IN VITRO STUDIES OF DRUG-ELUTING FILMS AND
COATINGS FOR INTRAOCULAR LENS

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SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

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“Feeling gratitude and not expressing it is like wrapping a present and not giving it” - William Arthur Ward (1921 – 1994)

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Abstract

Topical ophthalmic eye drops are prescribed to patients as a post-operative care against infections and inflammations after a cataract surgery. However, this mode of administration exhibits a poor drug bioavailability of approximately 5%, resulting in high drug wastage. In addition, topical administrations also have an issue of poor patient compliance.

The aim of this thesis is to investigate and develop a controlled release system for the administration of antibacterial agents over a sustained period of time. This delivery system can be used to deliver antibiotics such as Levofloxacin and Moxifloxacin to replace the eye drops and to improve the drawbacks faced by the current regimen.

This research work is divided into 2 broad categories: drug-eluting films and drug-eluting coatings.

First, in vitro studies were performed on Levofloxacin-loaded and Moxifloxacin-loaded PLC-based film formulations. Film characterization of formulation parameters, i.e. the effect of drug loadings and solvents were evaluated. The surface morphologies of the films fabricated with different drug loadings and solvents were investigated before and after the in vitro study. Surface morphology, drug loadings and solvent were found to affect the burst release and subsequent release from the systems. Both Levofloxacin and Moxifloxacin systems with THF solvent achieved the desired sustained release profile of 14 days.

Next, studies were performed to characterize the drug-eluting coating delivery system and to evaluate its efficacy in achieving a sustained release for each drug. The coating mandrel designs for the coating attachment and various fabrication process parameters i.e. flow-rate, translational displacement (TD) and number of loops were investigated. Investigation of the
coating morphologies as well as the drug loadings and their influence on the drug release profiles were also carried out. The results demonstrated that lower drug-loaded Levofloxacin coatings were able to lower the initial burst and achieve a sustained release for 14 days. Levofloxacin-loaded “sandwich” system managed to suppress the huge burst observed in single layer 25% coating and the thinner polymer-coated system was able to achieve release above targeted dosage for 9 days. However, due to the poor solubility of Moxifloxacin in DCM and THF, both coating systems suffered from low drug loadings and a sustained release above targeted dosage was unachievable.
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List of Abbreviations

ACN  Acetonitrile
°C   Degree Celsius
cm   Centimeter
DCM  Dichloromethane
DDS  Drug delivery system
DSC  Differential Scanning Calorimetry
EtO  Ethylene Oxide
g    Gram
IOL  Intraocular lens
IV   Inherent viscosity
kg   Kilogram
kV   Kilovolts
Levo Levofloxacin
mg   Milligram
min  Minute
ml   Milliliter
MIC  Minimum inhibitory concentration
Moxi Moxifloxacin
PBS  Phosphate Buffer Solution
PCL  Polycaprolactone
PLA  poly(lactic acid)
PLC  Poly(L-lactide-co-ε-caprolactone)
PLLA Poly(L-lactide)
rev/min Revolutions per minute
rpm  Rotations per minute
SA   Surface area
SD   Standard Deviation
SEM  Scanning Electron Microscopy
T_g  Glass Transition Temperature
T_m  Melting Point
TD   Translational displacement
THF  Tetrahydrofuran
v    Volume
v/v  Volume to volume ratio
w    Weight
wt%  Weight percentage
w/v  Weight to volume ratio
µm   Micrometer
Chapter One: Introduction

1.1 Background

“‘The eyes are the window to your soul’”. William Shakespeare (1564 – 1616)

Our eyes are regarded as one of the most vulnerable organs susceptible to damage. According to World Health Organization (WHO), the 3 major causes of blindness are cataract, glaucoma and age-related macular degeneration (AMD) [1]. For years, cataract is the leading cause of visual impairment worldwide and is deemed as one of the greatest health challenges to tackle even in this modern 21st century. It accounted for 47.9% of cases of blindness and is usually associated with ageing [2].

Cataract is treatable with a surgery. The surgery involves the removal of the cloudy natural lens that has developed opacification, commonly referred to as cataract and replacing it with an artificial intraocular lens (IOL). With advancement in medical technologies, IOL is even employed to correct astigmatism and optical aberrations. Before deploying the IOL, surgery had to be first performed to remove the cataract, and the types of surgical techniques used include Phacoemulsification, Extracapsular Cataract Extraction (ECCE) and Intracapsular Cataract Extraction (ICCE).

Nonetheless, even after the surgery, the risk of post-operative infections such as Endophthalmitis is ever present [3-6]. The current preventive treatment for infection and inflammation following cataract surgery is a short course of topical antibiotics or corticosteroids [7, 8]. The downside of topical eye drop administration is its poor intraocular drug penetration into the eye, resulting in a low 5% bioavailability. To make up for the low therapeutic concentration, eye drops have to be administered several times daily.
regular eye drop regimen leads to further complications such as patient non-compliance, particularly in developing countries as a result of poor accessibility and storage of medication. This ineffectiveness of the delivery method is its Achilles heel.

Polymeric biomaterials have so far proven to be efficient drug carriers for sustained drug delivery because of their excellent biocompatibility and mechanical properties. Therefore, polymeric biomaterials are often the selected choice as vehicles for controlled sustained drug release.

In hopes to optimize drug delivery to the targeted site, sustained drug-eluting IOLs are thus developed; whereby drug-eluting biodegradable film or coating is attached onto the IOL to deliver antibiotic drug sustainably without requiring eye drops and this solves the issue of patient non-compliance. By using IOL as the drug reservoir to deliver desired intraocular drug right into the anterior chamber of the eye, it enables a steady drug concentration and controlled delivery of drug. In addition, combining surgery with drug delivery allows everything to be done in a single surgical procedure without post-operative follow-up and reliance on patient for post-operative management. Hence, there is a tremendous potential to venture into drug-eluting IOLs and to study the drug release performance of them.

1.2 Problem Statement and Project Motivation

As mentioned earlier, the issue of topical eye drop regimen is its low bioavailability, resulting in necessary frequent dosing and patient non-compliance. Patient non-adherence to the regimen after cataract surgery has detrimental outcomes such as Cystoid Macular Edema (CME) [9]. Therefore, it is desirable to design a biodegradable drug-eluting device to release loaded drug in a sustained and controlled fashion.
Delivery of sustained drug to the anterior segment has its fair share of challenges and research to be done. Till this day, topical administration is still widely preferred since it is convenient and is a non-invasive route for treatment of diseases in the anterior segment of the eye. However, the presence of anatomical and physiological constraints exerted by the eye such as static and dynamic barriers, prevented deeper drug penetration to the targeted site [10].

Several approaches have been considered to develop a drug-eluting IOL. Our approach is to apply a drug-containing peripheral coating to the IOL that does not obstruct vision. Biodegradable coatings of thickness range within 200 to 250 µm were fabricated from poly(L-lactide-co-ε-caprolactone) (PLC, molar ratio 70:30) with different drug loading formulations. The stability of drugs and performance of drugs in the coatings at different loadings were studied. The use of biodegradable polymers eliminated the need for surgical removal of the drug carrier, thus reducing patient trauma while eliminating ocular infections.

1.3 Hypothesis

It is hypothesized that the biodegradable films and coatings can deliver antibacterial agents to the anterior segment of the eye as a post-operative treatment and can achieve a more effective therapeutic effect compared to topical administration. This is achieved by enabling localized delivery of drug to the immediate vicinity of the IOL and completely eliminating the need for rigid patient compliance.
1.4 Objectives

The main objective of this thesis is to investigate and optimize the drug release of antibacterial drugs from films and coatings on intraocular lenses.

First objective: To investigate and study the release of Levofloxacin (Third-generation fluoroquinolone) and Moxifloxacin (Fourth-generation fluoroquinolone) from films of the same thickness as the proposed coatings

Second objective: To optimize the IOL coating to obtain the desired coating structure

Third objective: To investigate and study the release of the same drugs from coated IOLs

1.5 Scope

This thesis work is categorized into 2 broad in vitro sections, in vitro study on films and in vitro study on IOL coatings. Both Levofloxacin and Moxifloxacin will be incorporated into the respective systems and characterized for their release profiles. The effects of drug loadings and solvents on the surface morphologies and the release behavior of the systems will be investigated. In addition, for the in vitro IOL coating study, the effect of fabrication process variants on the coating morphology as well as the release profiles will also be investigated.

1.6 Novelty

Levofloxacin and Moxifloxacin have not been evaluated for sustained release in the aspect of systems comprising PLC as the main matrix. This work provides insights on how solvents and drug loadings influence the in vitro drug release profiles. This thesis hopes to further the
understanding of controlled release of antibacterial agents: Levofloxacin and Moxifloxacin from films and coatings.

This is also the first attempt to fabricate a drug-eluting IOL coating as a sleeve over a commercial lens and to conduct a respective in vitro study on Levofloxacin and Moxifloxacin for sustained release in an IOL coating. The concept of a drug-eluting IOL coating that sleeves around commercial IOL is a novel concept that enables easy customization of coating to any existing IOL sizes, making integration of delivery system with IOL possible without significant changes in clinical work flow.
Chapter Two: Literature Review

2.1 Background

2.1.1 Anatomy of eye

Our eye is an important organ that gives us the privilege of vision and observation. The shape of our eye is not a perfect sphere but rather a slightly asymmetrical globe with a diameter of approximately 2.5cm [11, 12].

The eye can be divided into two anatomical segments, specifically the anterior segment and the posterior segment, with the lens acting as a separator (Figure 2-1). The anterior segment is the front one-third section of the eye. The components residing in the anterior segment are cornea, iris, lens and ciliary body. In addition, in the anterior segment, there are two chambers: anterior chamber and posterior chamber [13]. The anterior chamber containing the aqueous humor is located between the cornea and the iris, whereas the posterior chamber is between the lens and the iris. Aqueous humor provides necessary nutrients to the surrounding structures. Posterior segment is the back two-thirds of the eye and extends from the lens to the back of the eye. It comprises of macula, choroid, retina, optic nerve and vitreous humor. The vitreous humor is a gel-like substance the fills up the center of the eye, containing mucopolysaccharides and hyaluronic acids [14]. The function of hyaluronic acids is to maintain its shape and form. An opaque protective outer layer called sclera surrounds the entire eye.
2.1.2 Cataract Disease State

Cataract is a condition where the clear lens of the eye becomes cloudy, preventing adequate light rays from entering and thus, hinders our vision (Figure 2-2). Cataract occurs due to the accumulation of clumps of proteins that clouds the entire lens.

Cataract is usually associated with aging and can develop in one or both eyes. The lens in our eye helps to focus light or images on the retina and adjust the focusing power on the object to be viewed. In normal unaffected situations, light passes through the lens straight to the retina located at the back of the eye. The light-sensitive retina converts light into electrical impulses which is then transmitted to our brain by optic nerves [16]. Information received by the retina is transformed into an image through systematic transmission of electrical impulses along the neurons. Therefore, to receive a sharp image, the lens has to be clear; otherwise a blurry image will be formed.
Possible factors leading to cataract developments are prolonged exposure to ultraviolet radiation, prolonged consumption of mediation, diabetes and smoking [17]. Although cataract is common in elderly, it can also develop in young patients at birth [18, 19]. This condition is known as congenital cataract.

The other types of cataracts besides age-related and congenital cataract are secondary cataract, trauma cataract and radiation cataract. Secondary cataract may develop after eye surgery such as glaucoma, while trauma cataract can develop immediately or years later after an eye injury and radiation cataract may develop after prolonged exposure to certain types of radiation.

Figure 2-2: Cataract formation affecting vision [20]
2.1.3 Cataract Treatment Options

At early stages, surgical removal of cataract might not be necessary and might be corrected by measures such as brighter lighting and sunglasses to prevent glare from the sun. However, when those measures failed and vision is impaired, surgery is required to remove the cloudy lens and replace with an artificial lens implant known as an intraocular lens (IOL). Cataract surgery is believed to be one of the oldest surgical procedures, being documented in ancient times where the procedure was once called “couching”. Cataract surgery has then taken great leaps in advancement from antiquity to its current stage today. With the evolution of surgical procedures, the progression of lens replacement was too demanded. Currently, there are several surgical treatments for removal of cataracts: phacoemulsification, manual small incision cataract (MSICS), extracapsular cataract extraction (ECCE), intracapsular cataract extraction (ICCE) [21]. Although phacoemulsification is the most common performed surgical technique, surgeons can now use femtosecond lasers (FSLs) in cataract surgery for better precision and accuracy. FSL was first introduced into ophthalmic surgery in 2001 as a new technique for creating precise lamellar flaps in laser in situ keratomileusis (LASIK). The different procedures are summarized in Table 2-1.
Chapter Two: Literature Review

<table>
<thead>
<tr>
<th>Surgical Technique</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phacoemulsification</td>
<td>One or more incision (1.8 – 3.2 mm) is made in side of cornea and a high frequency ultrasound needle-like probe is inserted to emulsify the cataract and suction it out [22, 23]. IOL is then implanted and incisions heal without stitches.</td>
</tr>
<tr>
<td>Femtosecond laser (FSL)</td>
<td>Precise incision of tissue of 2.0 mm and eliminates heat generation and damage to surrounding tissues [24, 25]. Reduce ultrasound power requirement during phacoemulsification [26].</td>
</tr>
<tr>
<td>Manual small incision cataract (MSICS)</td>
<td>Evolves from ECCE. Entire lens is removed out of eye through a self-sealing sclera tunnel wound. Wound is smaller than ECCE but larger than phacoemulsification’s [27]. Cost is lower than phacoemulsification.</td>
</tr>
<tr>
<td>Extracapsular cataract extraction (ECCE)</td>
<td>Large incision (~10 mm) is made in cornea or sclera and another in front of lens capsule for removal of entire lens. IOL is then implanted in lens capsule before closing incision [28]. Mainly used for advanced cataracts where lens is too dense to use phacoemulsification</td>
</tr>
<tr>
<td>Intracapsular cataract extraction (ICCE)</td>
<td>Large incision required for removal of lens and surrounding capsule together [29]. IOL is implanted at the front of iris [30].</td>
</tr>
</tbody>
</table>

Table 2-1: Surgical procedures for cataract surgery

All surgical options will lead to the installation of the IOL into the eye for vision correction and improvement. The first IOL implant made of poly(methyl methacrylate) (PMMA) was created by Dr Harold Ridley in 1949 [31, 32] before soft foldable lens of silicon and acrylic are discovered. However, due to the insertion of foreign body into the eye, there are risks of post-operative complications. Endophthalmitis, a devastating intraocular infection is a result of bacterial infection originating from patient’s ocular surface and tissues or from external sources such as contaminated instruments or solutions. The norm after surgery is to prescribe
topical antibiotic eye drops to patients as a preventive measure against infections. Treatments to tackle Endophthalmitis are intravitreal antibiotics and even vitrectomy [33, 34].

2.2 Intraocular Lens

Intraocular lens (IOL) is a synthetic artificial lens implanted into the eye to replace the surgically removed natural lens during cataract surgery. IOL consists of a transparent plastic lens with 2 plastic struts at the sides, called haptics, to hold the lens in place within the lens capsule of the eye (Figure 2-3).

2.2.1 Post-operative Treatment Options

2.2.1.1 Topical Administration

Topical eye drop administration is the most preferred ocular treatment after IOL implantation, for its ease of application and low cost [35]. However, the issue of patient non-compliance to the regimen makes it undesirable [36]. Studies showed that besides being non-compliant, some patients did improper administration of the eye drops as well [37]. These two factors
stated will greatly affect the healing process after surgery as it relies strongly on the patients’ efforts.

These eye drops dominated roughly 90% of the ophthalmic market and are useful for treatment of eye disorders in the anterior segment. Nonetheless, the bioavailability is very low with less than 5% and much lesser drug actually reaches the deeper ocular tissues [38]. Topical application of antibiotics has poor level of intraocular penetration with less than 0.3% [39]. Several anatomical and physiological barriers such as tear turnover, nasolacrimal drainage, tear dilution, and other ocular barriers impede drug delivery and drug penetration to target tissues, especially in the back region of the eye [35]. Eye drops are rapidly drained from the ocular surface with a short duration of drug absorption. Due to the short contact time of eye drops with the pre-cornea, this results in lower drug concentration than the required therapeutic concentration. Hence, the patient has to administer droplets frequently each day [40].

2.2.1.2 Sustained Drug Delivery Systems

Based on the constraints mentioned earlier, topical administration may not be the most optimal type of post-operative treatment. Therefore, to overcome these constraints to achieve efficient drug targeting to sites and to improve on ocular drug bioavailability, various modes of drug delivery systems have been developed. These different options available to mitigate the risk of infection without the need for topical eye drops will be further elaborated.
i. **Drug-eluting Non-biodegradable Intraocular Implants**

Implants are inserted at site of disease to improve on duration of drug release and to provide localized, sustained and controlled release with effective therapeutic concentrations.

IOLs are used as drug delivery vehicles as a stand-alone drug reservoir, with a coating or with a separate reservoir attachment [41]. *Chomón et al.* states combining commercially available IOLs with drugs improves drug loading, and could also deliver efficacious release to prevent infection, inflammation or posterior capsular opacification (PCO) [42]. The different types of drug-eluting IOLs are specified in Table 2-2.

<table>
<thead>
<tr>
<th>IOL Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Soaked IOLs</td>
<td>Immerse IOLs in concentrated aqueous or hydroalcoholic solutions (pre-soaking) to load drug, likewise for contact lenses. Drug uptake dependent on affinity between polymer and drug.</td>
</tr>
<tr>
<td>Drug Inserts</td>
<td>Attached IOL with one or more drug-loaded inserts. Biodegradable devices allow efficient release of intraocular drug depots.</td>
</tr>
<tr>
<td>Supercritical Fluids Impregnation</td>
<td>Impregnating drug molecules without using organic solvents but using supercritical CO₂ (scCO₂). Higher drug loading yield than presoaked IOLs [43, 44].</td>
</tr>
<tr>
<td>Drug coated IOL</td>
<td>Coating IOL layer-by-layer (lbl) is an alternative to coating hydrophobic IOLs with drug-loaded film [45]. Spray-coated IOLs is a faster technique compared to lbl deposition.</td>
</tr>
<tr>
<td>Chemical Grafting</td>
<td>Binding drug chemically to IOLs to provide permanent therapeutic activity to IOL surface or prompt drug release when specific conditions are met.</td>
</tr>
</tbody>
</table>

*Table 2-2: Types of drug-eluting IOLs*
A common and cost-effective method is to soak IOLs in a drug solution to achieve drug performance comparable to other methods [41]. A drawback is this approach is more suitable for hydrophilic IOLs (hydrogels or soft acrylics) than hydrophobic ones as hydrophilic lens facilitate drug diffusion into the matrix [42]. In addition, it results in inefficient loading and drug wastage. By using scCO₂ supercritical impregnation method, drug is homogenously impregnated into polymers. For drug inserts, inserts are attached to IOL prevent dislodgement and the IOL needs no modification due to separate preparation of the inserts. Comparing this system to drug-soaked IOLs, this approach is much preferable as a higher loading and longer release period might be attainable.

ii. **Drug-eluting Contact Lens**

Drug-loaded contact lenses are used for ocular delivery of drugs like antimicrobials and antihistamines. The advantage of this system enables vision correction and drug elution simultaneously.

Drug is loaded into contact lenses by soaking them in drug solutions. Contact lenses being hydrophilic in nature absorb the drug. There are two ways to soak the lenses: presoaking or post-soaking [46]. For presoaking, commercialized lenses are soaked for hours in drug solution whereas for post-soaking, contact lens is first placed on the cornea before administering ophthalmic solution to it for prolong drug release. Soaked lens has better drug delivery efficiency but suffers from deficient drug loading and short drug delivery period. To overcome these problems, particle-laden contact lenses and molecular imprinted contact lenses are developed. In particle-laden contact lenses, drug is incorporated into vesicles like liposomes or nanoparticles and these vesicles are dispersed in the lens material such as poly-
2-hydroxyethyl methacrylate (p-HEMA) hydrogels. This provides controlled therapeutic drug levels over few days [47].

iii. Drug-eluting Biodegradable Gel

In-situ gels are another approach to substitute eye drops. Hydrogels are polymer solutions that undergo sol-gel transition to obtain viscoelastic gel. Temperature changes, pH and even ions activation are factors that trigger gelation. With a hydrogel system, residence time is enhanced considerably. Gelrite® (Kelco, Division, Merck & Co.), a polysaccharide gellan gum that forms clear gels in the presence of mono or divalent cations in lacrimal fluid [48], is able to enhance corneal penetration and ocular bioavailability.

2.3 Fluoroquinolones

Fluoroquinolones are a family of synthetic antibiotics with potent and broad-spectrum bacterial activity against numerous clinical pathogens responsible for a wide range of infections. They are effectively utilized in respiratory tract infections (RTI) and skin infections [49]. Structural modifications on the first generation fluoroquinolones result in subsequent second, third and fourth generation, with improved coverage of gram-positive and gram-negative bacteria. The classification of fluoroquinolones (Table 2-3) is based on their spectrum of activity and pharmacokinetic profiles [49, 50]. The type of administration routes are topical, intravitreal and systemic [51]. Fluoroquinolones were first used in 1990 to treat ocular infections after the introduction of topical Ciprofloxacin, Ofloxacin, and Norfloxacin [52].
Levofloxacin, Gatifloxacin and Moxifloxacin are favored topical ophthalmic prophylaxis agents for their enhanced activity against gram-positive organisms including *S. aureus* and some atypical mycobacteria, while maintaining their activity against gram-negative bacteria. In addition, the potential benefits of enhanced drug delivery into anterior segment of the eye, and lower predisposition for selecting resistant bacterial strains make them attractive [53].

For this project, Levofloxacin and Moxifloxacin are chosen as the antibacterial agents.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Drug</th>
<th>Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Nalidixic acid</td>
<td>Active against some Gram negative bacteria</td>
</tr>
<tr>
<td></td>
<td>Cinoxacin</td>
<td>Highly protein bound drugs</td>
</tr>
<tr>
<td></td>
<td>Oxolinic acid</td>
<td>Short half life</td>
</tr>
<tr>
<td></td>
<td>Pipemidic acid</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>Norfloxacin</td>
<td>Protein binding (50%)</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacina</td>
<td>Longer half-life than previous agents</td>
</tr>
<tr>
<td></td>
<td>Lomefloxacina</td>
<td>Improved activity against Gram negative bacteria</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enoxacin</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>Levofloxacin</td>
<td>Active against Gram-negative bacteria and Gram-positive bacteria</td>
</tr>
<tr>
<td></td>
<td>Sparfloxacina</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Balofloxacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pazufloxacin</td>
<td></td>
</tr>
<tr>
<td>Fourth</td>
<td>Trovafloxacin</td>
<td>Extended activity against both strains of bacteria</td>
</tr>
<tr>
<td></td>
<td>Moxifloxacin</td>
<td>Active against anaerobes and atypical bacteria</td>
</tr>
<tr>
<td></td>
<td>Gatifloxacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gemifloxacin</td>
<td></td>
</tr>
</tbody>
</table>

Table 2-3: Classification of fluoroquinolones
### 2.3.1 Levofloxacin

Levofloxacin is the L-isomer of racemic Ofloxacin. It inhibits bacterial DNA gyrase (topoisomerase II) and topoisomerase IV [54]. DNA gyrase is involved in replication, transcription and repair of DNA; topoisomerase IV catalyzes DNA unlinking activity [55].

Levofloxacin is a third-generation fluoroquinolone with increased gram-positive coverage and retained antibacterial activity against gram-negative and atypical bacteria compared to the second generation [56]. However, it has limited activity against most anaerobes. Fluoroquinolones, including Levofloxacin are valued for their broad spectrum and excellent tissue penetration. Levofloxacin ophthalmic eye drops are commercialized under Oftaquix® and Quixin.

The presence of the carboxyl group in Levofloxacin forms hydrogen bond readily with water and hence, this makes it a hydrophilic drug. It has great solubility in water at neutral pH and demonstrated greater penetration into the aqueous humor than second generation fluoroquinolones [57]. It is also lipophilic to penetrate well into the eye [58]. Table 2-4 compares the properties of Levofloxacin and Moxifloxacin.

Drug delivery system of fluoroquinolone loaded films were researched and developed for the treatment of periodontitis, an infection affecting the soft tissues surrounding the teeth and results in the loss of supporting bone. Prabhushankar et al. reported observation of rapid release in the first 3 days and a controllable release for subsequent 7 days using different formulations periodontal films containing Levofloxacin in PBS (pH 6.6) [59]. The average daily amount of drug release was reported to be above the minimum inhibitory concentration of Levofloxacin (MIC ≤ 2 µg/ml) [60, 61].
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<table>
<thead>
<tr>
<th>Fluoroquinolone</th>
<th>Levofloxacin</th>
<th>Moxifloxacin Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Generation</strong></td>
<td>Third- generation</td>
<td>Fourth- generation</td>
</tr>
<tr>
<td><strong>Chemical Structure</strong></td>
<td><img src="image1" alt="Levofloxacin" /></td>
<td><img src="image2" alt="Moxifloxacin" /></td>
</tr>
<tr>
<td><strong>Molecular Weight (g/mol)</strong></td>
<td>361.37</td>
<td>437.9</td>
</tr>
<tr>
<td><strong>Melting Point (°C)</strong></td>
<td>218 – 220</td>
<td>238 - 242</td>
</tr>
<tr>
<td><strong>Bioavailability</strong></td>
<td>99%</td>
<td>86 – 92%</td>
</tr>
<tr>
<td><strong>Half-Life</strong></td>
<td>6 to 8 hours</td>
<td>12 hours</td>
</tr>
<tr>
<td><strong>LD&lt;sub&gt;50&lt;/sub&gt; rat (oral)</strong></td>
<td>1478 mg/kg</td>
<td>1320 mg/kg</td>
</tr>
</tbody>
</table>

Table 2-4: Comparison between Levofloxacin and Moxifloxacin

2.3.2 Moxifloxacin

Moxifloxacin Hydrochloride is a fourth-generation fluoroquinolone. It has enhanced spectrum with increased potency against gram-positive bacteria, while retaining the broad spectrum of gram-negative bacteria observed in older generations [51, 62, 63]. It is said to have the highest potency against *Staphylococcus aureus* and *Staphylococcus epidermidis* [62]. It is commercialized as a topical ophthalmic eye drop under Vigamox and Moxeza.

The emergence of bacterial resistance prompted the introduction of fourth generation of fluoroquinolones like Moxifloxacin. Unlike the older generations, Moxifloxacin binds strongly to both DNA gyrase and topoisomerase IV, thus reducing the likelihood of bacterial resistance [64, 65]. Antibiotics like Levofloxacin and Moxifloxacin with relatively high lipid-water solubility coefficient can penetrate better into the deeper tissues within the cornea.
at more effective concentrations. Similar to Levofloxacin, Moxifloxacin too demonstrated good penetration into the eye.

Moxifloxacin was also loaded into films for treatment of periodontitis of *in vitro* studies. *Chinta et al.* reported that Moxifloxacin loaded cross-linked chitosan films were able to achieve a slow and sustained release for up to 15 days after the burst release [60].

### 2.4 Biodegradable Polymer

Polymeric biomaterials are highly sought after for biomedical applications, especially as drug carriers for sustained drug delivery. It possesses immense potential because of its flexibility in chemistry and thus, giving rise to biomaterials of diverse physical and mechanical properties. Biodegradable polymers are of interest as they can be broken down, excreted or resorbed without surgical removal or revision. Hence, this starts the revolution of biodegradable materials for biomedical applications.

All polymeric biomaterials have to be appraised based on their biocompatibility, mechanical properties and biodegradability to determine if they are appropriate for a specific medical application. For the implementation of biodegradable polymers in biomedical devices, the selected candidate has to be compatible and safe for implantation in human body. It should also not produce toxic degradation products and allow processing for applications [66].
The ideal characteristics are as listed:

1. **Biodegradable**
   The polymer chosen has to be biodegradable particularly for short-term applications. The biological activity facilitates the breakdown of these biodegradable polymers and is eventually metabolized in the human body; the necessity for secondary surgical intervention is eliminated.

2. **Biocompatible**
   As implantation is done *in vivo*, the polymer must not evoke inflammatory response and has to be well accepted in the body. Hence, the choice of non-toxic and non-immunogenic material is essential.

3. **Material Attributes**
   It should have suitable mechanical, physical and biological properties for the intended application. The material attributes play a role in material selection for different types of application. For ocular devices, flexibility of the polymer is one of the factors for consideration.

4. **Shelf-life and Sterilization**
   Final product should not degrade during the functionality period and has an acceptable storage life. Biomedical devices undergo Ethylene oxide (EtO) sterilization before implantation. It is carried out at room temperature, thus polymers selection will not be confined based on thermal properties and EtO will not cause changes to their properties. Although the above characteristics serve as a guideline for the selection of polymeric material, the feasibility of shaping the polymer into a biomedical device for the intended
application needs to be taken into consideration too. The preference of electing biodegradable polymers over non-biodegradable for implanted devices is also due to their key advantage of not requiring surgical removal after implantation and in the aspect of ophthalmic drug delivery; it can circumvent unwanted surgical complications.

2.4.1 Poly (L-lactide-co-ε-caprolactone)

Poly(L-lactide-co-ε-caprolactone) (PLC) is a biodegradable copolymer comprising of biodegradable poly(L-lactide) (PLLA) and polycaprolactone (PCL). Polylactide (PLA) is a hydrophobic polymer due to the presence of CH₃ side groups. PLA is synthesized from lactide, a condensed dimer of lactic acid. As a result of its chirality, PLA exist in two different enantiomeric forms, D- and L-lactide. PLA is a rigid and brittle polymer with low deformation at break [67]. PLLA is a linear and aliphatic semi-crystalline polyester obtained from the polymerization of lactide.

Compared to PCL, PLLA is stiffer with a glass transition temperature (Tₕ) of about 50 °C to 65 °C and a melting point around 180 °C. PCL is a ductile polyester with a Tₕ of -60 °C and a melting point of approximately 60 °C. According to Choi et al., PLLA shows low drug permeability and rapid hydrolysis. PCL, on the other hand, portrays good biocompatibility, biodegradability and drug permeability [68]. PCL is also extensively studied for its thermal stability and mechanical strength in scaffolding applications. As a result of its high hydrophobicity and crystallinity, the polymer undergoes slower degradation compared to PLLA [69]. Hence, PCL is considered an appropriate material for long-term drug delivery. By changing the PCL content in the copolymer, the mechanical properties, degradation rate, drug permeability and release profiles will change accordingly [70].
PCL having a low Tg, forms the flexible segment of the copolymer backbone in PLC. PLLA forms the hard segment of the copolymer due to its higher Tg. The flexible soft segments give enhanced elongation and elastic recovery, while the hard segments act as non-covalent physical crosslinks [71].

PLLA and PCL undergo hydrolytic degradation. PLLA hydrolyzes to its constituent lactic acid when implanted in human body. The by-products will be metabolized and excreted as water and carbon dioxide through tricarboxylic acid cycle [72]. The by-product of lactic acid invokes inflammatory response [73] and the blending of PLLA with PCL can reduce inflammatory response [74].

PLLA has shape memory effects but its recovery strain is low and its recovery temperature is considered high for usage in body [75]. Copolymer of PLLA and PCL improves shape memory properties and recovery temperature.

PLLA and PCL react with each other via ring opening polymerization. PLC (Figure 2-4), a copolymer of PLLA and PCL has traits of both polymers. It has enhanced elasticity due to shape memory effect, flexibility and high elongation. With a lower degree of crystallinity, PLLA/PCL copolymer exhibits faster degradation rate compared to PLLA and PCL.

It also has better permeability and mechanical properties. Since PLC is soft and elastic, it makes a better fabrication material than other biodegradable polymers such as PLGA. In addition, Liu et al. stated that a softer material is more suitable for implants fabrication as it minimizes chances of surgical trauma during implantation [76]. Therefore, with these reasons, it is justified as the choice of polymer in this study.
2.5 Solvent

Solvents used in film and coating systems need to have low boiling point for quicker evaporation since these samples come into contact with physiological environments. Therefore, it has to be ensured that solvents are removed or evaporated from dried samples, and usually pharmaceutically accepted organic solvents are chosen. Organic solvents are commonly classified into two specific categories: polar (hydrophilic) and non-polar (hydrophobic) solvents. Under the polar category, examples are dimethyl sulfoxide (DMSO) and acetonitrile (ACN). Non-polar solvents include dichloromethane (DCM), tetrahydrofuran (THF), chloroform, toluene and ethyl acetate.

In this study, two solvents used were THF and DCM. Both are widely used organic solvents and are clear, colorless liquids. PLC was soluble in THF and DCM and they were also chosen because of their low boiling points.
THF

THF has a couple of synonyms, such as diethylene oxide, tetramethylene oxide, 1,4-epoxybutane and oxacyclopentane. It is a cyclic ether with a distinctive odor reminiscent of acetone with a structural formula of C₄H₈O. THF demonstrates excellent miscibility with water, alcohols, ether and all common organic solvents such as ACN, methanol, DCM and chloroform. It also has excellent solvent power with several organic constituents.

THF is used extensively as a monomer in the production of poly(tetramethylene oxide) (PTMO), otherwise known as poly(tetramethylene ether glycol) (PTMEG) and polytetrahydrofuran (PTHF). PTMEG and PTHF are essential for the fabrication of thermoplastic and molded elastomers based polyurethanes, elastic Spandex fibers, copolyesters or copolyamides [77, 78]. As a versatile solvent, it is also used for natural and synthetic resins as well as poly(vinyl chloride) (PVC), adhesives, for pharmaceutical steroids; in vanish and film industries [77].

DCM

DCM is also known as methylene chloride. DCM is immiscible with water but is miscible in several organic solvents such as ACN, chloroform and ethanol. It is thermally stable to temperatures above 140 °C and in the presence of oxygen, is stable up to 120 °C (chlorinated hydrocarbons).

DCM is used in various industrial processes in many different industries. It is used as a cleaning agent for paint stripping, paint removal and pharmaceutical manufacturing. It is also utilized in extraction technologies such as paraffin extraction and decaffeination of coffee.
The physical properties of both solvents are compared in Table 2.5. The values inserted in Table 2-5 were obtained from Tedia’s material safety data sheet (MSDS).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dichloromethane (DCM)</th>
<th>Tetrahydrofuran (THF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>CH₂Cl₂</td>
<td>C₄H₈O</td>
</tr>
<tr>
<td>Molar mass (g/mol)</td>
<td>84.93</td>
<td>72.11</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>40</td>
<td>66</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>-96.7</td>
<td>-108.5</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>Miscible</td>
<td>Moderately soluble</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>1.33</td>
<td>0.89</td>
</tr>
</tbody>
</table>

DCM has a lower boiling point of 40 °C than THF (66 °C). This means that DCM has a higher volatility and hence, a faster rate of evaporation. The evaporation rate of the solvent can affect the morphology of films and coatings and influence the *in vitro* release profiles. This will be investigated in chapter 4 in the later section of the thesis.
2.6 Spray Coating

Spray coating is a procedure to apply drug-polymer solution onto the surface of drug-eluting devices. It is a state-of-the-art approach to achieve controlled and uniform coating for sustained release of drug, especially on stents. Other known techniques used are dip coating and gas-phase deposition [79].

Dip coating is an immersion method, whereby the entire stent or section of stent is dipped into a coating solution. However, due to the complex geometry of the stent and fluid nature of the coating solution, it is difficult to control specific regions to undergo coating and the thickness of the coating with this method [80]. There might be regions of non-uniform thickness, particularly the top and bottom regions because of sagging or wedge effects.

Ultrasonic spray technology, on the other hand, edges over its competitors because of its favorable advantages such as precision, thickness uniformity, repeatability and controllability. According to Sono-Tek Corporation, ultrasonic spray coating allows process variables controllability, giving greater efficiency and reduction of coating material needed. Spray coating uses ultrasonic atomization that is regarded as the best method to achieve desirable uniform coating. Predicate studies on coated drug-eluting stents have proven the usefulness of spray coating for biomedical devices [81, 82]. However, its drawback is the time-consuming fabrication time.

Although spray coating is widely adopted for drug-eluting stents but the idea of using it to fabricate drug-eluting coatings for IOLs is new. So far research done were direct spraying of coating solution onto commercial lenses. An example is commercial PMMA lenses sprayed with a solution of PLGA and rapamycin in chloroform [83]. However, spraying a layer of coating solution onto the IOL could lead to excess coating on the optical lens and hence,
affect the optical properties of the lens. Furthermore, drying the IOL above room temperature might affect the nature of the lens. Prior approaches like drug soaked IOLs and attachment of drug-loaded beads to the haptics were eliminated for two reasons. First, soaking may affect the optical properties of the lens and the therapeutic efficacy of this system is very short [84, 85]. Second, the attachment of the beads may affect the post-surgical orientation of the IOL because of the weight imbalance of heavier beads relative to lens. Therefore, this induces risk of in situ IOL de-centralization.

The creation of a drug-eluting coating attachment to IOL will benefit patients by eliminating topical administration and solving the issue of patient non-compliance. Moreover, drug will be delivered directly to the target site and this improves drug bioavailability with the absence of diffusion barriers.

2.7 Drug Release Mechanism

Controlled drug delivery systems are generally the interest of many researchers and the motivation for using biodegradable polymers. Controlled release is desirable to shepherd therapeutic agents such as antibiotics, antibodies or vaccines to target site in a sustained release manner and with appropriate degradation time of polymeric drug carrier. For drug delivery, the drug amount delivered over a specific period of time is pivotal and is subjected to the type of treatment. Therefore, the drug concentration has to be within the therapeutic window, where it delivers therapeutic benefits and not pose danger to patients. In view of that, controlled release systems can effectively reduce frequency of administration required, achieve desirable therapeutic efficacy and increase patient compliance [86, 87].
The characterization of release systems is determined by their principal release mechanism. 

Fredenberg et al. stated there are three modes for drug molecules to release from polymer-based drug delivery systems (DDS) (Figure 2-5): (1) diffusion through pores or channels filled with release medium, (2) diffusion through polymer matrix, and (3) erosion of polymer [88].

A more popular delivery system is a monolithic or matrix system, where drug is dissolved or dispersed throughout the carrier matrix. Drug release depends on the drug loading and the type of release mechanism. Although drug release is usually governed mainly by diffusion, we cannot eliminate the possibility of other mechanisms such as dissolution or erosion involved concurrently. There are two classes of systems. When the drug loading level is low and drug is fully dissolved in the polymer matrix, it is known as a monolithic solution. The second is known as monolithic dispersion. This system arises when only a fraction of drug is dissolved and the remainder is dispersed in the form of crystalline and/or amorphous particles (undissolved drug). In this system, the drug has limited solubility in the polymer or is at a high loading.

Baker classified monolithic dispersion into three categories depending on the volume fraction of drug contain in the polymer matrix: simple monolithic dispersion at low drug loading (0 – 5 w/w%), complex monolithic dispersion (5 – 10 w/w%), and monolithic matrix systems (> 15- 20 w/w%) [89]. In this work, the main focus will be on monolithic matrix system due to the high level of drug loading involved.
2.7.1 Burst Release

For matrix drug delivery devices, drug is either dissolved or dispersed within the polymer matrix. Drug diffusion through the polymer matrix is often the rate-controlling step of the system. For many DDS, drug present on the surface will first elute out into the release medium. This phenomenon of large surge of drug released upon immersion is denoted as “burst release”, and is commonly observed before the release profile reaches a stable rate. Although burst release occurs only for a short period, it leads to a high amount of drug released at the initial stage and hence, affecting the lifespan of the implanted device. This also leads to drug wastage and acute toxicity from high drug concentrations. Zero-order systems are of particular interest as they exhibit constant release of drug with time and hypothetically, result in the best control of plasma concentration. Figure 2-6 illustrates the effect of burst release on a zero-order controlled release profile.
In matrix DDS, the cause of burst release could be explained by four possible theories [86]:

1. During fabrication, some drug molecules get entrapped on the surface of the polymer matrix, particularly in the case of high drug loading, and elute out upon contact with release medium.

2. Formation of pores in polymer matrix and possibly channels during solvent evaporation. Increase removal rate of organic solvent increase porosity in matrices.

3. Migration of drug to the surface as solvent evaporates during drying and leaves some drug trapped on the surface.

4. Burst release is associated with small molecular weight solutes because they are highly soluble in aqueous systems and can pass through the porous matrix. Solubility of drugs and their partition coefficient can influence the driving forces of release.
2.7.2 Diffusion

Diffusion is the transport of molecules or particles from a region of high concentration to a region of low concentration. Diffusion of drug from polymeric systems is an essential aspect for controlled release and it can be explained using Fick’s laws of diffusion.

2.7.2.1 Fick’s Laws of Diffusion

Fick’s first law relates that diffusion flux (J) along the x-direction is proportional to the negative concentration gradient under the assumption of steady state, where concentration is time independent (Equation 2.1).

\[ J = -D \frac{\partial c}{\partial x} \]  
(Equation 2.1)

Diffusion flux (J) is expressed as the amount of substance diffusing through a unit area per unit time, while the diffusivity or diffusion coefficient (D) measures the molecule’s mobility in the medium.

On the other hand, Fick’s second law describes the diffusion process under non-steady state with concentration varying with time:

\[ \frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \]  
(Equation 2.2)

Crank developed an equation for sorption kinetics which interpreted the diffusion of small molecules in polymers. A polymer film having a thickness of 2l is immersed into an infinite bath of penetrant (Figure 2-7) and concentration, C_t at time t at any point within the film is given by Equation 2.3 [90].
Figure 2-7: Representation of a film immersed in infinite bath of penetrant

\[
\frac{c_t}{c_\infty} = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \exp \left[ \frac{-D (2n+1)^2 \pi^2 t}{4l^2} \right] \cos \left[ \frac{(2n+1)\pi x}{2l} \right] \]  
(Equation 2.3)

where \( c_\infty \) is the saturated equilibrium concentration. Integrating Equation 2.3 gives the mass of penetrant sorption by the film at time \( t \), \( M_t \) as illustrated in Equation 2.4.

\[
\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left[ \frac{-D (2n+1)^2 \pi^2 t}{4l^2} \right] 
\]  
(Equation 2.4)

\( M_\infty \) stated in Equation 2.4 is the mass uptake at equilibrium and the \( l \) here again is the film thickness. For short time approximation, Equation 2.4 can be shortened to

\[
\frac{M_t}{M_\infty} = \frac{2}{l} \left( \frac{D}{\pi} \right)^\frac{1}{2} \frac{1}{l} \]  
(Equation 2.5)

From Equation 2.4, it can be seen that besides diffusivity, the thickness of the film is also an important parameter that influences the kinetics of diffusion. As mentioned earlier, steady and non-steady states of diffusion are under the assumption of Fickian diffusion. However, there are cases that diffusion is non-Fickian. Take immersing a polymer film into penetrant medium as an example, the basic equation of mass uptake by the film derived from Fick’s second law is
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\[
\frac{M_t}{M_\infty} = kt^n
\]

(Equation 2.6)

Given \( n \) is the type of diffusion mechanism and \( k \) is a constant. Diffusion and transport in polymers are categorized based on the relative mobility rate of penetrant and polymer segments. These three categories are [90]:

(i) Case I (Fickian diffusion): Rate of diffusion is lesser compared to the mobility of polymer segment. Sorption equilibrium is rapidly attained, thus achieving time-independent boundary condition with no dependence on swelling kinetics. It is observed in polymeric system when temperature is well above the \( T_g \) of the polymer

(ii) Case II: Diffusion and penetrant mobility are heaps greater than the polymer segment relaxation processes, leading to a strong dependence on swelling kinetics

(iii) Non-Fickian: Occurs when the rate of penetrant mobility and polymer segment relaxation are equivalent. This diffusion is observed in hard and glassy polymers but disappears at and/or above the \( T_g \)

Case I and II can be distinguished by the shape of the sorption-time curve obtained using Equation 2.6. For Case I, \( n = 0.5 \), while for Case II, \( n = 1 \) and for Super Case II, \( n > 1 \). For non-Fickian (or anomalous) systems, \( n \) is between 1 and 0.5. Case II and anomalous diffusion are typically found in polymers whose \( T_g \) are higher than experimental values [91].
2.7.3 Degradation

Degradation or erosion is the next commonly reported mechanism for drug release from polymeric systems. Polymer degradation can be classified into two respective behaviors, namely bulk degradation and surface erosion. There is a distinctive difference between polymer degradation and surface erosion. Polymer degradation causes a change in the chemical structure due to cleavage of polymer chains to oligomers and monomers. Erosion, however, is a process of wearing away from the polymer or material losses from the polymer bulk, and this is the result of degradation. Polymers can degrade by different means, depending on their inherent chemical structure and conditions they are subjected to. There are five different modes of polymer degradation: thermal, mechanical, radiation-induced, enzymatic and chemical degradation.

The building blocks of PLC are comprised of ester bonds. Hence, in the presence of water, the ester bonds hydrolyze to form carboxylic acid and alcohol. In this work, the focus mechanism is chemical degradation via hydrolysis. Degradation can occur by bulk degradation, bulk erosion or surface erosion. Bulk degradation, bulk erosion and surface erosion are schematically presented in Figure 2-8.

Bulk degradation occurs in two stages: water first penetrates into the polymer, cleaves the chemical bonds in the amorphous phase and transforms longer polymeric chains into short and water-soluble ones; leading to molecular weight loss without loss of physical properties. This is soon followed by reduction of molecular weight with the polymer matrix disintegrating into fragments. Erosion occurs when water attack the covalent bonds in the polymer matrix. Homogenous or bulk erosion is characterized by a faster rate of water penetration than polymer hydrolysis, and at some critical point, molecular weight loss is
experienced. On the contrary, when the rate of water penetration is slower than hydrolysis, hydrolysis or mass loss is restricted to the surface and the interior of the polymer matrix remain unchanged. Over time, the dimensions will decrease and eventually, the matrix reduces in size. This is known as surface erosion. For surface eroding devices, the exposed external surface area is directly related to the erosion rate.

Figure 2-8: Mechanistic illustration of polymer matrix undergoing bulk degradation, bulk erosion and surface erosion [92]
Chapter Three: Experimental Materials and Methods

3.1 Materials

All the materials utilized in this research project are listed in the following Table 3-1.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Abbreviation</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(L-lactide-co-ε-caprolactone) 70/30, IV 1.5 dl/g</td>
<td>PLC 70/30</td>
<td>Purac (The Netherlands)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>Levo</td>
<td>Molekula Limited (UK)</td>
</tr>
<tr>
<td>Moxifloxacin Hydrochloride</td>
<td>Moxi</td>
<td>Molekula Limited (UK)</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>DCM</td>
<td>Tedia (USA)</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>THF</td>
<td>Tedia (USA)</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>ACN</td>
<td>Tedia (USA)</td>
</tr>
<tr>
<td>Phosphate Buffer Saline tablets</td>
<td>PBS</td>
<td>Sigma-Aldrich (Singapore)</td>
</tr>
</tbody>
</table>

Table 3-1: List of materials and reagents

The organic solvents used were of HPLC grade. PLC 70/30 represents the molar ratio of L-lactide to ε-caprolactone and has an inherent viscosity (IV) of 1.5 dl/g. Phosphate buffer saline (PBS) solution was prepared by dissolving five saline tablets in one litre of deionized water to achieve a pH of 7.4.

3.2 Methods

3.2.1 Films Preparation

For preparation of polymer films, PLC pellets were dissolved in DCM or THF at a weight-to-volume (w/v) ratio of 1:5 and left to stir continuously overnight to obtain homogenous solutions. For drug-loaded films, preparation step was similar. The chosen formulations of
study were 15 wt%, 20 wt% and 25 wt% of drug (Table 3-2). Levofloxacin or Moxifloxacin was dissolved respectively in DCM and THF, and the drug solutions were stirred for 4 hours before corresponding pre-weighed PLC were added to form drug-polymer solutions. The final solutions were stirred overnight at room temperature to allow homogenization.

Drug-polymer solutions were casted layer-by-layer on a glass plate using an automatic film applicator at a speed of 50 mm/min, forming three-layered films with thickness ranging from 200 to 250 µm. To achieve this final thickness, the height of the casting knife was adjusted to 60 µm (first layer), 70 µm (second layer) and 80 µm (third layer) with an interval of 8 minutes between each layer. Experiments have shown that one or two film layers were insufficient to achieve a thickness of at least 200 µm and therefore, three layers were required. As the thickness of the drug-eluting device is required to be 200 to 250 µm as advised by the clinician, the thickness of the films casted have to fall within that range.

Wet films casted were immediately covered to prevent air exposure and left to dry overnight under ambient conditions before drying in 37ºC vacuum oven for 2 weeks to allow complete solvent evaporation. Solvent residue analysis in films was conducted with Thermogravimetric Analyzer, TGA (TGA 2950, TA Instruments). The steps taken for the analysis was: samples of 10-20 mg were first heated from room temperature to 150 ºC at 10 ºC/min in nitrogen environment. Thereafter, it was held isothermally for 10 minutes before heating to 800 ºC at 10 ºC/min and held isothermally for 10 minutes. The resulting weight loss of the films revealed less than 1 wt% residual solvent left in all samples. The thicknesses of the dried films were measured using a coating thickness gauge (Elcometer 456, Elcometer (Asia) Pte Ltd).
Table 3-2: Films formulations

<table>
<thead>
<tr>
<th>Materials</th>
<th>Formulation</th>
<th>Polymer + Solvent</th>
<th>Wet Thickness (Knife)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>15%, 20%, 25%</td>
<td>PLC + DCM</td>
<td>15%: 50, 60, 70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20%: 40, 50, 60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25%: 35, 45, 55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLC + THF</td>
<td>60, 70, 80</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>15%, 20%, 25%</td>
<td>PLC + DCM</td>
<td>60, 70, 80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PLC + THF</td>
<td></td>
</tr>
</tbody>
</table>

3.2.2 Surface Morphology

Samples were dried in 37°C vacuum oven prior to the conduction of surface morphology study. Film samples were sputtered with gold for 60 s using a sputter coater, SPI-Module model. Coating samples were gold coated for 30 s. Samples then underwent analysis using Scanning Electron Microscopy (SEM) (JSM-6360 and JSM 5410, JEOL USA Inc.). SEM images were observed at 5.0 kV.

3.2.3 Films Thermal Properties

The thermal properties of polymer films and drug-loaded films were investigated using Differential Scanning Calorimetry (DSC). DSC (Q-10, TA Instruments) was used to measure the glass transition temperature ($T_g$) and the melting temperature ($T_m$) of the films. DSC data were obtained from both first and second heating profile of the films; in order to observe the thermal history imparted during the fabrication process and the thermal behavior in molten stage. The steps performed for thermal properties analysis were as mentioned:
Chapter Three: Experimental Materials and Methods

1) For polymer films: samples of 5-6 mg sealed in hermetic pans were first heated from -40 ºC to 180 ºC at 10 ºC/min. The samples were then rapidly cooled to -40 ºC at a cooling rate of 10 ºC/min before ramping back up to 180 ºC again at 10 ºC/min.

2) For drug-loaded films: samples of 5-6 mg sealed in hermetic pans were first heated from -40 ºC to 280 ºC at 10 ºC/min. The samples were then cooled down to -40 ºC at a cooling rate of 10 ºC/min before heating back up to 280 ºC again at 10 ºC/min.

3.2.4 In Vitro Drug Release Studies

Triplicate films of 1cm x 1 cm from each formulation were weighed before immersing in 5ml phosphate buffer saline solution (PBS pH 7.4). The sample vials were placed on a 3-way rotator set at 150 rpm and incubated at 37 ºC throughout the two-week release study. For in vitro release study of coatings, triplicate coatings were weighed before conducting the release and were placed in 5 ml PBS too. The procedures for both in vitro studies were based on the same protocol stated below.

On each predetermined time point, release samples were transferred to vials containing 5 ml of fresh buffer solution and release medium of that time point was collected. The collected medium samples were tested for drug concentration. Aliquots were diluted and filtered through 0.22 µm syringe filters into 2ml HPLC amber crimp vials for quantification using High Performance Liquid Chromatography, HPLC (Agilent 1100 series, Agilent Technologies). Zorbax Eclipse XDB-C18 column (Agilent Technologies) of 5 µm pore size and dimension of 4.6 x 250 mm was used.
Chapter Three: Experimental Materials and Methods

The following steps stated were the operating conditions of HPLC: 30 µl sample injection volume, 80% Phosphate buffer and 20% ACN (v/v) mobile phase (pH 2.5), and a flow rate of 2.0 ml/min. Excitation and emission wavelength for Levofloxacin was set at 295 nm and 440 nm while Moxifloxacin’s was set at 290 nm and 460 nm. Drug concentration was quantified using a Fluorescence Light Detector (FLD).

The detected drug concentration was calculated based on a standard calibration prepared specifically for each drug. For Levofloxacin, the concentration range was 0.25 µg/ml to 20 µg/ml, while the calibration for Moxifloxacin was ranged from 0.25 µg/ml to 10 µg/ml. The equation obtained from each calibration curve was then used to calculate the detected drug concentration and the amount of drug released. The results were plotted against time as the percentage cumulative release and daily amount of drug released.

3.2.5 Quantification of Drug

Drug quantification of Levofloxacin and Moxifloxacin was acquired by the following techniques:

**Levofloxacin**

A set of triplicate films (1cm x 1cm) or coatings was pre-weighed and dissolved in a definite amount of ACN. The solutions were then further diluted and analyzed by HPLC-FLD.

**Moxifloxacin**

A set of triplicate films (1cm x 1cm) or coatings was pre-weighed and left to dissolve overnight in 10ml of THF in 15ml centrifuge tubes to completely dissolve the polymer. The tubes were centrifuged the next day at 6,000 rpm for 10 minutes and were left seated for an
hour to allow suspended drugs to settle. The supernatant was removed; leaving settled drug at the bottom of the tubes and reconstitution was done with 10ml of water. Solutions were then vortex to dissolve the drug, diluted and tested for drug content.

The detected drug concentration from HPLC was then converted to weight using this formula:

\[
\text{Actual loading percentage} = \frac{C_{\text{drug}} \times D \times V}{W_{\text{film}} \times DL\%}
\]

where \(C_{\text{drug}}\) is the HPLC detected concentration, \(D\) is dilution factor, \(V\) is solvent volume, \(W_{\text{film}}\) is initial weight of film and \(DL\%\) is weight percentage of drug loaded in the film.

### 3.2.6 Drug Residual Test

A residual test was conducted at the end of the release study. Samples were taken out at the last time point and rinsed thoroughly with distilled water before drying in 37 ºC vacuum oven for a week.

The extraction and quantification of residual drug loading protocol is as described in section 3.2.5.

### 3.2.7 Coating Fabrication

Drug-eluting IOL coatings are fabricated using Sono-Tek ultrasonic spray coater. The spray solution is the prepared drug-polymer solution. This solution is prepared by dissolving weighed Levofloxacin or Moxifloxacin with PLC granules in THF or DCM to obtain the desired solution concentration of 6 mg/ml and left to stir overnight.
The drug-polymer solution was drawn up by a 10 ml glass syringe and pumped into the spraying channel (liquid inlet) at a flow rate of 0.05 ml/min. The solution was atomized into fine particles by the generator and nitrogen gas is fed through the nozzle orifice. The gas stream draws the spraying liquid to it, forming a narrow spray beam downwards.

The spray coating process is demonstrated in Figure 3-1. The designed disc or mold is mounted onto the mandrel and attached to the rotary drive to move the mandrel translationally and rotationally at 40 rev/min. The center of the disc is aligned to the spray stream before moving translationally and rotationally for a predetermined number of loops to achieve the desired uniformed thickness. Each loop cycle will move the mandrel back and forth from position (1) to position (2). This distance between position (1) and (2) is known as the translational displacement (TD). The coated mandrels were left to dry for a day under ambient conditions before drying in 37°C vacuum oven for a week.

Figure 3-1: Spray coating process
3.2.8 Statistical Analysis

All statistical analysis was performed using GraphPad Prism 5.0 and all *in vitro* data are expressed as mean ± standard deviation (SD).
Chapter Four: *In Vitro* Studies - Drug-eluting Films

4.1 Drug Release Study

The proposed application of drug-eluting device for the delivery of Levofloxacin or Moxifloxacin is to treat post-operative infection in patients; the ideal release profile to achieve is one that follows a zero-order release profile over a course of 2 weeks, with little or no initial burst. However, such a profile is difficult to achieve.

A typical release profile of a drug-eluting device will have an initial burst release phase and a diffusion phase before the onset of polymer degradation. A huge initial burst release is generally undesirable. Fluoroquinolones are generally well tolerated with most adverse reactions being mild to moderate. Common side effects after administration are gastrointestinal intolerance such as nausea, vomiting, and diarrhea, as well as headache and insomnia. However, rare cases of serious adverse events might occur; for instance, spontaneous tendon ruptures, irreversible peripheral neuropathy and anaphylaxis, succeeding the first dose. Therefore, it is vital to avert this situation by maintaining the initial burst release at a minimum level or to eliminate such burst release.

It is preferable if the release from the film or coating exhibits a zero-order release profile for two reasons. The first being, with the initial burst, a sudden burst of drug at any point during the release is deemed undesirable as it leads to overdosing effects that cause unwarranted discomfort to patients. Secondly, the initial burst is a waste of drug, as it exceeds the effective concentration by several-fold.
Targeted dosage

The targeted dosage required daily is calculated for both in vitro studies in Chapter 4 and 5. The concentration of Levofloxacin and Moxifloxacin 0.5% ophthalmic solution is 5 mg/ml. The treatment duration of topical administration is for 14 days. According to the prescription, patients are to apply 8 droplets daily. Assuming that the volume of each droplet is 0.1 ml, the total drug administered amounts to 20 μg. Taking into consideration the 5% bioavailability of eye drops, the amount of drug that reaches targeted site is 1 μg. Therefore, this is the targeted daily dosage for both the device and film.

Physiochemical properties of drug, characteristics of polymeric matrix and release environment are factors that affect the release profile of the system [93-95]. In this chapter, the effect of (1) drug loading and (2) solvent of the film were investigated. During fabrication, viscosity of the solution was also considered. If solution is too viscous, it will be difficult to fabricate and hence, making it unsuitable for applications.

All constituents in film and coating formulations stated in the following sections are expressed in w/w % unless otherwise stated. Films used in the release studies were 220 ± 20 μm.

4.1.1 Surface Morphology

In this section, the surface morphologies of blank PLC-DCM and PLC-THF films, along with drug-loaded films were compared. SEM images of the films’ surfaces were taken to observe the in-depth morphology unobservable by the naked eye. Macro examination or gross examination of the films (Figure 4-1) showed a transparent PLC-DCM film whereas PLC-THF film had an opaque outlook. Under SEM, both films showed no observation of surface
aggregation. The color of Levofloxacin is yellowish-white while Moxifloxacin is bright yellowish; hence the films casted appeared yellowish. Despite being very soluble in DCM, all Levo-DCM films gave rough and porous surfaces. On the other hand, Moxi that is quite insoluble in DCM was able to achieve a smooth film surface for all Moxi-DCM films. When the solvent was switched to THF, both drugs were insoluble in it. However, unlike the DCM films, Levo-THF films gave smooth surfaces. The same goes for Moxi-THF films. Hence, it was concluded that suspensions were preferable compared to dissolved solutions.

From SEM images of Levofloxacin-loaded films, all Levo-DCM films displayed significant porosity and drug aggregation on the surface. As this occurred for all three drug loadings, this suggested that it is not drug loading dependent. Levo-THF films had lesser surface drug aggregation and the morphology of 15% to 25% films looked similar. The surface morphologies of all Moxi-DCM and Moxi-THF displayed little drug aggregation.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>SEM Images (x500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank PLC-DCM</td>
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</tr>
<tr>
<td>Blank PLC-THF</td>
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## Chapter Four: In Vitro Studies – Drug-eluting Films

<table>
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<th></th>
<th>15%</th>
<th>20%</th>
<th>25%</th>
</tr>
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<tbody>
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<td><img src="353x527.png" alt="Image" /></td>
<td><img src="382x409.png" alt="Image" /></td>
<td><img src="409x290.png" alt="Image" /></td>
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<tr>
<td><strong>Levo-THF</strong></td>
<td><img src="258x216.png" alt="Image" /></td>
<td><img src="258x216.png" alt="Image" /></td>
<td><img src="258x216.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Moxi-DCM</strong></td>
<td><img src="258x216.png" alt="Image" /></td>
<td><img src="258x216.png" alt="Image" /></td>
<td><img src="258x216.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Moxi-THF</strong></td>
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<td><img src="258x216.png" alt="Image" /></td>
<td><img src="258x216.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Figure 4-1: Morphology and SEM images (x500) of blank PLC and drug-loaded films

Besides studying the surface morphologies of the formulations before PBS immersion, the surface morphologies after 14 days immersion were also investigated (Figure 4-2). Pores and pits were seen on surfaces of each drug-loaded sample. The images of Levo-DCM films after 14 days immersion showed increased porosity and creation of “cavities”. For Levo-
THF and both Moxifloxacin films, the images displayed slight surface morphological change with appearance of pores formed.

Figure 4-2: SEM images (x500) of drug-loaded films after 14 days immersion
4.1.2 Drug Analysis

High Performance Liquid Chromatography (HPLC) was established as an appropriate testing method and a HPLC method was developed for quantification of drug. Below states the HPLC method for both Levofloxacin and Moxifloxacin:

HPLC Column: Zorbax Eclipse XDB-C18, 5 µm and 4.6 x 250 mm

Mobile Phase: 80% Phosphate buffer: 20% ACN (pH 2.5)

Injection volume: 30 µl

4.1.2.1 Levofloxacin Quantification

For each release study, triplicate set of 13 standards was prepared. Triplicates were done to minimize any measurement errors induced. Calibration standards were ranged from 0.25 µg/ml to 20 µg/ml. The lower detection limit of Levofloxacin is 0.05 µg/ml [96]. Retention time for Levofloxacin was identified to be at approximately 2.3 minutes. Figure 4-3 shows the calibration curve for a single run.

![Levofloxacin Calibration graph](image)

Figure 4-3: Calibration curve of Levofloxacin 0.25 µg/ml - 20 µg/ml
Levofloxacin Stability in Buffer

To investigate the stability of the drug in buffer, the calibration standards were kept at 37 °C. Figure 4-4 shows Levofloxacin stability in buffer over a 4-week period. There were no significant changes in Levofloxacin concentration observed over the testing period. On day 21, drug stability dipped by 1.4%. Overall, this demonstrated that Levofloxacin is stable in buffer.

4.1.2.2 Moxifloxacin Quantification

For each release study, a triplicate set of 7 standards was prepared. Calibration standards were ranged from 0.25 µg/ml to 10 µg/ml. The lower detection limit of Moxifloxacin is 0.2 µg/ml [96]. Retention time for Moxifloxacin was identified to be at approximately 6 minutes. Figure 4-5 shows the calibration curve for a single run.
Moxifloxacin Stability in Buffer

To investigate the stability of the drug in buffer, the calibration standards were kept at 37 °C. Figure 4-6 shows Moxifloxacin stability in buffer over a 4 weeks period. There were no significant changes in Moxifloxacin concentration observed over the testing period. Overall, it demonstrated that Moxifloxacin is stable in buffer.

Figure 4-6: Moxifloxacin stability in buffer
4.1.2.3 DSC Analysis

Differential Scanning Calorimeter (DSC) is a thermo-analytical technique used to determine the modification of a drug during fabrication. Samples of Levofloxacin/Moxifloxacin was sealed in hermetic pans, heated and subsequently cooled before heating again. DSC of Levofloxacin (Figure 4-7) exhibited 2 endothermic peaks. The first peak observed at ~110 °C is due to the increase in pressure in the sealed pan, resulting in dehydration of Levofloxacin and water vapor buildup adsorbed to the sample [97]. A sharp melting point (T_m) peak was observed at 234.47 °C. Upon loading PLC to the drug, the peak measured became broader and smaller. A broader DSC peak suggests a wider distribution of drug particle size within the polymer matrix. Similar to the case of 25% Levo-DCM film, the peak for 25% Levo-THF was also broader.

Figure 4-7: DSC curve of Levofloxacin drug and 25% Levofloxacin films
Moving on to Moxifloxacin, a broad peak was observed at a $T_m$ of 229.60 °C. Comparing the $T_m$ peaks of drug and drug-loaded films, both drug-loaded films exhibited narrower and sharper peaks than the drug’s peak (Figure 4-8). The sharp peak suggested that the drug has crystallized and was in the amorphous phase of the polymer.

![Figure 4-8: DSC curve of Moxifloxacin drug and 25% Moxifloxacin films](image)
4.2  *In Vitro* Drug Release – Effect of Drug Loading

4.2.1 Levofloxacin-DCM Films

Changes in drug loading will affect the initial burst release of the system [93]. In this study, film formulations prepared in DCM were loaded with varying amounts of drug, namely 15 wt%, 20 wt% and 25 wt% of drug to investigate the effects of drug loading on the release profile. An *in vitro* release study was conducted for 14 days.

As depicted in Figure 4-9, all Levo-DCM films (15%, 20% and 25%) unloaded the entire reservoir within 24 hours with extremely high burst release; 99.4 ± 5.5% for 15%, 99.3 ± 2.6% for 20% and 99.9 ± 3.0% for 25%. The daily drug release amount after the initial burst was too little to achieve therapeutic effect. At day 14, the amount of drug released for 15% was 0.1 ± 0.1 µg, 20% was 0.5 ± 0.1 µg and 25% was 0.9 ± 0.2 µg. A sustained release was unachievable with this system, and as such the study was concluded after a week.
Figure 4-9: (a) Cumulative release and (b) Daily drug release of Levofloxacin-DCM films.
4.2.2 Moxifloxacin-DCM Films

Likewise, films of drug formulations, 15 wt%, 20 wt% and 25 wt% were prepared in DCM and an *in vitro* release study was conducted for 14 days. Figure 4-10 (a) shows the cumulative release profiles of Moxifloxacin from films. The results demonstrated that 15% Moxi-DCM film had an initial burst release of 5.6 ± 1.1%. When drug loading was increased to 20% and 25%, the initial burst exhibited increased to 12.9 ± 1.3% and 15.5 ± 2.5% respectively. At day 14, the films each achieved 7.1 ± 1.1%, 30.2 ± 0.8% and 52.0 ± 4.0% of loaded drug. 25% film that demonstrated the desired release profile was chosen for replication. The replicated 25% film exhibited a similar trend as the previous 25% film, with burst release of 28.2 ± 5.0% and 84.3 ± 3.5% of loaded drug released at day 14.

The daily drug releases (Figure 4-10 (b)) for all three formulations were above the 1 µg targeted dosage. Following the burst release, the daily amount of drug release for 15% film for day 2 to 14 ranged from 15 µg to 2 µg. For the 20% film, the drug release amount varies between 810 µg during the burst and 30 µg in the diffusion release phase for subsequent days. The daily drug amount released from 25% film ranges from 1250 µg during the burst release to 165 µg in the subsequent diffusion phase. The replicated 25% film too exhibited a daily drug release amount varying between 2260 µg during initial burst to 160 µg in the subsequent release phase, with a spike increase of drug observed on day 4.

In this case where all formulations meet the targeted dosage, a system that has a lower burst release is more favorable.
Figure 4-10: (a) Cumulative release and (b) Daily drug release of Moxifloxacin-DCM films
4.2.3 Discussion

Typically, in a matrix-dispersion drug delivery system, there are two phases in the drug release profile. The first is the initial burst release, which is present when the drug loading is above the solubility limit of the drug in the matrix. The second phase is the diffusional phase when the dissolved drug molecules diffuse through and out of the matrix.

Increasing the drug loading in the films will result in increase in burst release as demonstrated in the release profile of Moxifloxacin-loaded films. Therefore, a higher burst effect will be observed with the increase drug loading especially above saturation. This is also evident from the SEM images of the films in Figure 4-1. For this study, 15%, 20% and 25% were chosen as the drug loadings, to investigate the release behavior with increment of drug loaded for optimization of the system. When drug loading increased to 25% for both Levofloxacin and Moxifloxacin, there were more drugs aggregating on the surface of the films. Drugs all have a finite solubility in polymers so beyond the solubility limits, additional drug would phase separate from the polymer matrix and aggregate on the surface of the films. During the drying process in fabrication, these phase separated drug particles can migrate to the surface and with buffer infiltration, the surface-segregated particles would dissolve and eluted out quickly into the buffer. Hence, this created an initial burst effect in the release profiles and the burst effect becomes more prominent with higher drug loading.

As mentioned by Higuchi model, drug particles on the surface would diffuse out into the buffer first. Hence, with drug dissociation at the surface and continuous buffer infiltration into the polymer matrix, pores or “cavities” could be seen on the morphologies of the films as illustrated in Figure 4-2. The presence of pores enhanced the permeability of the buffer into
the films and increase in pore formations could increase the diffusivity of drug in the subsequent diffusion release phase following the burst release.

For Levo-DCM films, the morphologies of all three formulations exhibited surface roughness and were porous with visible drug aggregation on the surface. The extremely high burst implies that most of the drug was not dissolved in the film, and was present at the surface. From the results, it could be inferred that the solubility of Levofloxacin in PLC could be well below 15%.

In contrast, Moxi-DCM films did not display much drug aggregation on the surface and hence, the burst effect of all three formulations was minimal compared to Levo-DCM films. The solubility of Moxifloxacin in PLC appears to be between drug loadings of 15% and 20% as not much burst was observed below this solubility limit of loading. The 25% film did show some lack of reproducibility, which could be attributed to the variable solid drug distribution within and on the surface of the films. All drug loadings in this system were able to achieve daily releases above the targeted dosage. However, to optimize the system and to achieve a lower burst release, a lower drug loading will be preferred. As mentioned earlier, a lower drug loading will result in lower burst release. Therefore, from the results obtained in the study, 15% Moxi-DCM film will be the better choice out of all 3 formulations.

The effect of the solvent on the release profiles of both drugs will be discussed in the next section 4.3.
4.3  *In Vitro* Drug Release – Effect of Solvent

4.3.1 Levofloxacin-THF Films

To investigate effect of solvents on surface morphologies of films and their resultant release profiles, a second less volatile solvent is used. The volatility or solvent evaporation rate can influence the surface morphology of polymer films and surface roughness is influenced by the evaporation rate [98]. THF has a higher boiling point than DCM, is less volatile and should form a surface morphology with lesser porosity. The release profiles obtained using THF were studied.

All Levo-THF release profiles demonstrated suppressed burst release and sustained release till day 14 in Figure 4-11. The 15% film exhibited burst release of 18.5 ± 2.1% on day 1. It showed a gradual increase in cumulative release up to day 6 before starting to plateau from day 9 onwards till day 14. The initial burst release of 20% and 25% was 17.3 ± 0.7% and 33.0 ± 1.7%. By increasing the drug loading in films, burst release observed would increase too. The cumulative release of 25% film by day 14 was 74.2 ± 4.2%, 30% more than the final cumulative release of 15% film. This higher cumulative release for 25% is a result of a higher burst and amount of drug loaded, when drug loading is above saturation.

The daily drug releases for all three formulations were above the 1 µg targeted dosage. The daily release for 15% film from day 2 to 14 had amounts ranging from 255 µg to 25 µg. Likewise for the 20% film, the drug release amount after the initial burst had amounts varying between 210 µg and 70 µg. As stated previously, by increasing the drug loading, the amount of drug in the film will increase too. The daily drug release of the 25% film ranges from 2009 µg observed during the burst to 85 µg in the subsequent release days.
Figure 4-11: (a) Cumulative release and (b) Daily drug release of Levofloxacin-THF films
Effect of solvents on the *in vitro* release profiles was compared using the 25 wt% film. From Figure 4-12 (a), 25% Levo-DCM film eluted most of the drug within 24 hours whereas 25% Levo-THF film demonstrated significantly lower burst release of 33.0 ± 1.7% %; a third lesser. Following the burst release, drug released from Levo-THF was from 33.0 ± 1.7% at day 1 to 74.2 ± 4.2% at day 14.

Due to the unloading of the reservoir on day 1, the amount of drug released from Levo-DCM was approximately 6900 µg; thrice the amount released from Levo-THF (Figure 4-12 (b)). A plateau was then observed thereafter and the study was halted after day 7. In contrast, the daily release from the THF system exhibited daily drug amount above the targeted dosage for entire 14 days.
Chapter Four: *In Vitro* Studies – Drug-eluting Films

Figure 4-12: Comparison of (a) Cumulative release and (b) Daily drug release between Levo-DCM and THF films
4.3.2 Moxifloxacin-THF Films

Likewise for Moxifloxacin films, the effect of solvents on the release profiles was investigated. Figure 4-13 illustrates the cumulative and daily drug release from 15%, 20% and 25% loaded Moxi-THF films. The 15% film exhibited a burst release of $7.4 \pm 0.3\%$ and the burst release of 20% and 25% on day 1 was $14.4 \pm 0.9\%$ and $27.1 \pm 1.6\%$ respectively. As mentioned earlier, a higher drug loading would generally demonstrate a higher cumulative release; with the 25% film releasing 61% more than the 15% film. The 15% film displayed a gradual plateauing in the diffusion release phase after the burst release. By day 14, the respective cumulative drug release for 15%, 20% and 25% film was $11.5 \pm 0.6\%$, $32.8 \pm 1.3\%$ and $73.3 \pm 3.4\%$.

From Figure 4-13(b), the daily drug releases for all three loadings were above targeted dosage of 1 µg. After the initial burst, 15% film released daily amounts ranging from 30 µg and 4 µg. The burst release of 20% film released $757.2 \pm 16.3 \mu g$ on day 1 and subsequent releases ranged from $140 \mu g$ to $27 \mu g$, with the exception of day 6 (220 µg). 25% film exhibited a more desirable release profile and the system released daily drug amounts ranged from $1760 \mu g$ during the burst release to $45 \mu g$ in the subsequent diffusion phase.

Effects of different drug loadings in the THF also resulted in different release profiles. As reported in the previous section on effects of drug loading, the initial burst release and subsequent diffusional release phase are highly dependent on the amount of drug loading and drug solubility limits.
Figure 4-13: (a) Cumulative release and (b) Daily drug release of Moxifloxacin-THF films
The effect of solvents on the release profiles of Moxifloxacin-loaded films was compared using 25 wt% films.

![Moxifloxacin Release Profile](image)

**Figure 4-14:** Comparison of (a) Cumulative release and (b) Daily drug release between Moxi-DCM and THF films

Both 25% films in DCM and THF exhibited similar release trend as illustrated in Figure 4-14 (a). The initial burst release from both systems was similar too and they displayed only a
slight difference of 1%. By day 14, the 25% Moxi-DCM film released $84.3 \pm 3.5\%$ of loaded drug while Moxi-THF film released $73.3 \pm 3.4\%$ of loaded drug. The cumulative drug release difference was 11%.

The daily drug release from both systems was also compared in Figure 4-14 (b). Since the burst release from Moxi-DCM film was higher, the resultant amount released was also higher. The daily release from Moxi-THF showed a gradual decrement in the release diffusion phase. Moxi-DCM, however, a sudden spike in drug amount was observed on day 4 before displaying a downtrend for the subsequent days. The daily release amount of both Moxi-DCM and Moxi-THF was above the targeted dosage for full 14 days. From the comparison, it could be concluded that solvents in this case had a much lesser influence on the release profiles than in Levofloxacin films. This could be explained by the lower solvent evaporation rate and higher drug solubility in the polymer.

### 4.3.3 Discussion

Levofloxacin is highly soluble in both DCM and THF. The solubility limit of Levofloxacin in DCM and THF were investigated to be 68 mg/ml and 27.9 mg/ml respectively. As mentioned under the solvent section in the literature review, DCM is more volatile compared to THF. The lower boiling point of DCM lead to a faster evaporation rate and hence, resulted in the creation of drug aggregation and porosity on the film surface. It is postulated that the fast evaporation of DCM causes migration of drug particles to the surface and thus, entrapping the particles on the surface. In addition, it is also hypothesized Levofloxacin undergoes phase separation when the DCM evaporates. Therefore, with buffer infiltration, the surface-segregated particles elutes quickly into the buffer and result in a high initial burst
effect for Levo-DCM films. For higher drug loading, this effect is enhanced as more drugs accumulate on the surface of the films. Burst effect would be greatly lowered if the drug particles are entrapped within the polymer matrix and when drug aggregation and porosity are lesser on the surface of the films. From the results obtained from Levofloxacin films, burst releases from films are highly dependent on the surface morphology, solubility of the drug in the solvent and in the polymer.

The solubility of Moxifloxacin in DCM and THF was investigated to be 0.42 mg/ml and 0.016 mg/ml. For both Moxi-DCM and Moxi-THF, the amount of drug had exceeded their respective solubility limits; however from the results, it was demonstrated that solvents do not have much drastic influence on the release profiles. Unlike Levofloxacin films, the surface morphology of Moxifloxacin films obtained from both solvents did not differ greatly. The cumulative releases and daily drug releases from both 25% DCM and THF films demonstrated a trend akin to each other. Both films released drug amounts above the targeted dosage for 2 weeks. These findings could be supported by the presence of lesser surface-segregated drug particles and that the undissolved drugs were entrapped and distributed within the polymer matrix. The solubility of the drug in polymer could be drawn to be at a high level (~ 20%). Therefore, the release rates from the films are governed by diffusion of dissolved drug through the bulk to the surface since the duration of the studies were too short (2 weeks) to consider degradation as a major contributing factor for the release profiles.
Chapter Five: *In Vitro* Studies - Drug-eluting IOL Coatings

### 5.1 Coating Fabrication

In this work, the objective is to fabricate a ring-like biodegradable coating to sleeve over the commercial IOL, which can provide a sustained antibiotic release over a period of 14 days.

In this chapter, the coating morphology, the effect of the spray coating process variants i.e. flow rate, TD and loops and the *in vitro* studies will be discussed.

### 5.2 Drug-eluting Coating Design

The ideal IOL design is illustrated in Figure 5-1; the IOL coating will be looped around an IOL prior to insertion into the eye. This deployment of the IOL and the coating as a single entity solves the issue of patient non-compliance occurring from eye drops. The implantation at the localized site and the close proximity of the drug eluting coating around the IOL will improve drug availability and efficacy. Improvement in drug availability due to close proximity will also keep required therapeutic dose to a minimal.

![Figure 5-1: Schematic diagram of an ideal IOL coating attachment](image)
Chapter Five: *In Vitro* Studies – Drug-eluting IOL Coatings

The IOL coating consists of 3 key features as illustrated in Figure 5-2:

1) **Circular ring structure**

   The circular ring structure serves as the primary drug depot for the elution of sustained therapeutic drug dose over a 2-week period. The thickness of this ring structure has been pre-determined to be 200 µm to ensure sufficient drug loading while not obstructing the deployment process and patient’s vision once inserted into the eye.

2) **Hook structures that ring around the circumference of the ring structure**

   The coating is also designed with a hook structure that runs along the circumference of the ring structure. The hook structure is critical to ensuring the following:
   
   A) Coating is banded to the IOL throughout the drug release period
   
   B) To minimize accidental dislodgment of coating during deployment of the coating-IOL assembly

   With that in mind, two different tapered rings were designed to create a hook structure as shown in Figure 5-2 for the coating procedure.

3) **2 haptics holes to accommodate the haptics**

   The insertion of haptics holes allows proper securing of the coating to the IOL. It is to act as a safety precaution; in cases of adverse events such as coating dislodgement, the coating will still be around the IOL and not displace away from it. This will allow easy coating placement recovery or if deem appropriate, let the coating function as intended and degrade over time.
Figure 5-2: Schematic diagram of IOL features
### 5.2.1 Effect of Mandrel Designs

<table>
<thead>
<tr>
<th>Mandrel</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Mandrel A Schematic" /></td>
<td><img src="image2" alt="Mandrel B Schematic" /></td>
<td><img src="image3" alt="Mandrel C Schematic" /></td>
<td></td>
</tr>
<tr>
<td><img src="image4" alt="Mandrel A Cross Section" /></td>
<td><img src="image5" alt="Mandrel B Cross Section" /></td>
<td><img src="image6" alt="Mandrel C Cross Section" /></td>
<td></td>
</tr>
<tr>
<td>Diagrams of resultant coating</td>
<td><img src="image7" alt="Mandrel A Coating" /></td>
<td><img src="image8" alt="Mandrel B Coating" /></td>
<td><img src="image9" alt="Mandrel C Coating" /></td>
</tr>
</tbody>
</table>

Figure 5-3 shows the designs of the mandrels used. Mandrel A and B were designed with opposing mandrel edges to create a more defined hook structure while mandrel C was designed with a 90° straight edge. Following preliminary feasibility evaluation of the different mandrel design, A and B were unable to obtain a good and consistent hook structure. The two haptic extensions included in the design A and B were to account for the haptics of
the IOL so that the coating will banded not only to the circumference of the ring but also to the haptic optic junction for better securing of the coating to the IOL.

On the contrary, mandrel C was able to maintain a consistent formation of the hook structure. Although the hook structure was smaller than desired, preliminary trial of integrating the coating over the IOL suggests that this coating design was able to maintain a consistent securing of the coating on the IOL. Therefore, the ribbon ring (Figure 5-4) was proposed.

![Schematic diagram of a ribbon ring around IOL](image)

**Figure 5-4: Schematic diagram of a ribbon ring around IOL**

### 5.2.2 Coating Prototype

A proof of concept (POC) was conducted by assembling the coating to the IOL. The assembly was done in the following steps to sleeve the coating over an existing commercial IOL. Drug-polymer solution was first spray coated onto mandrel C to form the IOL coating attachment and the mandrel was left to dry. Before the removal of the dried attachment, 2 haptic holes were created on the attachment for the haptics of the IOL to protrude outwards and excess coating on the mandrel was trimmed off. This dried attachment was then sleeved
over the circumference of the IOL. The prototype is illustrated in Figure 5-5.

Figure 5-5: Assembly of coating to IOL

5.3 Coatings Morphology

In Chapter 4, the effect of solvents, DCM and THF on the films’ morphologies and release profiles were studied. In this chapter, coatings will be fabricated using similar solvent for release profile comparison between both systems. Similarly, coating morphology analysis will be carried out using SEM for comparison. There are several coating parameters that could affect the coating outcome and they have to be optimized. This will be elaborated in the following sub-sections. In the interest of this thesis, unless specified, only results pertaining to mandrel C will be discussed.
5.3.1 Effect of Spray Coating Flow-rates

Ideally, a low flow-rate is preferred to produce the best coatings as it reduces webbing and maximizes efficiency of material transfer. Overly high flow-rate is undesirable as it results in bulk and excess coating on the mandrel instead of a precise and uniform coating.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Flow-rate</th>
<th>DCM</th>
<th>THF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 ml/min</td>
<td><img src="image" alt="A" /></td>
<td><img src="image" alt="B" /></td>
</tr>
<tr>
<td></td>
<td>0.05 ml/min</td>
<td><img src="image" alt="C" /></td>
<td><img src="image" alt="D" /></td>
</tr>
</tbody>
</table>

Figure 5-6: SEM images (x400) of coating morphologies

The investigation of flow-rates and solvents on the coating morphology was fabricated using translational velocity of 0.004 cm/s and rotational speed at 40 rev/min. These 2 parameters were based on previous work on equipment process parameters characterization. In Figure 5-6, flow-rate of 0.1 ml/min resulted in webbing for both PLC-DCM (A) and PLC-THF (B) coatings. The higher flow rate also resulted in an “orange peel” outlook that is undesired for consistent drug release profiling. The flow-rate of 0.05 ml/min, on the other hand, presented better morphologies for both coatings C and D. Thus, a smoother and more uniform coating morphology was obtained. These findings affirmed that higher flow-rate results in unfavorable coating morphology and lower flow-rate should be employed to achieve optimal
coating conditions. After narrowing down the flow-rate to 0.05 ml/min, the morphology of PLC-DCM and PLC-THF coatings were evaluated. The surface morphology of PLC-THF coating was the better out of the two. This is consistent with the findings from previous chapter on film studies where films fabricated from THF solvents tends to form a smooth surface and thus, THF would be the preferred choice. THF was elected as the solvent of choice for the in vitro studies in this chapter.

5.3.2 Effect of Translational Displacement

Besides investigating the effect of flow rate on the coating morphology, the effect of another process variant, translational displacement (TD) on the coating thickness was investigated. TD will affect the width of the coating. This will have a direct effect on the formation of the hook structure i.e. longer TD will result in more sideward polymer deposition which will result in a larger and longer hook structure. Therefore, an appropriate TD is necessary for the formation of the desired hook structure. This study was conducted using mandrel B and 6 mg/ml solution of PLC in THF with the number of loops kept constant at 32 loops. The different TDs carried out were 0.1 cm, 0.2 cm and 0.3 cm (Figure 5-7).
As depicted in Figure 5-7, changes in TD affects the ring and hook thickness. When TD increased from 0.1 to 0.3 cm, the coating thickness and hook thickness increased. The process time and spraying coverage on the mandrel is greater with a higher TD. With a longer TD, more polymer deposition will occur on the top portion of the coating, resulting in a higher degree of thickness before gradually elongating the coating. From the coating, a correlation between the TD and coating thickness was established for THF. This is represented in Figure 5-8. Generally, as expected the longer the TD, the thicker is the resulting coating thickness. This is due to the longer spraying duration per coating cycle and the slow translational velocity and rotational speed.

Intuitively, the hook structure will also be thicker when the TD becomes larger. However, the hook structure, in this case, appeared to be independent of the TD. This could be due to the wettability of the coating solution. The polymer solution tends to agglomerate to the main matrix rather than spreading to the sides of the mandrel. This is evident from the small increment in hook thickness when TD increased from 0.2 to 0.3 cm and the thickening of the ring structure.
Figure 5-8: Effect of TD on (a) coating thickness and (b) hook thickness.
5.3.3 Effect of Coating Loops

The influence of the number of loop cycles on the coating thickness was explored. Predicate studies have shown that with increasing number of loops, the thickness of the coating increases too. This study aims to investigate the effect of coating loops on coating thickness and to establish a correlation between coating loops and coating thickness. This study also aims to identify the optimal coating loops required to achieve the desired 200 µm coating thickness. This study was conducted using 6 mg/ml solution of PLC in DCM.

![Figure 5-9: Effect of number of loops on coating thickness](image)

Figure 5-9 shows a direct correlation was established between the number of loops and the coating thickness. As expected, with increasing loop cycles, the amount of polymer deposited on the mandrel will increase and hence, a thicker coating was produced. With higher number of loops, the spraying duration is longer and this allows more polymer solution to accumulate on the surface of the disc rather than the sides of the mandrel. Hence,
this led to thicker resulting coating thickness. From this study, it is demonstrated that to achieve the desired thickness of 200 µm, a coating loops of 20 is required.

5.4  *In Vitro* Drug Release

Triplicates of coatings were immersed in vials containing 5 ml PBS and placed on a 3-way rotator set at 150 rpm and incubated at 37 ºC throughout the two-week release study. This *in vitro* section is divided into 2 sub-sections: Levofloxacin-loaded coatings and Moxifloxacin-loaded coatings.

5.4.1 Levofloxacin-loaded Coatings

5.4.1.1 Single Drug Layer Coating

Levofloxacin-loaded coatings were fabricated using THF as the solvent with a predetermined loop cycles of 25. In this study, coatings were loaded with varying amounts of drug, namely 20 wt%, 25 wt% and 30 wt% of drug. The selection of the drug loadings was for comparison with the film release data and these loadings were evaluated for 14 days.

Figure 5-10 shows the release profiles of the 3 formulations. It is notable that for all drug loadings, a high burst release was observed on the first day. Most of the drug encapsulated within the depot was released. The initial burst release of 20%, 25% and 30% were 78.4 ± 1.0%, 68.6 ± 3.8% and 75.5 ± 2.9% for respectively. The overall percentage release on the second day for 20%, 25% and 30% were as followed: 78.5 ± 1.0%, 68.7 ± 3.7% and 75.6 ± 2.9%, all giving a 0.1% increment from day 1 to day 2.
The amount of drug release on day 1 for 20%, 25% and 30% were 782.4 ± 6.3 µg, 920.3 ± 2.2 µg and 1230.0 ± 31.1 µg respectively. Following the burst, the amount of drug released from this system was very little to achieve a sustained release over 2 weeks. Moreover, the subsequent release after day 2 fell below the lower detection limit of the HPLC and thus, the study was concluded after two days.

Figure 5-10: Release profile of single 20%, 25% and 30% Levofloxacin layer: (a) Percentage cumulative release, (b) Daily drug release
A second study was conducted by decreasing the drug concentration to 3% and 5%. From the first study, it was shown that the burst release effect from the three drug loadings was huge and a sustained release system was not achievable. Therefore, a lower drug loading might be able to reduce the burst release effect and exhibit a desirable release profile.

Figure 5-11 illustrates the cumulative release and daily release from 3% and 5% drug loading. The initial burst releases on day 1 for 3% and 5% were 14.7 ± 0.7% and 51.6 ± 7.1%. Comparing the initial burst of 3% Levofloxacin coating to the 30% coating, the reduction in drug concentration greatly decreased the burst amount by approximately 60%. At day 14, both coatings released 36.4 ± 0.1% and 69.9 ± 3.0% of loaded drug respectively.

Moving on to the daily drug release profile, both 3% and 5% were above 1 µg targeted dosage. The initial burst resulted in an amount of 20.7 ± 0.9 µg for 3% and 119.0 ± 15.9 µg for 5% on day 1. Following the burst release, the subsequent drug release for the 3% coating from day 2 to 14 ranged from 15 µg and 1 µg. As for the 5% coating, the drug release amount varied between 120 µg during the burst and 1 µg in the subsequent diffusion release phase. Therefore, by decreasing the drug loading, burst release was lowered and a prolong drug release period of 14 days was achieved.
Chapter Five: *In Vitro* Studies – Drug-eluting IOL Coatings

(a) **Levofloxacin Release Profile**

(b) **Levofloxacin Release Profile**

In the next section, the effect of coating blank polymer layers on the top and bottom of the drug layer was investigated. The blank polymers layers are coated to suppress the huge burst release from high drug loading formulations and to achieve a sustained release system.
5.4.1.2 Levofloxacin-loaded Coating “Sandwich” System

To overcome the burst release presented in the high drug-loaded single Levofloxacin layer, a “sandwich” system was proposed. With the drug layer as the middle section, blank polymer layers were coated on the top and bottom of this drug layer. This is known as the “sandwich” layering system. It helps to suppress the burst release and to retard the rate of diffusion from the coating to the buffer. In this study, PLC was first coated on the mandrel before coating the middle 25% drug layer and coating another PLC layer on the top of it. The schematic diagram of the Levofloxacin “sandwich” system is illustrated in Figure 5-12. In order to achieve this sandwich configuration, fabrication was carried out over a span of 3 days. Each layer was allowed a day to dry before the addition of the subsequent layers.

![Figure 5-12: Schematic diagram of Levofloxacin sandwich configuration](image)

2 different sandwich configurations were evaluated against the single 25% Levo layer in this study. These 2 configurations are as follows: 30 µm blank-25% Levo-30 µm blank and 40 µm blank-25% Levo-40 µm blank. The initial burst release on day 1 for the 25% Levo layer was 79.7 ± 14.7%. On the other hand, the initial burst for 30 µm-25% Levo-30 µm and 40 µm-25% Levo-40 µm were 36.3 ± 3.9% and 26.8 ± 2.0% respectively. The difference in the burst effect between the 2 sandwich configurations was approximately 9.5%, with the thicker polymeric layer exhibiting a lower burst release. As the burst release from the 25% Levo layer was huge, the subsequent release after day 2 was lower than the HPLC detection limit.
and therefore, the cumulative release profile was only up to day 2. At day 14, both sandwich systems achieved 69.4 ± 1.4% and 41.1 ± 0.2% of loaded drug.

As shown in Figure 5-13, the drug release from 25% Levo on day 2 following the huge burst release was below the targeted dosage with an amount of 0.85 ± 0.5 µg. For 30 µm-25% Levo-30 µm, the amount of drug released varies between 500 µg during the burst and 0.9 µg in the subsequent diffusion release phase. In the diffusion phase, it was able to achieve drug amounts above the targeted dosage up to day 9, with day 10 to 14 giving an average of 0.9 ± 1.3 µg. Comparatively, 40 µm-25% Levo-40 µm had a lower burst release and was also able to meet the required dosage for 9 days. It is noticeable that there was a sudden increase in drug amount to 11 µg on day 7 to 9 with the remaining days releasing an average of 0.5 ± 0.4 µg.
Figure 5-13: Release profile of sandwich Levofloxacin coatings: (a) Percentage cumulative release, (b) Daily drug release

5.4.1.3 Discussion

i) Single Layer Levofloxacin-loaded Coatings

For the first release study of Levofloxacin-loaded coatings, single drug layer consisting 20 wt%, 25 wt% and 30 wt% of drug exhibited a huge burst release on the first day. In this study, a huge burst release on day 1 was observed and most drugs were unloaded from the depot within the first 24 hours. Following the burst, the drug release after day 2 from all 3 formulations was very little to be detected. The exhibition of such release behavior could be supported by two possibilities. The first postulation for the high burst release is the migration of drug to the surface of the coatings. As mentioned in the previous *in vitro* study, the evaporation rate of the solvent in during drying process can influence the release behavior of
the system. During the drying process, drug particles could migrate to the surface, leading to segregation of particles on to the surface of the coatings.

The second postulation is the solubility of the drug in the polymer. If the solubility of the drug in the polymer is poor, the additional undissolved drug not entrapped within the polymer matrix will phase separate to the surface of the coatings. Hence upon immersion in buffer, the huge amount of drugs on the surface will dissolved and released out quickly into the buffer and creates a huge initial burst (Figure 5-14).
For the second release study, the reduction of the drug loading to 3% and 5% was able to decrease the huge burst release observed in the higher drug loadings. Compared to 5% Levo coating, the 3% coating have significantly reduced the burst release. From the results, it could be inferred that the solubility of Levo in PLC is low (~ <5%). The reduction of drug loading helps to reduce and control the burst effect and a sustained release was achieved. The result from 3% loading was the best out of all formulations.
ii) **Sandwich Levofloxacin-loaded Coatings**

The “sandwich” system was employed to suppress the burst release presented in the single Levofloxacin layer and to obtain a sustained delivery of drug. From the results, the incorporation of blank polymer layers to the middle drug layer decreased the initial burst observed with just the single drug layer. The presence of blank polymer layers significantly retard the initial burst release by $43.5 \pm 10.7\%$ and $52.9 \pm 12.6\%$ for $30 \mu m$-25% Levo-30 $\mu m$ and $40 \mu m$-25% Levo-40 $\mu m$ respectively. These polymer layers slow down the diffusion of the drug into the medium and hence, reducing the burst effect on the first day. Theoretically, a thicker polymer layer will suppress the burst release more efficiently because the time the drug needs to diffuse into the medium is longer. Between the two formulations of $30 \mu m$-25% Levo-30 $\mu m$ and $40 \mu m$-25% Levo-40 $\mu m$, the thicker polymer layer lowered the burst to $26.8 \pm 2.0\%$. Although the blank polymer coatings were able to significantly reduce the initial burst release, it also impeded the subsequent drug diffusion. Drug diffusion retardation in the blank polymer systems was evident in the observed results. A total of 79.8% of drug was released from the 25% single layer while a lesser drug of 69.4% and 41.1% was released from the $30 \mu m$ and $40 \mu m$ system respectively.

While the “sandwich” system can help to retard the initial burst release, this mechanism is only effective when the coating is deployed immediately for treatment without extended storage condition. Studies have shown that during coating storage, the drug particles within the drug layer will diffused into the blank polymer layers and thus, reaching an equilibrium [86, 99]. Figure 5-15 shows the migration or diffusion of drugs into the polymer layers with time. This storage problem can be overcome by controlling the storage conditions such as freezing the coating. This method will significantly minimize this drug equalization phenomenon.
5.4.2 Moxifloxacin-loaded Coatings

5.4.2.1 Release Study I

An *in vitro* release study was also conducted for Moxifloxacin-loaded coatings. They were fabricated using THF as the solvent with different drug loadings of 15 wt%, 20 wt% and 25 wt%. From the coatings morphology study, THF was standardized as the solvent choice of all *in vitro* studies for coatings.

Figure 5-16 illustrates the release profiles of the 3 drug loadings. The initial burst release on day 1 for 15%, 20% and 25% were 1.0 ± 0.3%, 1.0 ± 0.6% and 1.9 ± 1.1% respectively. The overall percentage cumulative releases for all 3 formulations at day 14 were 2.2 ± 0.6%, 2.2 ± 1.2% and 3.0 ± 1.8%.

Moving on the daily drug release, all 3 formulations were unable to reach the targeted dosage of 1 µg. 15% Moxi coating was above the targeted dosage for 2 days, with amount varying from 0.95 µg to 0.2 µg. Both 20% and 25% coating only released above the targeted dosage for both day 1 and day 3. The drug release amount for 20% from day 4 to 14 ranged between 0.4 µg and 0.2 µg. As for 25%, after the initial burst, the drug release amount varied between 6.5 µg on day 3 and 0.27 µg in the diffusion release phase.
Chapter Five: *In Vitro* Studies – Drug-eluting IOL Coatings

(a) Moxifloxacin Release Profile

![Moxifloxacin Release Profile](image)

(b) Moxifloxacin Release Profile

![Moxifloxacin Release Profile](image)

Figure 5-16: Release profile of 15%, 20% and 25% Moxifloxacin-THF coatings: (a) Percentage cumulative release, (b) Daily drug release

The release behavior and large standard deviation in the release profiles could be due to the drug solutions. For all three drug solutions, the amount of drug has exceeded the drug’s solubility in the solvent and resulted in oversaturated solutions. As mentioned in the films discussions, Moxi solubility in THF was 0.016 mg/ml. Since the solubility of Moxi in DCM
was higher at 0.422 mg/ml, a second study using DCM as the solvent was conducted and will be discussed in the next section.

5.4.2.2 Release Study II

A second study was carried with the solvent as DCM and fabricated in the same fashion was the first Moxi release study. However, due to the solubility limit constraint of the drug in DCM, the release study was carried out with the drug concentration set at 5% drug loading.

(a) Moxifloxacin Release Profile

% Cumulative Drug Release

Timepoint/days
Unlike the previous study with THF, the release profile of this 5% coating demonstrated a sustained release; however unfortunately it was unable to achieve the desired release profile of releasing above 1 µg. From Figure 5-17, the initial burst release on the first day was 2.6 ± 0.5%. The final percentage cumulative release on day 14 was 6.7 ± 1.8%. Following the burst of 5.0 ± 0.8 µg on day 1, the daily drug amount released from this coating was too little to match the targeted dosage, with amount ranging from 1.26 µg to 0.45 µg in the diffusion release phase.
5.4.2.3 Discussion

In the first study, THF was used as the solvent. However, the drug loadings have far exceeded the drug’s solubility in the solvent. Therefore, very little amount of drug was dissolved in the solution and this resulted in an insignificant amount of drug coated onto the mandrel. This was supported by the low daily drug release in the previous section. Due to the variation of drug distribution in individual samples within the same formulation, a large standard deviation was found in all 3 systems. This greatly affected the release profiles and the higher burst release found in 25% could be due to the presence of more undissolved drug on the surface of the coating.

Due to the very poor solubility of Moxi in THF, the solvent for the second study was changed to DCM. Although Moxi has poor solubility in DCM too but its solubility limit is higher compared to THF and this allows more drugs to dissolve in the solvent. Moxi-THF and Moxi-DCM coatings were unable to meet the targeted dosage for the entire study. Comparing the release profiles between both systems, Moxi-DCM coating presented a more desirable profile. It demonstrated a slow gradual release and from both results, there appears to be an affinity between the drug and the polymer. Therefore, from the findings, the solubility of the drug in the polymer could be inferred to be high.
Chapter Six: *In Vitro* Studies Comparison

In the previous chapter 4 and 5, *in vitro* studies of films and coatings were evaluated individually. In this chapter, a cross comparison will be done between the films and coatings.

### 6.1 Films and Coatings Comparison

The discussion of the comparative study between the films and the coatings will be split into 2 parts: 1) Levofloxacin systems and 2) Moxifloxacin systems.

#### 6.1.1 Levofloxacin Systems

The *in vitro* release profile of the THF films and the coatings will be evaluated against each other. The film study was necessary to study the release behavior of the drugs and its affinity with the polymer and these could be translated to the coating work.
Chapter Six: *In Vitro* Studies Comparison

Figure 6-1 illustrates the cumulative release and the daily drug release of the films and the coatings. First, comparing the cumulative release between the films and the coatings, the higher drug-loaded films showed a more suppressed initial burst release than the coatings. In addition, they also demonstrated a gradual sustained release up to day 14. The higher drug-loaded coatings, on the other hand, unloaded most of drug in the reservoir within 24 hours and as such, a lower drug loading study was conducted for the coatings. 3% and 5% coatings exhibited similar release trend with lower burst effect compared to their peers. Only 3% has an initial burst that is lower than all 3 loadings in the films.
Similarly to the films, higher drug loading in coatings resulted in higher amount of drug released on day 1. However, higher drug-loaded coating formulations released amounts lesser than the target of 1 µg throughout the study and were unable to achieve therapeutic efficacy. Contrastingly, for films, all formulations were able to achieve above the target for whole 2 weeks.

The difference in the results obtained from both *in vitro* studies could be explained by 3 possible reasons. First, the fabrication process of the films and the coatings are different. For solvent casting, drug suspension in the THF-polymer solution can be casted into films. Undissolved drug was encapsulated within the polymer matrix and between each film layers. On the other hand, for spray coating process, oversaturation of drug within the THF-polymer solution is unacceptable. In the case of film fabrication process, the drug distribution within the films is heterogeneous, whereas the drug distribution in coating process will be more uniform and homogenous. Second possible reason for the difference could be the rate of solvent evaporation during drying. During drying, the rate of solvent evaporation for spray-coated samples might be quicker than in films to result in drug migration and aggregation of the surface. Thus, a huge burst release was observed.

The third reason could be explained using the ratio of drug over surface area (SA). The drug/SA ratio of films and coatings are calculated and tabulated in Table 6-1.

\[ SA_{\text{film}} = 2 \text{ cm}^2 \]

\[ SA_{\text{coating}} = 0.379 \text{ cm}^2 \]
From Table 6-1, it has shown that the drug/SA ratios for all drug loadings in coatings are higher than the values obtained by films. The percentage difference calculated between the films and coatings too demonstrated an increasing trend as the drug loading increased.

These findings supported the release profiles obtained. As demonstrated by 20% and 25% formulations, the 20% film exhibited burst release of 17.3% compared to 78.4% of burst release for 20% coating. Similarly in the case of 25% film, it exhibited 33.0% of burst release compared to 68.6% of burst release for 25% coating. As a consequence of greater drug accumulation on the surface that led to higher drug/SA ratios, higher burst effect was observed in all coating formulations. Hence, the results obtained in films were not translated into the coatings.
6.1.2 Moxifloxacin Systems

The *in vitro* release profiles of the Moxi-THF films and the coatings will be evaluated against each other too.

![Comparison of release profiles between Moxi films and coatings](image)

*Figure 6-2: Comparison of release profiles between Moxi films and coatings*

Figure 6-2 illustrates the cumulative release and the daily drug release of the films and the coatings. Comparing the cumulative release profile of films and coatings, it could be observed that the release trend from the films gave a more desirable profile and by day 14, the 25% film achieved $73.3 \pm 3.4\%$ while the 25% coating achieved $3.0 \pm 1.8\%$. The coating formulations displayed a plateau behavior after day 3. Moving on to the daily drug release, the amounts released from all 3 coatings were unable to meet the targeted dosage and were far lesser than the films. Noticeably for the 25% coating, the initial burst amount was 18 \( \mu g \).
whereas the 25% film released 1761 µg on day 1. In addition, all 3 film formulations are above the required dosage for the entire release period. It is also interesting to note the difference in release behavior of the 2 systems, despite having the same poor solubility issue in THF.

As mentioned in Levo comparison study, oversaturation of drug within the THF-polymer solution is not acceptable for spray coating. In the case of film fabrication process, despite the low drug solubility in THF, the oversaturated drug in the THF-polymer solution can be casted into films. Moxifloxacin, in this instance, was encapsulated within the polymer matrix and between each film layers. On the other hand, this drug suspension could not be effectively coated onto the mandrel. Most of the undissolved drug will agglomerate inside the coating syringe and adhere to the walls of the syringe. As a result, the solution coated on the mandrel will only consist of the drug loading that is equivalent to the drug solubility in the solvent. Therefore, the amount of drug released from Moxi-THF coating is significantly lower than what was expected.

### 6.2 Drug Comparison

As mentioned previously, the respective solubility of Levofloxacin in DCM and THF are 68 mg/ml and 27.9 mg/ml while for Moxifloxacin, the solubility in DCM and THF was investigated to be 0.42 mg/ml and 0.016 mg/ml. From these findings, it is concluded that Levofloxacin is more hydrophilic than Moxifloxacin and it is able to attain a higher drug loading that is still soluble in the solvents.

For the drug comparison, the release profiles obtained from films will be used for the evaluation (Figure 6-3).
25% Levo-DCM film unloaded its entire reservoir on day 1 and gave a huge burst effect. Comparing to 25% Moxi-DCM film, it was unable to achieve a sustained release. Since Levofloxacin had a higher solubility in DCM than Moxifloxacin, this result was unexpected. On the other hand, 25% Moxi-DCM was able to release drug amounts higher than the targeted dosage throughout the study, despite being a suspension.

Moving on to the films fabricated using THF; the drugs respectively formed a suspension in the THF-polymer solution. Both have exceeded their respective solubility in the solvent. Their cumulative release trend is relatively similar and both were able to release drug amounts higher than the targeted dosage. However, their difference lies in the initial burst;
25% Moxi-THF has a lower initial burst release of 27.1 ± 1.6% compared to 33.0 ± 1.7% for 25% Levo-THF.

The findings could be supported by the SEM images in Chapter 4. All Levo-DCM films presented pores and drug aggregation on the surface. Hence, a huge burst release was observed on day 1. The burst release in Levo-THF film was lower because its surface did not have severe drug aggregation. Moxi-DCM and Moxi-THF too did not exhibit severe surface drug aggregation and therefore, a huge burst release was not observed. Judging from the release profiles, Levofloxacin has poorer solubility in PLC than Moxifloxacin.
Chapter Seven: Conclusions and Recommendations

7.1 Conclusions

In this section, conclusions are drawn for both in vitro works done for this project in section 7.1.1 and 7.1.2.

7.1.1 In Vitro Studies – Drug-eluting Films

In this in vitro chapter, the method for both drug quantifications using HPLC quantification were developed and validated. The respective ranges of working linear drug calibrations for Levofloxacin and Moxifloxacin were 0.25 µg/ml to 20 µg/ml and 0.25 µg/ml to 10 µg/ml. Levofloxacin and Moxifloxacin were established to exhibit peaks at respective retention time of approximately 2.3 mins and 6 mins. Both drugs demonstrated to have good stability in buffer over a period of 4 weeks. Surface morphologies of blank PLC-DCM, PLC-THF films and drug-loaded films in both solvents were investigated. The SEM images of all Levo-DCM films displayed porosity and significant drug aggregation on the surface while Levo-THF films had lesser drug aggregation. The surface morphologies of Moxi-DCM and Moxi-THF films displayed little drug aggregation too. After 14 days immersion in buffer, pores and pits were seen on the surfaces of each drug-loaded sample.

In addition, the effects of drug loading and solvent were also investigated to assess their influence on the drug release profiles for both drugs. Increasing the drug loading in the formulation resulted in higher initial burst release and drug amount. This was due to the formation of more surface-segregated drug particles. For Levo-DCM films, all formulations had porous films’ surfaces that resulted in high initial burst and negligible release after it.
In contrast, Moxi-DCM films had no such issue and all 3 formulations system was able to deliver a sustained release above the targeted dosage for 14 days.

Solvent effect was also investigated for both drugs when solvent was changed to THF. Interestingly, suppression of drug burst and sustained release of 14 days was achieved in Levo-THF films. In the case of Moxifloxacin films, the influence of solvents did not greatly impact the release profiles. Both Moxi-DCM and Moxi-THF were able to achieve a sustained release system. These findings supported the postulations that the surface morphologies, volatility of solvents and solubility of drug in polymer can affect the drug release profiles.

### 7.1.2 *In Vitro* Studies – Drug-eluting Coatings

In this chapter, three mandrel designs were first evaluated for their feasibility in achieving the ideal IOL coating. Next, the effect of solvent and flow-rate of the spraying process was investigated. Findings showed that high flow-rates resulted in undesirable morphologies and the morphology using low flow-rate of 0.05 ml/min and THF solvent was the most promising. Translational displacement (TD) can too influence the coating and hook thickness. With a higher TD, both thicknesses would increase due to greater coverage on the mandrel.

**Levofloxacin-loaded Coatings**

For the single drug layer coating, all three formulations exhibited high initial burst release. This was likely due to the poor solubility of drug in the polymer and surface segregation/migration of the drug particles. Lower drug loadings of 3% and 5% were able to meet target dosage for 14 days.
The corporation of blank polymer layers on the top and bottom of the single drug layer to form a sandwich configuration effectively suppressed the initial burst. Increasing the thickness of the polymer layers decreases both the drug burst and the overall release system as the suppressive ability was enhanced as drug diffusion was impeded. This was proven with the release profile of 40 µm-25% Levo-40 µm that plateaued after day 2 and after day 9. On the other hand, the thinner polymer formulation of 30 µm-25% Levo-30 µm was enabled to deliver a sustained release above target dose for 9 days.

**Moxifloxacin-loaded Coatings**

In the first release study, the drug loadings were above the solubility limit in THF. The low amount of drug coated on the mandrel and the variation of drug distribution in each formulation resulted in huge standard deviations and low release amounts. Due to the poor solubility of the drug in THF and solubility constraints in DCM, a second study was carried out with a drug loading of 5%. A sustained delivery of drug was achieved, however, the drug amount released were below the targeted dosage after 2 days. There also appears to be an affinity between the drug and polymer.

### 7.2 Recommendations

#### 7.2.1 “Sandwich” Configuration Study

In the reported *in vitro* coating study, blank polymer layers were used to suppress the burst. After the addition of the blank polymer layers, the total thickness of the sandwich coating became quite thick and might be unfavorable to sleeve it over the commercial IOL. The number of loop cycles for the drug layer could be decreased to decrease the overall
“sandwich” system thickness. Therefore, it will be good to conduct a release study of the “sandwich” configuration with a thinner drug layer.

Secondly, the blank polymer layers and the drug layer are comprised of the same polymer matrix, poly(lactide-co-ε-caprolactone) (PLC). As mentioned previously, during storage, drug particles within the drug layer will diffuse into the blank polymer layers and reach drug equilibrium. From a commercial standpoint, the storage time of the coatings will be an issue and would be impractical to produce a product with a short lifespan. The usage of another polymer as the blank polymer layer might be able to delay this drug equalizing phenomenon. Hence, it would be useful to investigate the effect of different polymers for example, PLGA as the blank polymer layer matrix on the release of the sandwiched coatings and the storage duration.

### 7.2.2 Polymer and Excipient Study

For all fabrications, the polymer composition used was PLC 70/30. It might be interesting to use different molar ratio and understand how the change in polymer composition affects the release characteristics. In the case of coatings, a study with varying solution concentrations could be carried out. For the in vitro study, concentration of solution was established at 6 mg/ml. For Moxifloxacin-loaded coatings, a study could be conducted at higher solution concentrations.

An alternative to enhance drug release rates from drug delivery systems aside from increasing the drug loading, it has been reported that the use of excipients can enhance the release of drug from the polymer matrices. Excipients are additives that can also be used to increase the drug solubility. Hydrophilic excipients either synthetic or naturally occurring
can be utilized and these excipients can be added during the fabrication process. Examples of natural additives could be used are chitosan and cyclodextrin or polyethylene glycol (PEG), a widely used synthetic excipient.

7.2.3 Validation Study

For *in vitro* studies of coatings, 3% Levofloxacin was able to release above the targeted dosage was throughout the study. Replication could be done to confirm the findings from the study and due to time constraints, a validation study was not conducted to validate if the sustain delivery of drugs from this formulation is indeed sufficient to achieve therapeutic effectiveness. The validation could be done in the context of an *in vivo* study or a bacterial assay study.
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