetracomponent filament showing (a) levofloxacin and (b) dexamethasone release.

5.5. *Discussion*

Previously, we reported the formation of drug loaded filaments for the controlled release of drugs in the eye[4]. The data reported show that drug release and mechanical properties could be tuned. However, these filaments have several limiting characteristics. First, release profiles were triphasic, that is, they have an initial period of fast release, a plateau of slow release, and a final region of polymer-dependent, degradation controlled release. Second, a polymer chosen for a particular release profile may not have appropriate mechanical properties for a desired application. Finally, since polymer degradation facilitates both release and mechanical failure, filaments lose strength before complete release is achieved. Release and mechanical strength could not be independently tailored. Here, we describe the combination of multiple filament properties into a single device using side-by-side multicomponent spinning. In order for this methodology to be of value, the components of the filament must have decoupled properties so that each individual component retains the functionality of a single component filament of equivalent composition. Previously, we showed that for single component filaments, in-vivo performance correlated well to in-vitro results. Many of the same experimental techniques are used here to provide insight into the likely in-vivo performance of the multicomponent filaments.

During the extrusion process, drug retention varies depending on drug type. Dexamethasone content drops to approximately 85% of initial drug level over the first 15 seconds of coagulation, but is retained at the same level for longer coagulation times. Levofloxacin content drops steadily during coagulation, with 90% retention after 15 s decreasing to 37% after 60 s. DMSO is rapidly eliminated from both filaments in a drug-independent manner. A bicomponent

filament prepared by joining dexamethasone and levofloxacin loaded phases displays the same formation mass flux characteristics as the individual components (Figure 5.3). This result allows for the same high dexamethasone retention and moderate levofloxacin retention that is seen in individual filaments to be incorporated into a bicomponent filament.

After formation, each individual component largely retains the properties of a single component filament of that composition. The morphology of the components is retained as shown by the images in Figure 5.2. As previously reported, a filament spun from a solution containing precipitated drug particles had discrete protuberances on the filament surface. A filament spun from a homogenous solution of drug and polymer has a rough surface, but discrete particles are rarely observed. A bicomponent filament comprising a particle containing component and a homogenous component has split morphology. Under SEM, half of the filament has observable particles, while the other half does not (Figure 5.2(c) and 1.2(d)). In addition, there is a clear dividing line between the two components, indicating that each component is retaining specific morphology based on the physical characteristics of the polymer/drug solution. Other filaments can be prepared where the morphologies of the different phases are difficult to observe, e.g. different PLGA copolymers are not differentiated by SEM.

Thermal and mechanical properties of the individual components are also decoupled in a bicomponent filament. Thermal transitions of each component are retained (Figure 5.5), with the best example of this being filament AF. The observation of the individual components is possible in AF since the melting point of PCL is separated from the glass transition of RG 506 by 18°C. The glass transitions of the various PLGA copolymers are very close to each other

(maximum separation of 9°C) and the DSC traces of the transitions tend to overlap for these bicomponent filaments. Filament AD shows a dual glass transition, but the difference between the glass transitions of the two PLGA copolymers is not large enough to fully separate. The result is a broad, “two-humped” transition that suggests separation between the two components, but is not conclusive.

Filaments made of component A only lose mechanical strength rapidly, and become unstable after only 7 days of incubation in PBS at 37°C[4]. Figure 5.6 and Figure 5.7 demonstrate how a component made from a different polymer can elongate the mechanical life of a component spun from solution A. Filaments AD and AF were formulated to stabilize component A with a second component with a slower degrading polymer. Filaments AD and AF shows two breakage events at the early stages of testing, but after 5 days shows an additive effect between the two components, with the longer-lasting component (D) dominating the mechanical behavior. Because PCL degrades much more slowly than PLGA, component F can mechanically stabilize filament AF. A PCL component allows the retention of mechanical strength over long time lengths. Ultimately, the PCL component will degrade. However, filament AF is promising because it shows that mechanically stabilizing components can be used to elongate the lifetime of a drug releasing filament.

Filaments made from single components display a characteristic, triphasic drug release profile when using PLGA. One goal here was to combine individual component release profiles by multicomponent spinning to better control the drug release from a single filament. Precise control of drug release can be achieved if release from one component is decoupled from the

release of a second component (as demonstrated in Figure 5.4). In other words, release from any individual component should not be affected by the release from neighboring components. Decoupling of release is especially important since previous bicomponent filaments have not necessarily shown a combined release profile[14]. The release of levofloxacin and dexamethasone can be independently controlled from a single filament. This could allow for the release of different therapeutics at different rates, depending on the necessary time course for a particular drug. An example of this may be after surgery, where a short time course of antibiotics may want a short period of antibiotic release to prevent immediate infection, with a slower period of steroids to prevent inflammation as the surgery fully heals. Alternately, steroids or other anti-inflammatory drugs could be released throughout the lifetime of any degradable filament to prevent inflammation associated with device degradation. For a single drug, fast-releasing components (component B with large drug particles) can be combined with slower-releasing components. This leads to the therapeutically desirable profile of fast initial release followed by a long, sustained period of slow release.

Different components of different polymer compositions have additive release profiles, allowing extended release of a single therapeutic. This is a critical finding since single component filaments display a triphasic release profile that does not allow for extended, sustained release of a drug. Polymer-dependent release from various components can be combined to give a pseudo-first order release profile over a desirable time course. The plateau region of the polymers could be manipulated to delay release until long after device implantation.

The fabrication of a tetracomponent filament demonstrates the generalization of the methodology beyond bicomponent devices. Figure 5.8 demonstrates how levofloxacin can be released continuously over two months, while dexamethsone release is controlled from days 30 to 60. Filament A releases most of its drug over 2 weeks, D releases between days 14 and 40, and E releases between days 40 and 80. Because drug is split 1/3 in each phase, the timeframe for release is skewed slightly towards earlier times. In future filaments, the release could be smoothed by using more components or having some components contain a larger fraction of the total drug than others. Release of dexamethasone from component G is unaffected by the three levofloxacin containing phases and follows the same release profile as monofilament G. A wide variety of PLGA copolymers, of varying lactide to glycolide ratios, are available commercially so this multicomponent spinning technique can easily be extended to provide many different drug release profiles.

Wet spinning is a useful way of processing drug containing filaments and has been used in recent studies for applications including tissue engineering[3, 9], cancer therapy[2], and ophthalmic drug delivery[4]. These devices all employed slightly different processing conditions, with some being prepared from homogenous solutions, some from suspensions, and some from emulsions. Here, we describe a way of furthering these products by a simple combination of polymer streams to fabricate multicomponent filaments. The properties of the individual components are decoupled from the properties of other components and can be used to tune the specific release and mechanical properties of a device. We believe that this method can be useful to a wide range of wet-processed filaments and can be applied to many different biomedical applications.

5.6. *References*

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Chapter 6: Conclusions and Future work

The overall objective of this thesis was to produce, by wet-spinning, a class of biodegradable filaments that had mechanical properties suitable for implantation into the eye and could deliver drugs over a long period of time. In the introduction to this thesis, several objectives were laid out. An analysis of success in meeting these objectives, along with some conclusions drawn from achieving these objectives, are summarized below.

6.1. *Conclusions*

6.1.1 Wet-spinning is a useful and flexible method for encapsulating drugs in biodegradable polymer filaments

Our first objective was to develop a procedure for encapsulating therapeutics by wet-spinning. The bench top scale procedure that we ended up using adequately allowed for variation in coagulation time and was sufficient for producing a wide range of filaments. One of the greatest benefits of using a syringe pump based extrusion setup, as opposed to some commercially available wet-spinning module, was that low volumes of solutions could be used. Since a variety of filaments were produced from expensive starting materials (drug and PLGA), it was necessary to use as little material as possible for each extrusion. For most extrusion, less than 2 grams of total material (polymer, DMSO, and drug) was necessary to produce a length of filament sufficient for release, thermal, mechanical, and compositional analysis.

6.1.2 Solubility of drug in solvent/antisolvent system is critical for drug encapsulation and controlled release

A key objective was to determine what system parameters lead to highly loaded filaments that can control the release of therapeutics. In chapter 2, the drug encapsulation process during wet-spinning was examined while chapter 3 explores how formulation affects drug encapsulation and release. Dexamethasone, which is almost 50 % soluble in DMSO, can be efficiently loaded into filaments relative to levofloxacin. When 7 % drug solutions are coagulated, dexamethasone is highly retained after an initial loss of ~10 - 20 % while levofloxacin is steady depleted, resulting in only ~30 - 40 % retention after 60 s. The levels of drug retention were found to be linked to solution-state properties, as solid-state solubility was too low to justify the levels of drug loading. Furthermore, it was found that if a formulation of 0.7% levofloxacin was used (a drug concentration small enough to ensure that levofloxacin stayed dissolved during polymer coagulation), then high loading (~80 %) could be achieved.

Whether drug is dissolved or suspended proved to be important to release properties as found in chapters 3 and 4. Filaments formulated from suspensions of levofloxacin had higher drug loading, but most of the therapeutic was released over a single day, in-vitro and in-vivo. This demonstrates the necessity of drug dissolution during formation.

Solid-state solubility was also not a good indicator of controlled release from the filaments. Crystalline drug was detected for both levofloxacin and dexamethasone after extrusion and drug loadings were significantly higher than solid state solubility. For a levofloxacin filament, crystalline drug could not be detected after one day of release, suggesting that only drug that is amorphously trapped in the polymer matrix is released in a controlled fashion. But this

conclusion is directly contradicted by dexamethasone release, where crystalline drug is detected for at least 25 days.

6.1.3 Swelling with water dominates in-situ properties of filaments

It was important to determine if any entrapped chemical species changed the properties of the filaments and to determine what aspects of the chemical composition is most important, especially for mechanical properties. This is especially true for a wet-spun filament, where residual solvent will always be present to some extent. Moreover, some drugs are known to plasticize polymers used for controlled release[1]. For the filaments in this study, levofloxacin and dexamethasone did not seem to have any effect on glass transition temperature. Drugs had some effect on mechanical properties but, with the exception of a filament loaded with 43% dexamethasone, these differences were small. The highly loaded filament probably only had different properties due to the sheer amount of solid drug in the filament, not due to drug interacting with the polymer. Residual solvent did have an effect on both thermal and mechanical properties, lowering T_g and making the filaments easier to deform.

6.1.4 In-vivo tear concentrations correlate to in-vivo drug release

In-vitro studies of drug delivery devices are of very little use unless the in-vitro data correlates well to in-vivo results. Implantation technique, meaning filament placement and method of securing, had a large impact on the detection of levofloxacin in the tears. Eventually an implantation technique was settled upon, but this experience is of note because it exemplifies how in-vivo methodology can be as important as in-vitro experimentation. In the end, the

filament had to be in contact with the tears and the end had to be knotted, but if several iterations of implantation had not been attempted, the conclusions from the animal experiments could be greatly different. Once an implantation technique was developed, several different experiments were run in-vivo to examine drug and these experiments largely confirmed the in-vitro results. The correlation between in-vivo and in-vitro drug release justifies the remaining in-vitro experiments, even for filaments that were not implanted. In-vitro experiments can now be run on different filament formulations and designs, with confidence that these experiments will translate well into in-vivo results.

6.1.5 Proper mechanical testing mimics in-situ conditions

The mechanical properties of the filaments were just as important as the release properties, as filaments that broke after in-vivo implantation were usually expelled from the eye soon thereafter. Again, in-vivo implantation technique was critical to understanding in-vitro experiments. Filaments implanted by technique #2 in chapter 4, without secured ends, would sometimes last several days, while others would fall out almost immediately. This data seemed to contradict in-vitro experiments, which showed that filaments gradually lose mechanical strength. The solution to the problem was to tie the ends of the filament. This solution ended up demonstrating how in-vitro experimentation can lead to in-vivo innovations, as the discrepancy between in-vitro and in-vivo results led to a change in implantation technique. In-vivo results led to a re-evaluation of in-vitro methods as well. Originally, filament mechanical properties were tested under ambient conditions, but it was noticed that filaments would stretch and comply more with movement while implanted in rabbit eyes. This observation led to the filament

mechanical tested under PBS at 37°C and very different mechanical properties results were achieved.

Side-by-side multicomponent spinning can be used to combine the properties of individual filaments

Single component filaments had two limitations, polymer dependent drug release and mechanical failure before full drug release, that were addressed by using a side-by-side, multicomponent spinning technique in chapter 5. The individual properties of the components were retained during spinning, allowing a combinatorial approach to creating new devices. Release properties could be predicted from single component filaments and mechanically stable components could be used to make filaments with long life spans. Side-by-side wet-spinning has now been shown to be a promising platform technology that can be potentially extended to many drug release applications.

6.2. *Future work*

6.2.1 Exploration of bath-side conditions during wet-spinning

One feature that sets wet-spinning apart from other manufacturing processes for drug containing polymer devices is the coagulation bath. For melt, compression, and solvent evaporation processes, the external environment is typically air (or some other gas) that does not allow for drug egress from the device. In-situ forming depot solutions do lose drug to their surroundings, but the external conditions are set by physiology, and are not easily manipulated to control drug release[2]. For wet-spinning, the outer conditions of the filament can be controlled. Using the

techniques described in Chapter 2, the coagulation process can be examined with various external conditions.

Sometimes, solvent is added to a coagulation bath to slow the precipitation of polymer in the bath[3-5]. Solvent content in the coagulation bath is known to affect the internal morphology of the filament and to change the mass transport properties of solvent during coagulation[4]. It is unknown at this time how changing the kinetics of polymer coagulation will affect drug retention for drug containing filaments, since it was shown that drug content had little effect on DMSO/water transport rates and only pure water baths were used in these studies. Since close to full solvent removal is desirable for a final product, multiple baths with progressively lower solvent content may ultimately be used if solvent presence in the bath gives any advantage for drug loading or release properties. Having solvent in the coagulation bath may also help to provide insight into the early stage of coagulation. It was mentioned in Chapter 2 that early time points for filament coagulation are hard to obtain, since the bath residence times are so short and the product is unstable once removed from the bath. Instead of trying to obtain very short coagulatioin time points, it may be more convenient to slow down the solvent/antisolvent diffusion process by adding solvent to the coagulation bath. This could be particularly useful in understanding how water moves from the bath to the protofilament during the early stage of coagulation.

Bath conditions, like pH and temperature, may affect drug encapsulation during wet spinning, depending on drug type. Charged drugs, like levofloxacin, are often pH sensitive. Levofloxacin is more soluble at high and low pH than at neutral pH, with a maximum solubility reported at

~pH 6. Commercially, both as pill and eye drop, levofloxacin is often sold as a salt with HCl to improve the solubility in aqueous media. Based on what was learned in Chapter 2, changes in solubility of drugs in DMSO/antisolvent could improve drug loading. The drawback to this approach could be the acidification of the polymer matrix, which would speed polymer degradation. Temperature control in the bath could be interesting to study, particularly since temperature should affect the solubility properties of both drug and polymer, and can influence the diffusion coefficients of DMSO and water[6]. Bath temperature may not make any difference in the early stage if the temperature considerations are dominated by the heat released by mixing DMSO and water. In later coagulation, it would be interesting to study filament formation above the glass transition temperature of PLGA in water. Having a non-glassy polymer during all stages of filament formation would have a significant effect on the thermodynamics and kinetics of solvent and antisolvent flux, but the effect on drug loading is not known.

Placing additives in the coagulation bath that directly affect the solubility properties of the formulated drugs is another path that deserves attention in the future. Drugs are often formulated with excipients that change the solubility of the drug in a particular media. One example is hydrophobic ion pairing (HIP), where a charged drug is prepared with a hydrophobic molecule of opposite charge. HIP has been used to increase drug loading in controlled release devices[7, 8] and to increase ophthalmic bioavailability by making a drug more likely to partition through the cornea[9-12]. Use of HIP in the coagulation bath may change the solubility of drugs in a way that would encourage drug loading. A major problem could be that HIP agents are typically acidic or basic, which could cause polymer degradation for PLGA.

Cyclodextrins are cyclic oligosaccharides that form a cup with a hydrophobic interior and hydrophilic exterior[13]. Cyclodextrins can be used to increase the water solubility of small, hydrophobic molecules, such as steroids[14-16], chemotherapy agents[17-20], and poorly soluble antibiotics[21, 22]. During the course of our investigations, cyclodextrins were examined to increase the solubility of some drugs in DMSO (data not shown). This route was ultimately abandoned due to the mass considerations, since cyclodextrins have a molecular weight of ~1200 and drugs ~300-400, even a 1:1 cyclodextrin:drug pairing means that most of the therapeutic mass comes from the cyclodextrin. Even though cyclodextrins were not practical for most formulations, they could be of use on the bath side of coagulation given what was learned in Chapter 2. Several modified β-cyclodextrins (cyclodextrins with 7 sugar units) are highly soluble in both DMSO and water. These could be added to the water bath to keep hydrophobic drug solubilized during polymer coagulation. If benefit was found, this could open up a whole new line of research, as transport of cyclodextrin, cyclodextrin/drug affinity, and cyclodextrin/PLGA interactions would all become important.

6.2.2 Filaments for glaucoma therapy

One drug that is promising for filament encapsulation by our described technique is latanoprost, a prostaglandin analogue used to decrease intraocular pressure for the treatment of glaucoma[23]. Glaucoma is an excellent target for a controlled release therapy, since daily medication is required and the symptoms of skipped doses are not usually discernable for a patient. It was for these reasons that the Ocusert was developed for pilocarpine release.

Latanoprost is an extremely potent drug, with eye drops containing only 0.005% drug instilled a single time each day[24]. This means that the average patient is receiving only ~1.5 μg/eye/day of drug. This means that, for a 10 cm implant over 6 months, a drug loading of ~27 μg/cm would provide the same amount of drug as drops over that time span. This level of drug loading was achieved and surpassed for both levofloxacin and dexamethasone. If higher drug loading was possible, the length of implanted filament could be shortened. Replacing daily drops with a twice yearly implant could be of significant value for glaucoma patients and could be worth the temporary discomfort associated with implantation surgery.

It was shown in Chapter 5 that side-by-side, mulitcomponent spinning could provide drug release over extended periods of time. Specifically, levofloxacin was released over three months at a relatively steady rate. The encapsulation and release of latanoprost over 6 months would be an extension of the current technology, but also could bring up new difficulties. First, new polymers may have to be used to control release, but this barrier can probably be addressed by choosing from the wide range of commercially available controlled release polymers. A greater problem could be drug stability during release. A 6 month target for release means that significant drug degradation may occur and could undermine the efficacy of the latanoprost. This could be especially true since latanoprost contains an ester bond, which could potentially cleave by hydrolysis. However, various cyclodextrins have been shown to stabilize prostaglandin analogues and specifically slow the rate of ester bond hydrolysis[25]. In fact, a cyclodextrin containing formulation has been shown to essentially halt degradation of latanoprost over at least 3 months at 40°C[26].

A new animal model will have to be developed in order to fully explore the potential of filaments as anti-glaucoma implants. Release from latanoprost in an in-vivo model is exciting because the efficacy of the drug can be monitored by measuring intraocular pressure. For drug concentration, a commercially available latanoprost ELISA detection kit can be purchased with sensitivity on the order of ng/mL, so both effect and drug concentration can be measured at the same time. The major problem with model development is animal species. Rabbits, typically used for ophthalmic models and used in our study, do not show decreased intraocular pressure after latanoprost administration[27]. This would necessitate the use of dogs, cats, or another animal type for in-vivo trials, a significant step. Animal model development is typically a slow, laborious process involving repeated in-vivo attempts before an adequate result is achieved, but by building on what we already know from this thesis, the ultimate number of large animals could be minimized. To reduce the number of animals needed for these experiments, in-vitro work, which we have shown to correlate to in-vivo release, should be used extensively. Preliminary in-vivo experiments could then be performed in rabbits to verify drug release and only when every other test has been done should larger animal in-vivo work be considered.

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