

**DESIGN, DEVELOPMENT AND EVALUATION OF AN EXTENDED RELEASE
VAGINAL FILM PLATFORM FOR HIV PREVENTION**

by

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The human immunodeficiency virus (HIV) is a global health pandemic and over 70% of those living with HIV are found in Sub-Saharan Africa. In this region, the main method of transmission is through heterosexual contact and women account for the majority of those living with the virus. Topical pre-exposure prophylaxis is the vaginal or rectal application of a microbicide product prior to sexual intercourse to protect against HIV infection. Microbicides can be formulated into dosage forms such as; tablets, gels, films and rings. Acceptability studies have identified the film, a traditionally fast release, coitally dependent formulation as an acceptable dosage form to women. Although this dosage form is found to be safe, effective and preferred among women, there are barriers to daily and coitally dependent product usage for some women. In efforts to overcome barriers of both dosage form and dosing regimen acceptability, we propose that the vaginal film can provide a platform for extended drug delivery. We hypothesized that vaginal residence time of polymeric films could be manipulated through physical film properties and polymeric makeup of the film, which can be used to extend the dosing interval of films for the delivery of potent antiretrovirals. Increased volume films were manufactured through altering film thickness. These films were able to accommodate a hydrophilic agent, tenofovir (TFV), a hydrophobic agent, dapivirine (DPV) and a combination of dapivirine and MK-2048. Model films containing TFV and DPV were assessed in the non-human primate and showed that increased volume films can extend vaginal retention time compared to their thin film counterparts. Another approach to formulate extended release films

was through a design of experiments using a combination of polymers to enhance vaginal retention. A panel of films was manufactured and characterized. A lead film platform was selected and when tested in the non-human primate was able to deliver MK-2048 vaginally at levels above the IC_{50} for this compound for up to 30 days. Overall, this work provided strong evidence for the application of the polymeric film as an extended release platform for the vaginal delivery of antiviral agents for HIV prevention.

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PREFACE

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Dr. Lisa Rohan has been a constant in my life for the last 6 years. From my first day as a summer intern and throughout my entire graduate career, she has pushed me to become the best independent scientist that I can be. She has shaped the way that I approach scientific thinking and problems and has continued to invigorate my love of science and my passion for public health. It is impossible to express the gratitude and appreciation that I have for her. I will carry the knowledge that I have gained from her in the lab, as well as the many life lessons that I learned from her, throughout my entire life.

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This PhD is dedicated to my parents, Sally Folan and Bud Grab. Everything I am
and all that I am capable of, is because of them.

LIST OF ABBREVIATIONS

EFdA 4'-Ethyne-2-fluoro-2'-deoxyadenosine

AIDS Acquired Immunodeficiency Syndrome

ACN Acetonitrile

ARV Antiretroviral(s)

ASPIRE A Study to Prevent Infection with a Ring for Extended Use

CAP Cellulose Acetate Phthalate

CD4 CD4 T lymphocyte

CSIC 5-chloro-3-phenylsulfonylindole-2-carboxamide

CVL Cervicovaginal Lavage

DPV Dapivirine

FDA Food and Drug Administration

FTC Emtricitabine

HEC Hydroxyethyl Cellulose

HHS US Department of Health and Human Services

HIV Human Immunodeficiency Virus

HME Hot Melt Extrusion

HPC Hydroxypropyl Cellulose

HPLC High Performance Liquid Chromatography

HPMC Hydroxypropylmethyl Cellulose

IAS-USA The International Antiviral Society-USA

IC₅₀ Half Maximal Inhibitory Concentration

ICH International Conference on Harmonization

IVR Intravaginal Ring

LOD Limit of Detection

LOQ Limit of Quantification

MTN Microbicide Trials Network

N-9 Nonoxynol-9

NRTI Nucleoside/Nucleotide Reverse Transcriptase Inhibitor

NNRTI Non-Nucleoside Reverse Transcriptase Inhibitor

PBS Phosphate Buffered Saline

PEG Polyethylene Glycol

PrEP Pre-Exposure Prophylaxis

R&D Research and Development

RH Relative Humidity

RT Retention Time

STI Sexually Transmitted Infection

TAF Tenofovir Alafenamide

TDF Tenofovir Disoproxil Fumarate

TDF/FTC Truvada

TFA Trifluoroacetic Acid

TFV Tenofovir

UNAIDS Joint United Nations Programme on HIV/AIDS

UPLC Ultra Performance Liquid Chromatography

US United States

VCF Vaginal Contraceptive Film

VFS Vaginal Fluid Simulant

VOICE Vaginal and Oral Interventions to Control the Epidemic

WHO World Health Organization

1.0 INTRODUCTION

1.1 THE GLOBAL HIV PANDEMIC

One of the most devastating viruses the world has ever seen, the human immunodeficiency virus (HIV) now has a global prevalence of 0.8% and currently over 36.7 million people are living with the virus worldwide [1]. HIV infects mainly activated CD4⁺ T lymphocyte cells, although CD4 independent infection can also occur. Once HIV infects these cells, it replicates its genetic material and spreads throughout the body while destroying the host's CD4⁺ immune cells. Over time, as these cells are destroyed, immunological immunodeficiency develops [2]. HIV can spread through infected fluids such as semen and seminal fluids, blood, vaginal fluids, rectal fluids and breast milk. Transmission from the host with HIV to an uninfected individual can occur through exposure to these fluids through sexual intercourse (vaginal or rectal), intravenous drug usage, or from mother to child during pregnancy, birth and breastfeeding [3, 4]. Geographical, cultural, and economic factors contribute significantly to the skewed distribution and prevalence of HIV across the globe.

Sub-Saharan Africa, a region which encompasses the majority of the African continent, is the most heavily HIV burdened region in the world. Over 70% of those living with HIV are found in this region and the main method of transmission in this area is through heterosexual contact [5]. Due to the heterosexual nature of transmission in this region, women account for the

majority of those living with HIV in sub-Saharan Africa. Of the total population of women living with HIV aged fifteen and older, 80% of them live in this region [6]. Cultural norms, as well as physiological factors, contribute to the disproportionate infection rates seen in this specific population [7]. Global efforts to curb new incidence rates, including an aggressive initiative to provide antiretroviral therapy, have helped to control the number of new infections, but progressive declines in incidence rates have leveled out since 2015 [1]. While treatment strategies have helped to slow new infections rates and prolong the lifetime of infected individuals, great efforts in disease prevention are desperately needed to achieve the global goal of ending HIV/AIDS by 2030. Further development of prevention strategies is paramount to halt the progression of this deadly virus. Said prevention methods can also ease the costly, and often crippling, burden of treatment costs. Current prevention strategies include oral Pre-Exposure Prophylaxis (PrEP), or microbicides, and are intended especially for individuals at high risk. Even if these products are only partially effective, they have the potential to greatly reduce the spread of HIV and the growing healthcare costs that overwhelm developing nations. It has been estimated that in a 3 year period a microbicide product that is only 60% effective has the potential to reduce up to 2.5 million new HIV infections, all while helping recover billions of dollars in healthcare costs [8].

1.2 CURRENT PREVENTION STRATEGIES

Both topical and oral PrEP use antiretroviral agents aimed to halt various stages of the HIV lifecycle. HIV infection and replication has been broken down into seven primary stages. These steps are 1) binding, where the virus attaches to a target cell using a receptor like CD4 2) fusion

which allows HIV to enter into the cell 3) reverse transcription in which the genetic material of HIV (RNA) is converted to HIV DNA so that it can enter into the nucleus 4) integration, in which the replicated genetic material of HIV is then integrated in to the host-cell DNA 5) replication where the HIV genetic material is replicated 6) assembly, where HIV genetic material and proteins assemble together and move towards the cell surface and finally 7) budding, which allows the newly assembled HIV to be pushed out of the cell [4]. Earlier generation microbicide agents were non-specific agents. These early generation microbicide candidates were intended for topical use and were surfactants and agents that blocked HIV binding [9]. Surfactants like nonoxynol-9 (N9) and SAVVY, which can disrupt the viral HIV membrane, were tested but failed to have any preventative effects against HIV infection, and even increased infection rates due to tissue disruption and genital lesions [10-13]. Large charged polymers were attractive candidates for blocking HIV binding and these included agents like PRO 2000, carageenan, and cellulose sulphate [9]. None of these agents, nor those that worked to maintain the acidic pH of the vagina, showed promising clinical results [14-16]. Later generation microbicide candidates targeted specific points during the HIV lifecycle. These agents include antiretrovirals which target HIV fusion with host cell (gp 41 inhibitors), those which block HIV attachment (gp120 and CCR5 antagonists), those which inhibit viral reverse transcriptase (reverse transcriptase inhibitors), those which block the integration of the viral genetic material into the host genetic material (integrase inhibitors), and agents that block the processing of HIV proteins in the final stages of host cell infection and replication (protease inhibitors) [17]. These HIV target-specific microbicides can be formulated into various dosage forms which can be taken orally for systemic exposure or applied topically for targeted local drug delivery.

1.2.1 ORAL PrEP

The first Food and Drug Administration (FDA) approved medication, intended for individuals who are at a high risk for contracting HIV, was Truvada[®]. This oral medication, taken daily, is a combination of two antiretroviral agents, emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF), which inhibits the activity of the HIV-1 reverse transcriptase (RT). Truvada has been shown to reduce the risk of HIV infection by over 90% when taken daily as prescribed [18]. There have been several landmark studies with daily oral Truvada[®] mainly the iPrEx, FEM-PrEP, Partners PrEP, TDF2, VOICE, IPERGAY and PROUD trials. Populations varied for these trials; iPrEx, IPERGAY and PROUD trials were conducted in men who have sex with men (MSM), FEM-PrEP and VOICE was conducted in reproductive aged women, Partners PrEP was conducted in serodiscordant couples aged 18 and older, and TDF-2 was conducted in heterosexual men and women of reproductive age [19-25]. Results from these studies showed that there was the highest reduction of HIV risk in the PROUD and IPERGAY studies (86% risk reduction), where adherence – as measured by drug levels in the plasma – was good [24, 25]. The lowest risk reduction was seen in the FEM-PrEP and VOICE trials which showed no risk reduction for those on oral daily PrEP [19, 22, 26]. Drug levels, which were directly related to adherence to the study product, were low in these studies and showed less than 40% of all participants had detectable drug levels in the plasma, a direct indication of study product usage [22, 27]. While this medication can be extremely effective in preventing the acquisition of HIV, there have been concerns raised with the adherence to the daily regimen of dosage administration.

1.2.2 TOPICAL PrEP

Unlike oral PrEP, topical PrEP generally results in limited systemic exposure of antiretroviral drugs. Topical PrEP can be divided into two main categories, vaginal and rectal, based on the route of delivery for these dosage forms. Vaginal dosage forms currently in development include gels, creams, films, capsules, inserts, nanofibers and intravaginal rings [28, 29]. In large scale phase IIb and III clinical trials with gels and rings, an association between product adherence and clinical efficacy was seen [26, 30-32].

Rectal-specific dosage forms for HIV prevention which are at various stages in the research development pipeline, include gels, enemas (douches), and suppositories [33-35]. Rectal gels and enemas are the most advanced rectal microbicide dosage forms and have been tested for safety, acceptability, pharmacokinetics and pharmacodynamics [36-38]. These trials have shown the importance of the design of rectal-specific products and adherence data suggested that daily dosing may not be ideal for rectal gel products [36].

1.2.2.1 Coitally Dependent and Independent Dosage Forms for Topical PrEP

Topical PrEP can further be divided based on how they are intended to deliver the microbicide; coitally dependently or coitally independently [28]. Based on how drug is released from a dosage form will govern how often and when a microbicide product needs to be administered. On-demand, coitally dependent products like films and gels, are used around the time of sex (either before or after intercourse). Other on-demand microbicide dosage forms in development are ovules, tablets, suppositories, and enemas [39]. These dosage forms are designed for immediate drug release [39]. Unlike on-demand products, coitally independent products do not need to be used around the time of sexual intercourse to provide protection to the user. Dosing of these

products can range from daily to monthly [39]. Vaginal rings are the most clinically advanced coitally independent dosage form to date. The DPV microbicide ring, tested in the clinic, provided sustained release of DPV for up to a month and this ring requires less frequent application than vaginal films and gels [40].

1.3 THE DEVELOPMENT OF TOPICAL MICROBICIDES

1.3.1 *In Vitro* Development and Evaluation of HIV-Preventative Products

There are extensive preclinical evaluations required before any of these microbicides in development can enter the clinic including rigorous *in vitro* assessment as well as *in vivo* testing in animal models. Only after these experiments have been conducted and have been shown to be safe can a microbicide candidate continue on the path of clinical development. Current microbicide products are formulated with antiretrovirals that target specific stages of the HIV infection cycle. An active pharmaceutical ingredient (API) can target any point of this HIV lifecycle to halt the infection process of HIV. Combining more than one agent, with the same or different HIV inhibitory mechanisms, within a single product is an emerging strategy under investigation to increase efficacy. This strategy, combining multiple active agents, is currently used in HIV treatment regimens [41-43]. Multiple active agents can provide synergistic efficacy and reduce the potential for the development of viral resistance [44].

In 2010 there were over 70 microbicide drug candidates in the pipeline in either clinical or preclinical development. Of these, only a few will make it to human studies after being formulated into dosage forms, evaluated in animal models, and finally moved to clinical studies

[45, 46]. Candidate APIs should be potent, have a good therapeutic index (TI), have a favorable resistance profile, exhibit stability over time, be non-toxic at administered concentrations, and be cost effective to manufacture and produce [47]. Doncel et al. wrote a comprehensive review on the common evaluations microbicide drug substances and drug products undergo *in vitro*. This will be used as a reference to describe these tests. Pre-formulation studies for drug candidates mainly test the physio-chemical properties of the compound such as solubility, stability, compatibility with excipients, and degradation of the API. After these studies, the API can be formulated into a dosage form (gel, ring, tablet, etc.) which will also undergo extensive testing. Dosage form mechanical and physical properties are crucial to these evaluations and differ depending on specific delivery system. Microbicide products are subject to a number of evaluations dependent on dosage form type. Some examples of physical product parameters which are commonly assessed *in vitro* include mechanical testing such as tensile strength, disintegration, puncture strength, osmolality and elongation. Chemical properties such drug content and drug content uniformity, and dissolution of the API are also studied [44, 48-52]. Biological testing includes assessment of drug permeability, anti-HIV activity, cellular toxicity, and compatibility with [47, 53]. A thorough preclinical product evaluation scheme will aim to establish the variability within the product and overall functionality of the product.

1.3.2 Animal Models for Microbicide Evaluation

After rigorous physical, chemical and biological *in vitro* product testing, the next step in evaluation is the assessment of the product in animal studies. Animal models are crucial in the evaluation of microbicide product safety, pharmacokinetics, pharmacodynamics, and efficacy. Small animal models; rats, mice, and rabbits, are commonly used for drug product safety

evaluation [54]. Compared to other large animal models such as the non-human primate (NHP), these animals are much lower in cost, have greater availability, and can be used to narrow product and candidate selection [53]. However, there are numerous differences between the reproductive anatomy of these animals and humans [54]. Cycle type is one of the main differences between humans and rodents. Humans have menstrual cycles while rodents undergo estrous cycles and they also undergo periods in which they do not cycle at all [54]. Cell type in the vagina is another main difference found between humans and rodents. The human vagina is lined with squamous epithelial cells while columnar epithelial cells are present in the vagina of rabbits and mice [54-56].

Regardless of these differences, these small animal models are useful in preclinical microbicide evaluations. Humanized and engineered strains of murine models have been implemented to evaluate product efficacy since they can be infected with HIV [54]. To this end, the humanized bone marrow/liver/ thymic (BLT) NOD/SCID mouse has been used in microbicide testing [54]. This murine model has been used to demonstrate that various routes of microbicide delivery can prevent rectal, vaginal and systemic HIV exposure [57-59]. In addition, the rabbit vaginal irritation model is one of the most commonly used safety evaluations in small animals for microbicides [53, 54]. Rabbits can also be used for dermal and penile irritation studies for products intended for vaginal application [60].

Sheep present a larger model which can be used for toxicity studies of microbicides, mainly topical vaginal microbicides. Sheep are widely available, inexpensive, easy to handle and, like humans, have stratified squamous epithelium lining the vagina [61]. The dimensions of the vaginal tract between humans and sheep are also similar [62], therefore, products do not need to be scaled when used in this model. However, as with rodents and small animals, sheep have an

estrous cycle as opposed to a the human menstrual cycle [62] . The sheep model has been used mainly for safety which has been is established through imaging methods, histology, colposcopy and also for pharmacokinetics and plasma testing [53, 61].

The non-human primate (NHP) has been used to study various aspects of HIV transmission, prevention, and treatment. Macaques, considered the ‘gold standard’ in vaccine research [63], are the most common NHP used. The rhesus, pigtailed and cynomolgus species are the three most common species of macaques used due to anatomical and reproductive similarities to humans [54]. Patton et al. noted similarities in the menstrual cycles of pigtailed macaques and humans, both 28 days, and similarities in the bacterial species which colonize the vagina. The dominant gram-positive *Lactobacilli* and gram-negative prevotella found in the vagina of humans was also found in similar levels in the macaque vagina [64]. The epithelium of the macaque vagina was shown to be on average approximately 30 layers thick, which is similar to the human vagina [65]. Additionally, Patton et al. looked at the rectal histology and microflora of the pigtailed macaque, and found distinct similarities between the human and the macaque. The major bacterial species of the rectal microflora were similar for both humans and macaques and the stratified squamous epithelium and lamina propria of the rectum were consistent with that found in the human [65]. These findings further validate the use of this model for the development of microbicides, specifically those rectally and vaginally applied.

This animal model has a robust history of use and continues to provide significant information regarding safety for vaginal and rectal specific drug delivery, as well as systemic drug delivery. Primates, due to their distinct similarities to humans, also give valuable and predictive data regarding drug pharmacokinetics and pharmacodynamics. These studies can be used to push a microbicide candidate or product further into development or highlight pitfalls

that could arise in costly human studies. Unlike smaller animal models, whose anatomical and physiological differences limit correlations and predictions to human data, non-human primates can be more extrapolative of *in vivo* drug functionality and performance. There have been numerous studies in the NHP model for the delivery of oral, vaginal and rectal microbicides. García-Lerma et al. studied oral PrEP regimens using different routes of administration (oral and subcutaneous) of emtricitabine (FTC) with tenofovir-disoproxil fumarate or tenofovir at doses similar to that of Truvada® in rhesus macaques who were then subsequently exposed to rectal viral challenges [66]. Studies that examined oral dosing, daily and intermittent, followed by vaginal challenge in NHP models have also been conducted [67]. These studies together provided evidence and support for the advancement of oral PrEP regimens to human clinical trials. Heneine et al. compiled a comprehensive review of vaginal, rectal, oral, subcutaneous, and intraperitoneal administration of antiretroviral agents in rhesus, pigtailed and long tailed macaques which have been used to support microbicide development [68]. Tenofovir vaginal and rectal gels were tested in pigtailed macaques prior to and/or after HIV exposure by various groups and showed ranging degrees of protection [69-71]. Dapivirine and maraviroc containing vaginal gels have also been studied in primates for pharmacokinetics and efficacy after administration [72, 73]. Numerous intravaginal rings (IVR) containing microbicides have also been studied in primates, namely the pigtailed or cynomolgus macaque, including an IQP-0528 containing ring [74], a tenofovir disoproxil fumarate (TDF) ring [75] and combination rings containing the following combinations: TDF and FTC; TDF, FTC, and maraviroc (MVC) [76]; and DPV and darunavir [77]. Taken together, these studies show the application of the non-human primate in microbicide product development and the usefulness of this model in preclinical testing.

1.4 CLINICALLY ADVANCED VAGINAL MICROBICIDE STRATEGIES

Animal testing in primates can be highly predictive of product performance and is needed to advance to human trials, but as years of clinical research has taught us, there is no true replacement for human subjects. Human free will and ability to adhere or not to adhere to study regimen, attitudes towards products, adverse reactions, and many other details related to human product usage makes clinical trials the true pinnacle of drug development. Clinical data regarding the most currently advanced microbicide products will be presented in the following sections. While oral and both vaginal and rectal topical PrEP have been discussed in depth, the focus of this dissertation work is vaginal microbicides.

1.4.1 Anatomy and Drug Delivery to the Vagina

As previously discussed, women, especially those in sub-Saharan Africa where over 70% of those living with HIV reside, are disproportionately infected with HIV compared to their male counterparts [5, 7]. Women now also constitute the majority of those living with HIV [78]. Because of the severity of the HIV pandemic in the female population, vaginal microbicide development is at the forefront of prevention research. The vagina provides a unique, but challenging, environment for drug delivery. The vagina is a fibromuscular, collapsible tube that can vary in length from 6-10 cm and has an area ranging from 65.73 to 107.07 cm² [79, 80]. There are several distinct advantages associated with vaginal drug delivery; circumventing first pass metabolism, ease of insertion through a non-invasive route of administration, potential for systemic drug delivery because of large blood supply, and potential for local delivery [79, 80]. The vagina is also a dynamic environment with complex biological processes and innate barriers

which impact drug delivery. Vaginal secretions can be composed of vaginal cells, leucocytes enzymes, enzyme inhibitors, proteins, carbohydrates, amino acids, alcohols, hydroxylketones and aromatic compounds. The amount of fluid present at any time is dependent on age, menstrual cycle and arousal and can impact a delivery system introduced into the vaginal track [80]. It is estimated that there is approximately 6 grams of fluid are produced per day in the vagina and that 0.5-0.75 grams of fluid are present at a time in the vagina [81]. A major component of this fluid, is mucus. Mucus present in the vaginal compartment introduces additional considerations when developing vaginal drug delivery systems. This mucus is composed mainly of large glycoproteins and can be a target for drug delivery systems, but this can be permeability barrier for drug candidates as well [80]. Another factor in the dynamic vaginal environment is the maintenance of a healthy, acidic, vaginal pH which ranges between 4-4.5 [79]. *Lactobacillus* produces lactic acid for pH maintenance but can also produce hydrogen peroxide to help prevent the overgrowth of harmful pathogens [79]. Because of the importance of these bacterial species, compatibility with *Lactobacillus* is a crucial characterization measure for dosage form development.

1.4.2 Gels

Because of the advantages of the vaginal route of administration, vaginal gels were attractive candidates for vaginal microbicides since it was found that large amounts of local drug could be delivered with limited systemic exposure [82]. Gels are semisolid, highly viscous dosage forms which can be applied vaginally or rectally using an applicator in varying volumes depending on drug potency. Gels can spread in the anatomical compartment to which they have been applied allowing drug delivery to these tissues without significant removal or washout [83]. Magnetic

resonance imaging (MRI) studies have shown that gels can be spread in the vagina and rectum during sexual intercourse and normal movement at volumes varying from 3-5 mL in the vaginal compartment and 5-10 mL in the rectal compartment [84, 85]. There is a familiarity with gel products because they have been marketed over-the-counter (OTC) as rectal and vaginal lubricants, contraceptives and moisturizers, and they have also been prescribed for the treatment of infections such as bacterial vaginosis and candida infections (metronidazole vaginal gel), for the delivery of progesterone (progesterone vaginal gel), and for seizure control (diazepam rectal gel).

Gels provide advantages over other dosage forms; they can provide both vaginal and rectal lubrication during sexual intercourse, provide a physical barrier to HIV entry, are low cost to manufacture, can be retained in the vaginal or rectal compartment due to bioadhesion, and can spread in the vaginal or rectal compartments [28, 79]. User familiarity and market availability of gel formulations have also allowed these dosage forms to be perceived as easy to use. Their acceptability to women for vaginal use has been demonstrated in vaginal studies [30, 86-88]. Bunge et al. compared a microbicide containing vaginal film and gel, and while some users reported difficulty with film insertion, none of the gel users reported difficulty with insertion [89]. The majority of gel-product users (62.5% and 73.4% for placebo and drug-loaded gel product, respectively) in this study said they were “likely” to use this product if it would protect them from HIV acquisition. Gel users did not have product-related adverse events or changes in the vaginal microbiota, thus proving safety of this product [89]. Another clinical trial also reported favorable acceptability data. In this large scale clinical trial conducted by Karim et al. (CAPRISA 004) tested a TFV vaginal gel. 97.4 % of participants reported that this gel was

acceptable and 97.9% of participants reported that they would use this gel if it prevented against HIV [30].

There are numerous advantages with gels, but there are also challenges associated with their use as a vaginal or rectal delivery platform. Topical microbicide delivery is a targeted form of drug delivery aimed at increasing site-specific drug concentrations while limiting systemic exposure to the active agent. In a clinical study comparing vaginal administration of DPV in either a gel or film form, site-specific concentrations were similar for the two delivery systems. Further, the gel had a larger impact on the innate anti-HIV activity in the vagina compared to the film which caused little disruption to this protective mechanism [89]. When tested in large scale clinical trials, pericoital administration of vaginal TFV gel showed an overall 39% reduction in HIV infection in the CAPRISA 004 trial [30] but showed no reduction in HIV acquisition compared to control in the VOICE trial [26]. Efficacy was tied closely to adherence in both studies. In the CAPRISA trial, efficacy was 54% in users who adhered to the study regimen more than 80% of the time [30] and it was found that adherence, as assessed by mean proportion of quarterly plasma samples with TFV detected (%), was low (25%) in the VOICE study [26]. Clinical trials with gels have shown that when used correctly they can be effective in prevention, but certain barriers to adherence to gel usage may be too great a hurdle to overcome as evidenced in the VOICE trial.

1.4.3 Films

Vaginal films were developed as an alternative delivery system to vaginal gels, as they resolved some of the issues seen with gels, specifically the messiness, leakiness, and impact on innate protective factors. Polymeric films are a delivery system that has been around in the

pharmaceutical industry for years. The thin polymeric film is a versatile dosage form that can be used to deliver a wide range of pharmaceuticals. There has been wide application of this technology for oral drug delivery. It has also been applied in topical wound care, diagnostic devices, and pH dependent dissolution in the gastrointestinal tract [48]. Currently, there are many marketed prescription oral thin film products for indications including nausea from cancer and chemotherapeutic treatments, opioid dependence, and pain management. These products include Zuplenz[®] (Galena Biopharma) for the delivery of ondansetron; Bunavil[®] (BioDelivery Sciences International, Inc.) and Suboxone[®] (Indivior, Inc.) for the delivery of buprenorphine and naloxone; Onsolis[®] (BioDelivery Sciences International, Inc.) for the delivery of fentanyl; and Belbuca[®] (Endo Pharmaceuticals, Inc.) for the delivery of buprenorphine. Numerous over-the-counter oral thin films are also marketed such as Listerine[®] Breath Strips and Chloraseptic[®] Relief Strips. There are over-the-counter vaginal films such as the Vaginal Contraceptive Film[®] (VCF), VCF Scented Film[®] and VCF Lubricating Film[®] all manufactured and distributed by Apothecus Pharmaceutical Corporation. Vaginal films represent an emerging technology in the field of topical pre-exposure prophylactics (PrEP) [79] and various film platforms are currently being developed in an attempt to reduce the HIV infection rate in women. The most clinically advanced films contain either the antiretroviral tenofovir or dapivirine [35, 89-91], and a film containing the monoclonal antibody Mapp66 has recently entered into the clinic and this trial is expected to be completed in October 2018 [92-94]. The tenofovir and dapivirine film products have been shown to be safe and acceptable in Phase I clinical trials. In these same studies they were shown to deliver adequate levels of drug to inhibit HIV infection in an *ex vivo* challenge model and showed favorable pharmacokinetic data from these Phase I trials.

Vaginal films have many advantages for delivery and studies have shown that women find this dosage form acceptable for vaginal use. As a delivery system in general, the polymeric films have many inherent positive qualities compared to other dosage forms. Films as a solid dosage form have the capacity to stabilize drugs susceptible to hydrolytic degradation which can occur in aqueous based hydrogel formulations. In addition, films are inexpensive to manufacture, convenient to administer non-invasively, do not require an applicator for administration, and have limited leakage potential [90, 95]. Films can offer a quick dissolving delivery platform which requires only the fluid within the vagina to disintegrate and dissolve. This limits product leakage and also allows for the API to be release quickly upon disintegration. Further, because of the small dosage form volume and lack of product leakage and messiness, it is possible to use a film discretely and without ones sexual partner's awareness of product usage. The discrete nature of the film is especially attractive for users who want to control their own prevention method and who may have difficulty negotiating condom or product usage with a sexual partner. When targeting resource poor areas where the HIV pandemic is most severe, cost-effective manufacturing as well as cost-effective dosing (no applicator required) will greatly influence product development and usage [96].

A study conducted by Nel et al. assessed the acceptability of three different vaginal dosage forms; a vaginal tablet, vaginal film, and vaginal soft-gel capsule in sexually active, reproductive aged women in different countries in Africa [97]. The film was one of the preferred dosage forms and it is important to note that product preferences varied by country. Characteristics which led to this preference included; ease of insertion, absence of product leakage, dissolving time of the dosage form, comfort of the product, as well as physical texture and color [97]. Specific reasons that women preferred vaginal films were highlighted in a recent

study conducted by Fan et al. in Pittsburgh, PA using focus groups and questionnaires in sexually active, reproductive aged (18-30) women of diverse ethnic, educational, marital and sexual backgrounds [98]. This study reviewed user perceptions of film products and specific traits that women found desirable in a vaginal film. Regardless of familiarity with the film dosage form, women were receptive to potential usage for HIV prevention [98]. Focus groups were recorded and common themes which were highlighted as positive about the vaginal film were, “minimal perception of use by user or her partner, likelihood of physical comfort, potential for less embarrassment compared to condoms, skin-to-skin genital contact, and female control of use” [98]. To better inform product design, a study was conducted by Morrow et al. to examine user perceptions of films regarding specific film attributes. Participants evaluated vaginal films with varying colors, textures, and sizes, and expressed attitudes regarding willingness to try a product, impact of these physical parameters on product efficacy, ease of insertion, perceived awareness during use, as well as views. Data collected from these focus groups provided detailed insights regarding visual and tactile properties of films [99]. Another study, conducted by Bunge et al. in HIV-negative women at Magee-Womens Hospital at the University of Pittsburgh Medical Center, which examined the acceptability of a vaginal film compared to a vaginal gel, found that the film was overall more comfortable to use and less leaky than the vaginal gel product [89]. In this same study, the small dosage form volume (110 mg) was hypothesized to have a smaller impact on the innate anti-HIV activity in the vagina when compared to a gel applied larger volume gel (4 g). Bunge et al. confirmed this hypothesis and found that the vaginal film was less disruptive to the intrinsic anti-HIV protection mechanism of the vagina when compared to the gel, thus offering another advantage to this film product [89]. This same study

found that the film was as effective as the gel when challenged with HIV *ex vivo*, showing that the film is an effective strategy against HIV infection.

Some of the characteristics of the film which make it so favorable (quick disintegration time, small volume, no applicator needed) have also unfortunately contributed to some of the disadvantages seen with this product. Film insertion remains one of the most commonly observed drawbacks to film usage. Without an applicator there can be difficulty getting the film to reach the correct anatomical location in the vagina which is fully inserted near the cervix. Women have reported issues with insertion in various studies. In the clinical study evaluating dapivirine containing films, it was found that 5 out of 30 women randomized to receive film product had film misplacement with film product remaining at the introitus instead of within the vagina [89]. Distrust of a film product is another common perception that has come to light with microbicide and contraceptive studies in women. The characteristics of the film (appearance, size, etc.) do not instill confidence in some women that it would actually be able to offer protection from STIs or pregnancy [100]. Lack of confidence in a product or perceived product efficacy (PPE) is an important measure as it can dictate product usage and impact user product adherence, regardless of if there is scientific fact to base these assumptions. Understanding which specific characteristics of films dictate these user perceptions regarding perceived efficacy can be crucial in future pharmaceutical vaginal film product development efforts.

1.4.4 Rings

In contrast to gel formulations and the fast dissolving film, other strategies are being developed for long-acting or extended use. These long-acting or extended release delivery strategies are intended for use over a longer period of time, rather than daily or pericoital usage

as utilized with film, gel or oral Truvada[®]. Intravaginal rings are the current most clinically advanced and well-known long-active dosage form in the field of microbicides. Two clinical trials (ASPIRE (MTN-020) and The Ring Study (IPM 027)) with rings containing dapivirine, a non-nucleoside reverse transcriptase inhibitor (NNRTI) which works by preventing HIV from replicating its genetic material, showed promising efficacy and safety results in women. The ASPIRE and Ring studies evaluated a dapivirine IVR intended for monthly use. The HIV incidence reduction for the ASPIRE and Ring studies respectively were 37% and 31% and there were and there was an association with adherence and efficacy [31, 32]. In the ASPIRE (MTN-020) study, the efficacy of HIV protection was 56% with an adherence rate of greater than 80%, and the efficacy of HIV protection of -27% in those with an adherence rate of less than 80% [31].

Vaginal rings are typically polymeric, circular, and inserted and removed by the user every 21-28 days. However, as this dosage form does not disintegrate or dissolve, it has to be removed and replaced on a regular basis and there are concerns that it could interfere with sex or that a partner could feel it during sex. Traditionally, these rings have been used in hormone replacement therapy and for contraception [80], so there is already a familiarity within the female reproductive-aged market. This can be a benefit when it comes to user perceptions and awareness of insertion method. Rings offer a user-controlled, continuous method of drug delivery over the course of at least a month. There are three main types of rings; matrix, reservoir and pod, which differ based on release profile and drug distribution within the ring. The API is homogeneously distributed throughout the ring in the matrix type ring therefore release from this ring is highly dependent on surface area and drug loading [80, 101]. Reservoir rings a drug loaded core surrounded by and non-drug loaded layers which can be used to control the rate of

drug release [102]. Finally, the pod-type ring utilizes compressed drug substance inserts (pod) which may or may not incorporate a thin layer coating that acts as a rate controlling barrier for drug release. Pods are placed into the vaginal ring and delivery channels for each pod allow for controlled drug release [103]. The pod construction, number of pods, and pod window size all impact drug release rate [101, 102].

Release rate from vaginal rings can be manipulated to allow the slow release of an API over time and keep drug levels at the target site consistent. There are numerous advantages with long-acting vaginal rings and they have shown to be acceptable and have favorable attributes regardless of indication. Acceptability measures differ from study to study but in general some of the common themes found were that neither women nor their partners could feel the ring during sex, they felt that it was easy to insert and remove, and they would recommend the ring [104-107]. Some of the common positive attributes highlighted from clinical data found that the ring did not interrupt spontaneous intercourse or normal sex and that there was the option to disclose or not disclose usage [107]. Unlike the film or gel which needs to be inserted around the time of sex, the ring is worn continuously. Women also found that the ring was easy to use, discrete, and comfortable, [107, 108] and rings are also safe for the user when formulated with microbicide candidates and did not cause harm to the vaginal microflora or change the vaginal pH [108-110].

With respect to adherence, although the ring was safe, women reported adhering to vaginal ring usage and there was a low rate of ring expulsion, it is interesting that adherence was still cited as a potential issue in the two clinical trials testing microbicide rings. In the ASPIRE study, adherence and efficacy was further analyzed based on age group. Adherence, as assessed through plasma dapivirine levels, was lowest in women aged 18 to 21 and higher in the women aged 22 years and older. This age group (18 to 21) also showed the lowest product efficacy,

which highlights that there are still some barriers to product usage and that adherence is correlated with efficacy [31].

1.4.4.1 Novel Extended Release Strategies in Development

Through data obtained from numerous clinical trials, the direct correlation between correct product usage and efficacy cannot be denied or ignored. There seems to be a distinct challenge with pericoital and daily use products (gel, film and oral pill) and longer acting products (vaginal ring) may offer a more convenient option to those women who wish to utilize product which require less frequent administration. It is suggested that longer-acting products that women can control may be the key to developing strong prevention products for a population which is hardest hit by the HIV pandemic [31].

In efforts to increase product uptake and usage, especially in women, a population which is disproportionally infected with HIV, other longer acting products are in development. Safety, acceptability, as well as pharmacokinetics and pharmacodynamics of long acting injectable ARVs are currently under investigation. Injectable ARVs were some of the early strategies explored for microbicide development and as the field has shifted towards long acting methods, intramuscular injections of rilpivirine and S/GSK1265744 are now being investigated [111, 112]. Nanoparticles are also attractive candidates for extended drug delivery. They have been shown to achieve higher concentrations at the site of action because of their ability to penetrate the vaginal mucosa in addition to protecting the active agent from metabolism, and therefore prolonging the duration of action [113]. Nanoparticles formulated with mucoadhesive polymers allowing mucoadhesion in the vaginal track and nanosystems have also been shown to have favorable release profiles for microbicides, including sustained release of a compound over several days [114]. Implants are another long-acting strategy under development. Gunawardana et al.

demonstrated that an implant can deliver sustained levels of the prodrug tenofovir alafenamide (TAF) over 40 days in dogs [115]. Electrospun nanofibers can also be used to sustain release from solid microbicide dosage systems, and represent another emerging as a strategy to prolong drug release from vaginal films [28, 114, 116].

With all of the products in development, there are individual encouraging aspects and properties which have allowed further development and advancement of these products. Favorable acceptability, safety, efficacy, manufacturability, cost, user perceptions, and other parameters distinguish these products from each other. Due to varying social, economic and cultural differences, a ‘one size fits all’ model is not likely to work globally, so there are many dosage forms in development. Cultural practices influencing familiarity or willingness to touch the genitalia, preference for wet vs. dry sex, and partner communication about issues relating to sexual intercourse are just a few of the widely varying attitudes surround microbicide product usage [117]. It is the responsibility of the research community to try to combine as many positive parameters as possible in a single product to make the most attractive microbicide candidate to help women protect themselves from HIV acquisition. This responsibility does not mean developing one single product and abandoning all other avenues, but rather working to create a hybrid of creativity and innovation in many different product types so that women can have unique and exciting choices for ways to protect themselves.

1.5 RESEARCH HYPOTHESIS AND SPECIFIC AIMS

The term acceptability has become a replacement measure or marker for adherence, especially in the field of topical microbicides where there is currently no approved and marketed product.

Morrow et al. best described this phenomenon by stating, “In the absence of an approved product with which to study “acceptability” as a phenomenon in its own right, the field has morphed the constructs of “acceptability” and “adherence” (i.e., the degree to which a product is consistently and correctly used, as per instructions), using the latter as a surrogate by which to presume the former: if a product were “acceptable,” women would use it (and/or recommend it to others). Thus “acceptability” has been presumed to have a near-perfect positive correlation to reported use, or to potential users' intentions to use a given candidate” [118]. While there are flaws to this logic, as highlighted by clinical data where highly acceptable products still showed issues with adherence, there is great merit to this idea. Also, in this field of topical microbicides there has to be some measure to set as an ultimate goal, and at this point, acceptability is the most logical.

The polymeric thin film offers an advantageous dosage form for the delivery of anti-HIV microbicides. This dosage form is discrete to use as it does not require an applicator, it can be used without one's partner knowing, it is low cost to manufacture and easily scaled for large volume manufacturing, it is a versatile dosage form which has been shown to be able to accommodate a range of hydrophobic and hydrophilic active agents including small molecular drug, potent peptide drug and bacteria [44, 49]. Even over some of the more traditional vaginal delivery systems, women have shown to prefer this dosage form over other vaginal delivery systems and clinical data has shown that it is safe and effective for microbicide delivery. In resource poor areas where the HIV pandemic hits the hardest and women have little power to negotiate condom usage or other protection methods with their sexual partners, films are an extremely attractive candidate to help curtail high HIV infection rates. A potential drawback of quick-dissolve films is their need to be used in a pericoital manner to offer adequate protection to the user which can lead to lower adherence rates. Reviews of daily vs. intermittent (weekly or

monthly dosing) have shown greater adherence to intermittent dosing schedules [119]. Therefore, we believe that extending the vaginal residence time of this widely acceptable dosage form, the polymeric thin film, could provide a more acceptable microbicide drug delivery platform for women by removing the need for coitally dependent dosing. **We hypothesize that vaginal residence time of polymeric films can be manipulated through physical film properties and polymeric makeup of the film, which can be used to extend the dosing interval of films for the delivery of potent antiretrovirals.**

This hypothesis will be tested through the following specific aims.

Specific aim 1: To establish the use of the vaginal polymeric film as an extended release dosage form for delivering potent ARVs through geometric manipulation (i.e. increased dosage form volume).

- a) Hydrophobic and hydrophobic model compounds (tenofovir, dapivirine and MK-2048) will be used alone or in combination in increased volume dosage forms for extended release film development.
- b) A model hydrophobic and hydrophilic extended release film will be evaluated *in vivo* in the pigtailed macaque model.

Specific aim 2: To develop an extended release film through modifications in polymeric makeup to slow release rate from films and increase mucoadhesive interactions between the film and mucosal environment. The overall goal being to develop a week-long film product for the delivery of a model compound for HIV prevention.

- a) A Design of Experiments (DOE) will be conducted using three extended release polymers and a model compound MK-2048. *In vitro* film properties will be assessed and a lead extended release film candidate will be selected for *in vivo* study and clinical development.
- b) The lead extended release film will be studied *in vivo* in the pigtailed macaque model and developed for clinical usage in women.

2.0 DEVELOPING METHODS TO EVALUATE FILMS FOR QUALITY CONTROL AND PERFORMANCE

2.1 INTRODUCTION

Indications for pharmaceutical films are widespread and the field continues to grow especially with concentrations in areas of public health, wound care and diagnostic devices [48]. As this dosage form gains more traction in the field of microbicides and drug delivery in general, it is important to establish certain test methods to standardize evaluations of the physical attributes of this dosage form. In addition, as the development of the polymeric film field expands, it is crucial to have biologically relevant methods in place to evaluate film performance.

There are numerous preclinical *in vitro* physical and chemical tests to evaluate product functionality and uniformity. Common testing protocols for films exist regardless of the route of administration and chemical entity in the product. Standard testing for polymeric films in general include drug content, and drug content uniformity, contact angle, water content, dissolution, disintegration, and mechanical properties such as tensile strength, puncture strength, elongation, Young's modulus, and folding endurance [44, 48-52]. These evaluations are commonly used in stability assessments. Dosage form disintegration and dissolution are two of the most critical parameters that dictate achieving efficacious levels of drug at the desired site. In general, for solid dosage forms like tablets, disintegration is a process in which, the dosage form breaks

down into smaller particles after coming in contact with the physiological fluid. The nature of disintegration (e.g. time taken to disintegrate) impacts the downstream dissolution process and ultimately drug dissolution. *In vitro* disintegration is a valuable tool used to evaluate a crucial dosage form parameter that can predict the behavior of film *in vivo* and provide a means of comparison between other products. While this test can be extremely valuable, there is not a well-defined, bio-relevant, quantitative method that introduces no user bias. In 1997, The Food and Drug Administration (FDA) issued a guidance for industry for orally disintegrating tablets [120], which refers to the United States Pharmacopeia (USP 701) disintegration testing methods [121]. The disintegration apparatus for this testing can be seen in Fig. 1.



Figure 1: USP Capsule and Tablet Disintegration Apparatus

This method uses a 1 L beaker in which a basket and rack consisting of six plastic tubes with a wire mesh basket at the bottom of the tube. A mechanical arm consistently raises and lowers the tubes in and out of the fluid. Fluid volume in the beaker must be enough so that at the highest point, the wire mesh is still 15 mm below the surface of the fluid and at the lowest point the

mesh is at least 25 mm from the bottom of the beaker [121]. This test uses volumes of fluid which are logs greater than what is found in the vagina [81]. Further, the constant motion of the test may simulate gastric movement and is not necessarily applicable for vaginal films. Therefore, these test are not applicable for film disintegration.

In lieu of an acceptable method specific for polymeric films, other methods have been adapted for use for this dosage form as there is no official guidelines for films [122]. These methods can be classified based on volume of media used throughout the experiment. Small volume methods include visual methods and the slide frame method [123]. Visual disintegration methods use a holder for the films (i.e. petri dish) and a known amount of media is applied to the films, ranging from 2mL- 25 mL [49, 123]. Disintegration time is user-defined by when the film disintegrates. There are no standardized guidelines for this endpoint. The slide frame method places the film in a slide frame which is then laid on a Petri dish. Media is added to the film and time until the film disintegrates is measured. This test has been performed with amount of media ranging from drops to 2 mL [124-126]. Again, this is a visual method and there are no specific indications which can be used across users to define film disintegration. Larger volume tests are mainly modifications to the USP disintegration method for tablets and capsules as described previously. The disintegration apparatus (Fig.1) as described previously and 500mL- 1L of media are used for these tests [123]. In one method using this setup, one end of the film is clamped to a weight and the other end to a sample holder. Films are then submerged in media, or continuously dipped in and out of media, and the time until the weight drops to the bottom of the vessel is measured [127]. Other tests using the disintegration apparatus mimic what is done for tablets and capsules, but include a way to secure the film in the basket or to the arm of the apparatus [123]. There are pitfalls with both the large and small volume methods as they have

user defined endpoints, use non-biologically relevant volumes of media or introduce a large amount of user bias into the final measurement of disintegration. In addition, these tests can be difficult to replicate and control (i.e. droplet size) and can have large deviations [123, 124]. With this wide range of *in vitro* methods, it is difficult to draw meaningful comparisons between film products.

There are also limitations with *in vivo* methods used to evaluate pharmaceutical film performance and functionality, integral for the successful development of any product. Relating specifically to microbicide development, there are numerous standardized *in vivo* tests to examine safety, irritation, efficacy as well as pharmacokinetics and pharmacodynamics [47]. These parameters though are highly dependent on topical product functionality; that the applied product can distribute and coat the target tissues and effectively deliver the formulated agent [128]. Without effective spreading and coating of the vaginal or rectal tissues, drug delivery to target tissues will be stunted or ineffective. As these tests of functionality are not required, though highly informative, they are not always done as part of the development process [47]. However, testing the spreading, coating or retention of a product over time can be extremely informative when evaluating different formulations or developing extended release products. Common performance characteristics which can be evaluated *in vivo* as part of topical microbicide development include; dispersion behavior, bio-adhesion, retention, spreading, and rheology [129]. These parameters are commonly tested using gamma scintigraphy, optical scanning, colposcopy [129], but there is no standard method used for microbicide film development. Magnetic resonance imaging (MRI) has been used *in vivo* for microbicide gel product tracking to confirm product placement and retention in either the rectal to vaginal cavity

[130, 131], however these studies are costly and to date, gel specific. There has been no film-specific, standardized tracking studies.

As the overall goal of this dissertation is to develop extended release films, we first wanted to put in place methods which can be used to evaluate critical parameters of these films. In this chapter we aim to establish methods which can be used for the evaluation of crucial film attributes both *in vitro* and *in vivo*. These tests aim to fill critical gaps in the current field of microbicide film development, which will not only aid in the development of extended release films in this project, but also in future pharmaceutical film and microbicide product development. Standardizing the disintegration test to characterize film dosage forms would allow for comparisons between various films that range in application, delivery site, and release profiles. Therefore, one of the goals of this project is to establish a standard testing method for *in vitro* film disintegration that provides objective and quantitative data. It will be of high importance to design and qualify this testing method through Quality by Design (QbD) approach [132, 133]. QbD is a concept centered on the idea that quality must be built into a product and is widely used in pharmaceutical development and highly encouraged by the FDA [134]. This approach focuses on the following principles as stated by Yu et al. which are:

1. A quality target product profile (QTPP) that identifies the critical quality attributes (CQAs) of the drug product
2. Product design and understanding including the identification of critical material attributes (CMAs)
3. Process design and understanding including the identification of critical process parameters (CPPs) and a thorough understanding of scale-up principles, linking CMAs and CPPs to CQAs
4. A control strategy that includes specifications for the drug substance(s), excipient(s), and drug product as well as controls for each step of the manufacturing process
5. Process capability and continual improvement [134].

These principles can be applied to pharmaceutical process development and the disintegration method will therefore aim to follow a QbD structure as described by Borman et al. in which these principles are applied to process development. This method focus on the establishment of: the design intent or the performance requirements of the method, the design selection or method development, control definition and control verification [135, 136]. The goal of this method is to establish a quantitative disintegration method that introduces less user bias than visual methods. Specific parameters utilized for this test were tailored towards vaginal films. It is anticipated that these parameters can be modified for other film applications. A second goal of this project is to establish *in vivo* methods which can be used to track film spreading, retention and drug disintegration *in vivo* using the macaque model. The use of colposcopy imaging methods are proposed an alternative to MRI for study of film product functionality. In the development of these *in vitro* and *in vivo* methods, we utilized two of the most clinically advanced vaginal film formulations to evaluate the ability of these methods to measure disintegration and *in vivo* distribution and drug release.

The development of an *in vitro* disintegration method and an *in vivo* tracking method aim to add valuable steps to the development process for polymeric films, specifically for vaginal microbicide delivery. Standardizing these testing parameters can be used to evaluate film quality and performance and establish useful new methods to develop lead products and formulations.

Throughout these studies, two model microbicide compounds were utilized; dapivirine (DPV) and tenofovir (TFV). Dapivirine, (4[4-[(2,4,6-trimethylphenyl) amino-2-pyrimidinyl] amino] benzonitrile) or (TMC120), targets viral reverse transcriptase and is a non-nucleoside reverse transcriptase inhibitor. It is a hydrophobic compound (logP of 5.27) with a molecular mass 329.4 [49]. Characteristic of an ideal drug candidate for vaginal or rectal HIV prevention,

DPV is extremely potent with an IC_{50} of 7.9 nM [49]. Akil et al. successfully formulated this compound into a polymeric thin film, which was then evaluated clinically [35, 49, 89]. The film was formulated into a polyvinyl alcohol base which dissolved quickly (less than 10 minutes) and allowed the drug to be released rapidly [49]. DPV retained its anti-HIV activity after being formulated into a solvent-casted film and was found to be non-toxic to innate vaginal bacteria, *L. crispatus* and *L. jensenii*, *in vitro* [49]. Further, DPV has been formulated for HIV prevention as a microbicide ring and gel and clinical studies have shown that the gel, film, and ring when administered correctly, are safe and effective against HIV acquisition [31, 35, 89]. Similar to DPV, TFV targets viral reverse transcriptase. Although both compounds target similar points in the HIV lifecycle, there are many differences between the two. TFV is a hydrophilic ($\log P$ of -1.6), nucleoside reverse transcriptase inhibitor [44] with an IC_{50} of 2 μM [137]. The prodrug of TFV has been formulated along with emtricitabine and marketed as Truvada[®] for oral PrEP and is the only FDA approved PrEP medication. TFV was successfully formulated into a cellulose based film formulation intended for the quick dissolution and release of the compound [91]. One of the first, large-scale microbicide trials to show efficacy was using a TFV gel and it was found to reduce HIV infection by 39%. TFV has also been formulated in combination with DPV and has shown to provide a synergistic effect in combination [44, 138].

DPV and TFV were formulated into previously developed film formulations for use in studies throughout this chapter. These two films are the most clinically advanced films and will be used throughout this chapter to evaluate the developed disintegration and imaging methods.

2.2 MATERIALS

Tenofovir was graciously provided by CONRAD (Arlington, Virginia, USA) and dapivirine was graciously provided by the International Partnership for Microbicides (IPM, Silver Spring, MD, USA). Film excipients used in the formulations were; polyvinyl alcohol (PVA) (Emprove[®], EMD Millipore), glycerin (Spectrum Chemical), hydroxymethylcellulose (HPMC) (Dow Pharmaceutical Solutions), propylene glycol (Spectrum Chemical), polyethylene glycol (PEG) 8000 (Dow Pharmaceutical Solutions), hydroxyethyl cellulose (HEC) (Ashland), carboxymethylcellulose sodium (NaCMC) (Spectrum Chemical), polyvinylpyrrolidone (PVP) (Fluka) and sodium hydroxide (Spectrum Chemical). MultiHance[®] (gadobenate dimeglumine), 529 mg/mL (Bracco Diagnostic Inc.) was used for MRI studies. Food grade blue dye containing water, glycerine, FD&C Blue #1, citric acid and sodium benzoate (as a preservative) (Target Brands, Inc.) was used for film tracking studies. Films were cast onto an Elcometer[®] 4340 Automatic Film Applicator using an Elcometer[®] Doctor Blade. A TA.XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA/Stable Micro Systems, Godalming, Surrey, UK) and associated TA.XTPlus Texture Analyzer probes and accessories were used for the disintegration testing. The TA-108S5 fixture with five 10mm openings was used to secure films and the TA-8A: 1/8" diameter rounded end ball probe was used to apply force to the films.

2.3 METHODS

2.3.1 Film Manufacture

The DPV film formulation was composed of a base polymer of PVA as well as glycerin, HPMC, propylene glycol and PEG 8000. The TFV film formulation was composed of three cellulose-based polymers; HEC, HPMC and NaCMC, glycerin and sodium hydroxide. Films were made using the solvent cast manufacturing method [49]. Briefly, polymers, excipients, plasticizer and APIs were either dissolved or dispersed in water using a Caframo Ultra Torque overhead mixer and an IKA bladed propeller stirrer. Solutions were mixed until homogenous and cast on a heated polyethylene terephthalate (PET) substrate (Amcor Flexibles, Mundelein, IL, USA) secured to an Elcometer[®] 4340 Automatic Film Applicator using an Elcometer[®] 3700 Doctor Blade. Films were allowed to dry and were then peeled, cut, and packaged in aluminum foil packaging material. DPV films were scaled to a 1" x 1" size for the macaque vaginal studies. Films were made to mimic the standard thickness film which is currently in development [139] and these films were poured at a thickness of 110 μm . To evaluate if the disintegration method developed was sensitive enough to detect changes in formulation, a superdisintegrant (sodium starch glycolate) [140] and a mucoadhesive thiomers were incorporated into the DPV film formulation for additional studies. Tenofovir films were also scaled to a 1" x 1" size for the macaque vaginal studies. Films were made to mimic the film which is clinical development as a fast dissolve, coitally dependent film [141] and were poured at a thickness of 100 μm . For tracking and visualization, DPV and TFV films were made with the incorporation of Gadobenate dimeglumine (GD-contrast agent) for MRI tracking studies or a water soluble blue dye (composition listed in the Materials section above) for colpophotography visual retention studies.

Gadobenate dimeglumine was added to formulations at a ratio of 1 to 400 (Gadobenate dimeglumine: total formulation) and blue dye was added to formulations at a ratio of 0.75g / 5 grams of formulation.

2.3.2 Film Characterization

Films were characterized for mass (mg), thickness (μm), puncture strength (g/mm), water content (% w/w) and drug content (mg/film). Mass was measured using an analytical balance and thickness was measured using a Mitutoyo Absolute electronic thickness gauge. Puncture strength was determined using a TA.XTPlus Texture Analyzer and the force required to break the film per cross-sectional area was measured. Residual film water content was measured using a Karl Fischer Titrando[®] instrument with oven setup. DPV content was measured by first extracting the drug from the film using a 50% acetonitrile in water solution, followed by reverse phase Ultra Performance Liquid Chromatography (UPLC) analysis using a Waters Acquity[®] UPLC System with a Waters Acquity[®] TUV dual wavelength detector using Waters Acquity[®] BEH C18 1.7 μm 2.1x50mm column at a wavelength of 290 nm. Mobile phases of 0.08% trifluoroacetic acid (TFA) in water (v/v) and 0.05% TFA in acetonitrile (v/v) at an initial ratio of 90:10 were used, and DPV retention time was 9.7 ± 0.6 min. TFV content was measured by first extracting TFV from the films by dissolving the film in water, diluting with formic acid and then using solid phase extraction (using solvents; 2% formic acid and 5% methanolic ammonium hydroxide) to extract TFV from the film solution. After extraction, drug content was determined using a Waters Acquity[®] UPLC System with a Waters Acquity[®] TUV dual wavelength detector using Waters Acquity[®] BEH C18 1.7 μm 2.1x50mm column at a wavelength of 260 nm. Mobile

phases of 0.1% TFA in water (v/v) and 0.1% TFA in acetonitrile (v/v) at an initial ratio of 100:0 were used, and TFV retention time was 2.6 ± 0.4 min.

2.3.3 *In Vitro* Release

In vitro release was measured using a USP 4 flow through SOTAX CE 7 dissolution system followed by UPLC analysis as previously described for API quantitation. Films were loaded into 12mm cells maintained at a temperature of 37°C with a flow rate of 16ml/min and sample aliquots were taken. 0.5 mL aliquots were taken at the following time points: 0, 3, 6, 10, 15, 30, 45, 60 minutes. Media used was either 50% acetonitrile in water (DPV analysis) or 1X Phosphate-buffered saline (PBS) (TFV analysis). Aliquots were analyzed using UPLC methods as described.

2.3.4 Disintegration

2.3.4.1 Visual Disintegration Testing

Visual disintegration tests were performed as previously described [49, 52]. Briefly, a film was placed in 1 mL of water and set on an orbital shaker. A timer was started when the film came in contact with the fluid and ended when the film had complete structural loss, which was observed visually. This was used as a control throughout this study.

2.3.4.2 TA.XTPlus Texture Analyzer Disintegration Testing

A TA.XTPlus Texture Analyzer and associated TA.XTPlus probes and accessories were used for the disintegration testing. The TA-108S5 fixture with five 15 mm openings was used to secure films and the TA-8A: 1/8" diameter rounded end ball probe was used to apply force to the films.

Design Intent

Precision: Precision limit is set initially within a relative standard deviation less than or equal to 15%. *Sensitivity:* The method will be able to measure time in seconds to at least two decimal places. *Selectivity:* The assay can differentiate between films prepared with distinct film forming polymers, thickness or excipients.

Design Selection/ Method

A TA.XTPlus Texture Analyzer instrument was utilized. Films were secured in the TA-108S5 fixture and the TA-8A: 1/8" diameter rounded end ball probe was affixed to the TA.XTPlus Texture Analyzer instrument. A "Hold Until Reset" test was developed using the Texture Analyzer software, Exponent. Test parameters were as follows: Test Mode: Compression; Pre-Test: 0.5 mm/sec; Test: 0.2 mm/sec; Post Test: 10 mm/sec; Force: 5-15g; Auto Trigger: 5g; Max tracking Speed: 5 mm/sec; Proportional Gain: 50; Integral Gain: 20; and Differential Gain: 5. Films were secured in the TA-108S5 fixture, the probe applied a constant force to the film product, and a biologically relevant amount of fluid was applied to the film surface where the probe interacts with the film. The probe was able to penetrate the film upon disintegration resulting in an applied force of zero at that point. A curve of force vs. time was plotted, and disintegration time was defined as the time from initial fluid addition until the probe force

reached zero. Test parameters such as fluid volume and probe force were optimized to reduce error.

Data Analysis

Data was collected and analyzed by Texture Analyzer software, Exponent. For analysis, a specific macro was used to measure the time between fluid addition and zero force application on the film, defined as disintegration time.

Control Definition

As this method is not a standard analytical method, controls for this method were determined through vigorous testing and assessment of various parameters to ensure that the intentions set for the method are achieved. These parameters included the impact of crucial testing factors (volume and force) on measured disintegration time, reproducibility and variability of the method through validation with a commercially available film (VCF[®]), and selectivity of the method through testing with a series of vaginal films with various modifications.

2.3.5 Statistical Analysis

Disintegration times were measured in seconds. Data is represented as average disintegration time, and variability is represented as the standard deviation (SD). Statistical analysis was performed using unpaired t-tests and one-way ANOVA (GraphPad Prism 6.07). Tukey's test was used for post-hoc analysis. P values less than 0.05 were considered statistically significant.

2.3.6 *In Vivo* Characterization

Dr. Dorothy Patton and Yvonne Cosgrove Sweeny at the University of Washington conducted the *in vivo* NHP studies at the Washington National Primate Research Center (WaNPRC).

2.3.6.1 *In Vivo* Film Retention

One GD-labelled film was intravaginally delivered to each of four pigtail macaques at the Washington National Primate Research Center (WaNPRC). MRI (3 T Philips Achieva MRI) used to track the film dispersal from the point of instillation, throughout the vagina, the upper reproductive tract (URT) and rectum of the pigtail macaques. Sagittal and axial sections of each macaque's pelvic cavity were obtained at 4 and 24 hours post film insertion. MRI scans were scored on a binary scale signal for presence in the vaginal canal, ectocervix, endometrium, fallopian tubes, rectum, urethra and periurethral tissues.

2.3.6.2 *In Vivo* Drug Release

Visual film disintegration and swab collection for drug quantification was conducted at the Washington National Primate Research Center (WaNPRC). A speculum was inserted vaginally and colpophotography was used to examine the film presence or absence. A vaginal swab was taken using a Dacron polyester-tipped swab and was analyzed for drug content using Waters Acquity[®] UPLC and a Thermo Quantum Advantage MAX TSQ triple quad mass spectrometer in positive electrospray ionization mode. The DPV method used a 5mM ammonium formate and acetonitrile mobile phase on a Phenomenex Hyperclone 3 μ BSD C8 150x4.6 mm column, running at 1mL/min from 60% acetonitrile to 80% acetonitrile over 6 minutes. The mass spec monitored the mass transition of DPV from m/z 330.2 to m/z 158. An internal standard (d4-

DPV) was used for quantitation; its transition is m/z 334 to m/z 145. For TFV, an Agilent ZORBAX XDB-C18 5 μ m, 4.6x50 mm column was used with a 0.1% formic acid and methanol mobile phase running at 0.5mL/min from 5-50% methanol over 5 minutes. The transitions were m/z 288 to m/z 176.1 for TFV, and m/z 293 to m/z 181.1 for the internal standard ^{13}C -TFV.

2.4 RESULTS

2.4.1 Film Manufacturing

2.4.1.1 Dapivirine and Tenofovir Containing Films

DPV and TFV films were successfully manufactured using the solvent cast method described. Excipient and polymer percentages which were included in the formulations of the DPV and TFV films can be found in Tables 1 and 2, respectively. These films were made to mimic the standard thickness film which is currently in development and are therefore termed TFV Clinical and DPV Clinical Films.

Table 1: Dapivirine Film Formulation

Component	Percent (w/w)
Polyvinyl alcohol	7.02
Polyethylene glycol 8000	2.34
Hydroxypropyl methylcellulose (HPMC) E5	1.75
Dapivirine	0.34
Propylene Glycol	0.73
Glycerin	0.73
MilliQ Water	87.26

Table 2: Tenofovir Film Formulation

Component	Percent (w/w)
Hydroxyethyl cellulose (Medium Viscosity)	6.00
Hydroxypropyl methylcellulose (HPMC) E5	6.00
Carboxymethylcellulose Sodium (Low Viscosity)	2.00
Tenofovir	4.00
Glycerin	2.00
Sodium Hydroxide	0.56
MilliQ Water	79.44

2.4.2 Film Characterization

After manufacturing, films were characterized for weight, thickness drug content, puncture strength, water content and disintegration time (after method development). These parameters can be found in Table 3. Disintegration time was most significantly impacted by the inclusion of the mucoadhesive polymer, which also impacted the puncture strength of the films. Water content was below 6% for all films manufactured.

Table 3: Tenofovir and Dapivirine Film Characterizations

Film	TFV Clinical Film	DPV Clinical Film	DPV in the Cellulose Film	DPV Clinical Film w/Super Disintegrant	DPV Clinical Film w/ Mucoadhesive
Weight (mg)	88.92 ± 3.64	76.68 ± 3.44	76.41 ± 2.80	69.2 ± 1.76	71.50 ± 11.76
Thickness (µm)	87.67 ± 5.37	98.91 ± 6.67	120.00 ± 8.34	155.90 ± 9.75	131.37 ± 11.94
Drug Content (mg/film)	18.17 ± 1.18	1.76 ± 0.13	1.83 ± 0.11	1.41 ± 0.13	1.64 ± 0.19
Puncture Strength (g/mm)	8.58 ± 0.83	12.01 ± 0.50	4.87 ± 1.31	3.77 ± 0.58	13.55 ± 0.48
Water Content % (w/w)	5.94 ± 0.28	3.52 ± 0.42	4.43 ± 0.66	4.87 ± 0.2	5.89 ± 0.057
Disintegration Time (sec)	41.93 ± 2.39	88.36 ± 10.61	36.20 ± 5.30	56.63 ± 6.49	167.61 ± 21.20

Data shown is the mean ± standard deviation

2.4.3 *In Vitro* Release

To ensure drug release from film matrices, *in vitro* release was conducted using a USP 4 Flow Through system (SOTAX) followed by UV analysis. The percent of drug release over time was evaluated and both films showed greater than 75% drug release within the first ten minutes (Fig. 2).

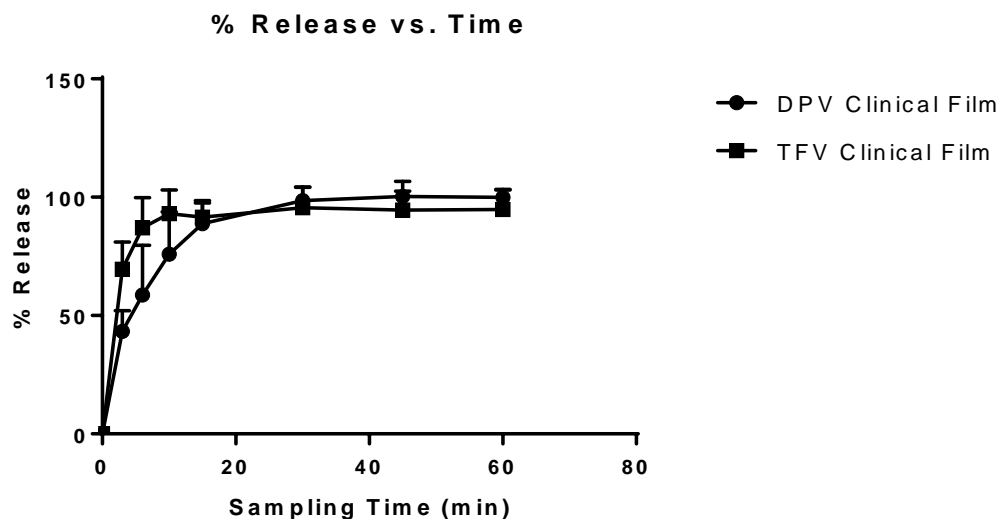


Figure 2: Tenofovir and Dapivirine *In Vitro* Release

Tenofovir (TFV) and dapivirine (DPV) release profiles evaluated using the USP 4 SOTAX *in vitro* release method. Release media was different for each film: TFV, 1X phosphate buffered saline; DPV, 50% acetonitrile. Data shown is the mean \pm standard deviation of three replicates.

2.4.4 Quantitative Disintegration Development

This method was developed and tested following QbD framework, by establishing control definitions and design intent with precision, sensitivity, and selectivity limits. The design intent of this method was develop a quantitative disintegration method which can be used as standard testing method for polymeric films. The Texture Analyzer instrument was selected because it can be configured to have a discriminatory, non-user defined endpoint for the disintegration time of solid dosage forms. It can provide quantitative measures (up to 2 decimal

places) and has a desirable data acquisition rate (up to 500 points per second (pps)). Previous disintegration methods, as discussed, introduce user bias and large variability. Ham et al., when developing a topical microbicide product using the solvent cast method of film manufacture, used visual disintegration methods to evaluate disintegration time of a range of polyvinyl alcohol based formulations. Disintegration times of these films ranged up to 36% RSD demonstrating that visual methods used to evaluate solvent cast films have a wide range of variability [52]. Another group, Garsuch et al., compared different film forming polymers and evaluated disintegration using the slide frame method of film disintegration. Variability was also present in this method and RSD values ranged up to 50% [124]. Therefore, selecting a 15% RSD precision limit is seen as a significantly improved variability target compared to current disintegration methods.

The method was created using the standard template provided by the Textual Analyzer software, Exponent, using a “Hold Until Reset” test. This test applied a small amount of force constantly to the film in the presence of a biologically relevant volume of media, and the time required for the probe to penetrate the film completely, reaching zero force, was measured. The test was reset manually and repeated as necessary. The disintegration time was measured from the time the fluid was added to the film until the force reached zero value. The probe force alone, without fluid addition, was not significant enough to break through the film. Further, the time between when the probe first comes into contact with the film until fluid addition, was controlled for. The parameters that impact the disintegration time were identified and optimized. These included probe force and the volume of fluid applied to the film. The TA-108S5 fixture and the TA-8A: 1/8" diameter rounded ball were selected for set up because this fixture securely holds the film for testing and the ball probe fits within the 15 mm opening. Fig. 3 shows the actual

setup of the Texture Analyzer instrument (a), a graphic of the test setup (b), and a typical disintegration plot produced with the Exponent software (c).

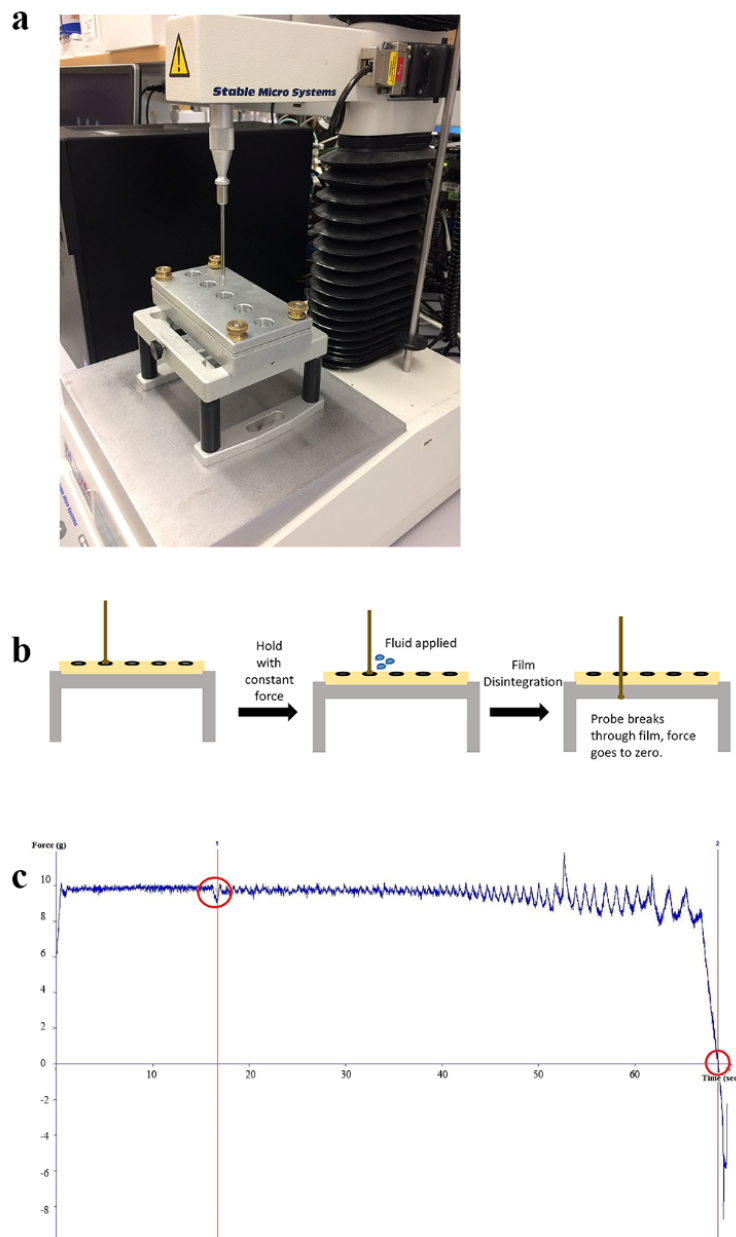


Figure 3: Texture Analyzer Instrument Setup

(a) Instrument setup with TA-108S5 fixture and the TA-8A: 1/8" diameter rounded end ball probe. (b) Graphical schematic of setup and test positions of the Texture Analyzer disintegration technique. (c) Typical plot of force vs. time graph produced with Exponent software. Event at 15 seconds and force to zero (disintegration test end) marked in red.

As part of the control section of QbD, three different parameters were evaluated: the impact of crucial testing parameters on outcome, reproducibility and variability of the method through validation with a commercial film, and selectivity of the method through testing with a series of vaginal films with various modifications. The goal of the test was to use a minimal, consistent force that can puncture the film only when the integrity of the film structure is lost in the presence of fluid. It is to be noted that there is no external, consistent force applied *in vivo*, but squeezing forces within the vagina exist which could aid in product disintegration. For initial volume and force experiments, the Vaginal Contraceptive Film (VCF[®], Apothecus Pharmaceutical Corporation) was used. VCF[®] is a commercially manufactured product therefore less variability exists when compared to film products manufactured through hand-poured methods on the laboratory scale. Three initial forces of 5, 10 and 15 grams were tested on the instrument when applied to a film. The disintegration test was performed using 15 μ L of fluid. Disintegration times between the 5 and 10 gram forces showed no statistically significant differences, but there were significant differences between the 5 and 15 gram and 10 and 15 gram disintegration times (Fig. 4). All deviations were within the set precision limit.

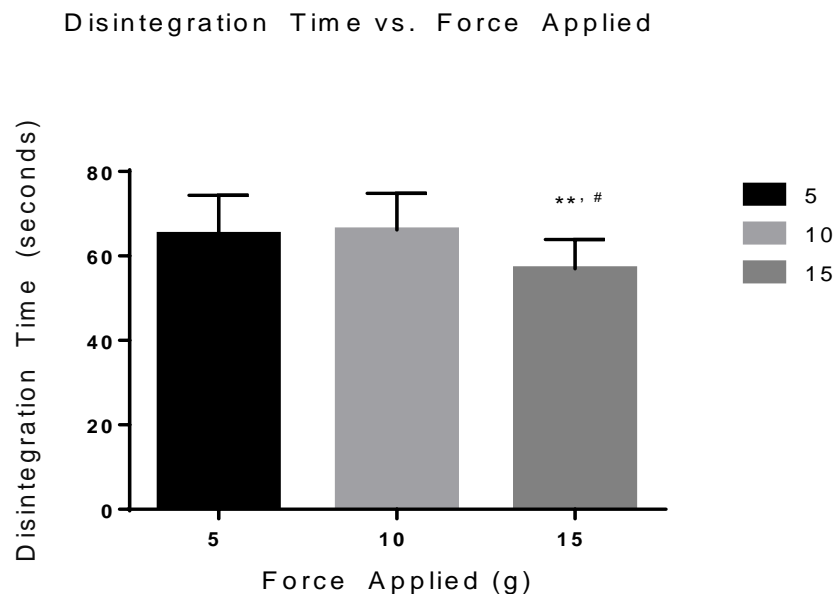


Figure 4: Force Testing using Commercially Available Vaginal Contraceptive Films[®]
 Disintegration times of Vaginal Contraceptive Films (VCF)[®] (GMP product) with varying force obtained using the TA.XTPlus Texture Analyzer disintegration method. Data shown is the mean \pm standard deviation of sixteen replicates. (** 15 g vs. 10 g, # 15 g vs. 5 g)

Due to the goal in developing this method, the 10 gram force was chosen and can be rationalized by the following explanation. The average vaginal squeezing force is estimated to be 4.45 N to 44.5 N over a surface area of approximately 100 cm² [142]. Based off the area of the film exposed to the fluid (1.767 cm²) and the conversion of N to grams, the resultant force for that area would be approximately 8 grams. The 5 gram force may be more applicable for oral applications, as this force more closely mimics the force which a human tongue applies when licking a probe [127]. Volume of fluid added to the film was the other critical parameter assessed. Based on the probe size and the 15 mm opening of the film holder, the following volumes were selected for testing; 5, 10, 15, 20, 30, 45, and 135 μ L (Table 4). Post-hoc analysis using a Tukey test of multiple comparisons for results from volume testing showed there were statistically significant differences between disintegration times when using the following

volumes ; 5 μL and 15 μL (p value = 0.0040), 15 μL and 30 μL (p value = 0.0498) and 15 μL and 135 μL (p value = 0.0001). These tests show that fluid volume impacted disintegration time and that at higher volumes, 45 μL and above, the relative standard deviation exceeded 15% (Table 4). The mean surface area of the vagina is 87.46 cm^2 [79] and average amount of fluid in the vagina is 0.5–0.75 mL [81]. Based off of this, and the area of film exposed to fluid in the holder, 15 μL was selected for our testing of films formulated for vaginal use.

Table 4: Volume Testing using using Commercially Available Vaginal Contraceptive Films[®]

Volume	Average Disintegration Time (seconds)	Standard Deviation (seconds)	% Relative Standard Deviation (RSD)
5 μL	51.37	7.19	14.00
10 μL	56.19	6.07	10.80
15 μL	66.19	8.61	13.02
20 μL	56.86	6.46	11.37
30 μL	54.55	3.99	7.32
45 μL	59.26	13.47	22.73
135 μL	47.82	20.53	42.92

Reproducibility was evaluated with a marketed product, the Vaginal Contraceptive Film (VCF[®], Apothecus Pharmaceutical Corporation). Testing with VCF[®] performed on separate days and by different users to assess reproducibility and variability of the test. As highlighted in the data from Table 5, disintegration times are reproducible with average deviations within the desired QbD precision range. The average disintegration time for the VCF[®] was found to be 57.88 seconds and on average, regardless of user or time, the average % RSD fell within the set precision limit ($\leq 15\%$).

Table 5: Disintegration Testing using Commercially Available Vaginal Contraceptive Films® Evaluated by Different Users

User	Trial Number	Disintegration Time (seconds)	Standard Deviation (seconds)	%Relative Standard Deviation (RSD)
A	1	59.60	4.81	8.07
A	2	60.77	4.36	7.18
A	3	59.21	6.73	11.36
B	4	63.89	7.35	11.50
B	5	53.82	9.38	17.42
B	6	46.27	2.40	5.18
B	7	61.61	6.82	11.08

Following this, the selectivity of the method was evaluated with a series of films containing one of two drugs under investigation for HIV prevention. Two clinically advanced antiretroviral compounds, tenofovir (TFV) and dapivirine (DPV), have been formulated into polymeric films for microbicide delivery. These films differ with respect to polymeric base, with the TFV film being a cellulose-based film [91] and the DPV film being a polyvinyl alcohol based film [49]. Both visual and quantitative disintegration testing were performed on a series of TFV and DPV containing films. Disintegration testing using the Texture Analyzer method could distinguish between these two formulations and showed that they differed significantly with a P-value less than 0.0001 (Fig. 5).

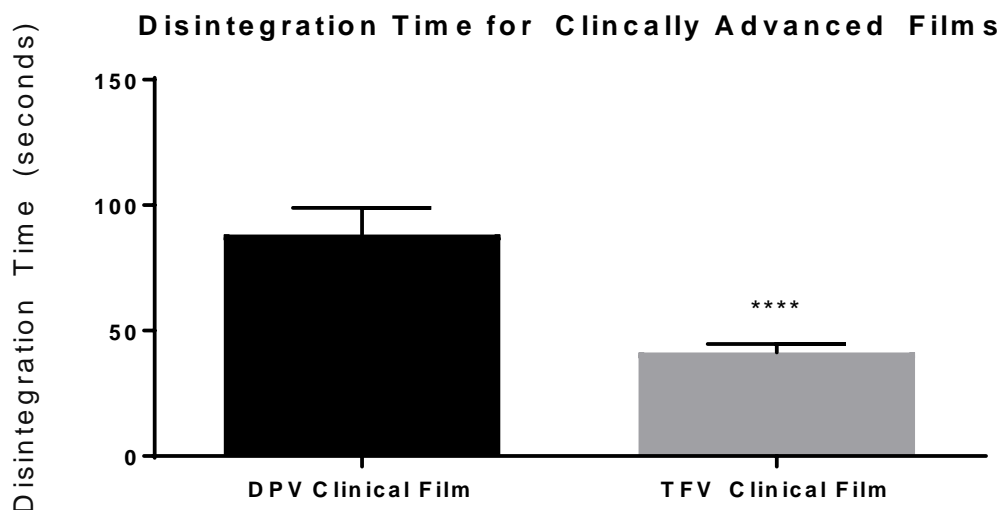


Figure 5: Tenofovir and Dapivirine Clinical Film Disintegration

Comparison of disintegration times of two clinical film products (tenofovir (TFV) and dapivirine (DPV) films) obtained using the TA.XTPlus Texture Analyzer disintegration method. Data shown is the mean \pm standard deviation of eighteen replicates.

Visual disintegration was conducted for these films by submerging film in 1 mL of water and measuring time until complete structural loss while rotating on an orbital shaker. Compared to visual disintegration for these same films (Table 6), the Texture Analyzer method produced lower deviations ($RSD < 15\%$) and the endpoint was not defined by the user. The trend towards longer disintegration time for the DPV clinical film compared to the TFV clinical film was seen, but the p value had lower significance ($p = 0.0013$).

Table 6: Tenofovir and Dapivirine Clinical Film Visual Disintegration

Film	Average Disintegration Time (seconds)	Standard Deviation (seconds)	% Relative Standard Deviation (RSD)
TFV Clinical Film	124.50	23.95	19.24
DPV Clinical Film	227.00	40.88	18.01

Initial film formulations of these compounds were modified by alternate film-forming polymers. TFV films were formulated with a polymer base of polyvinylpyrrolidone (PVP) (as opposed to

hydroxyethyl cellulose) and DPV films were modified from a polyvinyl alcohol base to a cellulose based film. The disintegration results for the modified TFV and DPV films are shown in Figs. 6 and 7, respectively.

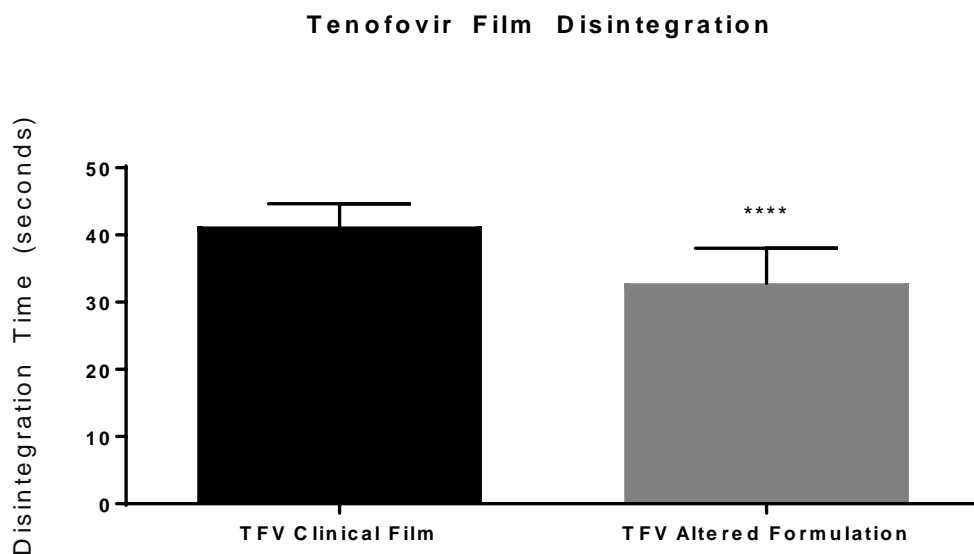


Figure 6: Texture Analyzer Disintegration for Tenofovir Films

Disintegration times obtained using the TA.XTPlus Texture Analyzer disintegration method for tenofovir (TFV) films which have been modified using different base polymers. Data shown is the mean \pm standard deviation of eighteen replicates.

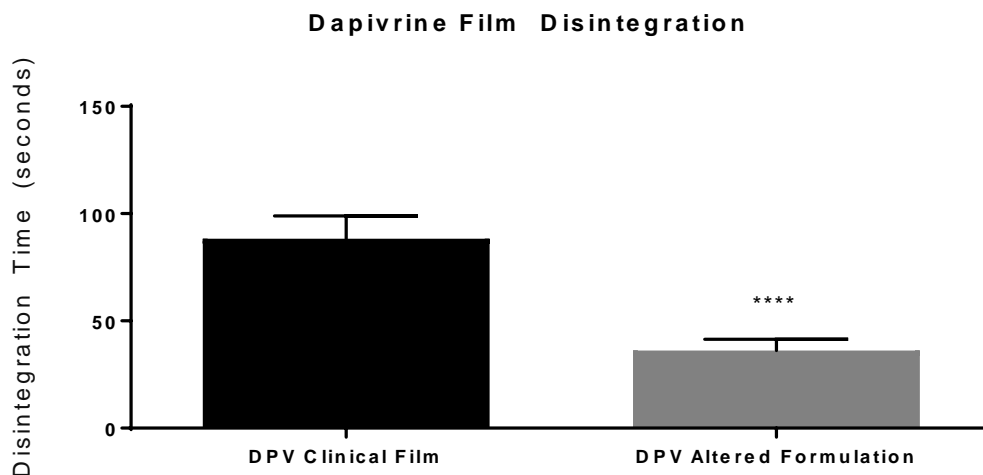


Figure 7: Texture Analyzer Disintegration for Dapivrine Films

Disintegration times obtained using TA.XTPlus Texture Analyzer disintegration method for dapivrine (DPV) films which have been modified using different base polymers. Data shown is the mean \pm standard deviation of eighteen replicates.

The Texture Analyzer method was sensitive enough to be able to distinguish between changes in formulation ($p < 0.0001$) for both TFV and DPV films (Figs. 6 and 7). To see if the method could distinguish between other formulation variations, the DPV formulation was altered through the addition of a mucoadhesive polymer to extend disintegration, or through the inclusion of a super-disintegrant, sodium starch glycolate, to enhance disintegration. Comparisons of average disintegration times between these film formulations showed that they both differed significantly from the quick-dissolving clinical DPV film disintegration time and from each other ($p < 0.0001$) (Fig. 8).

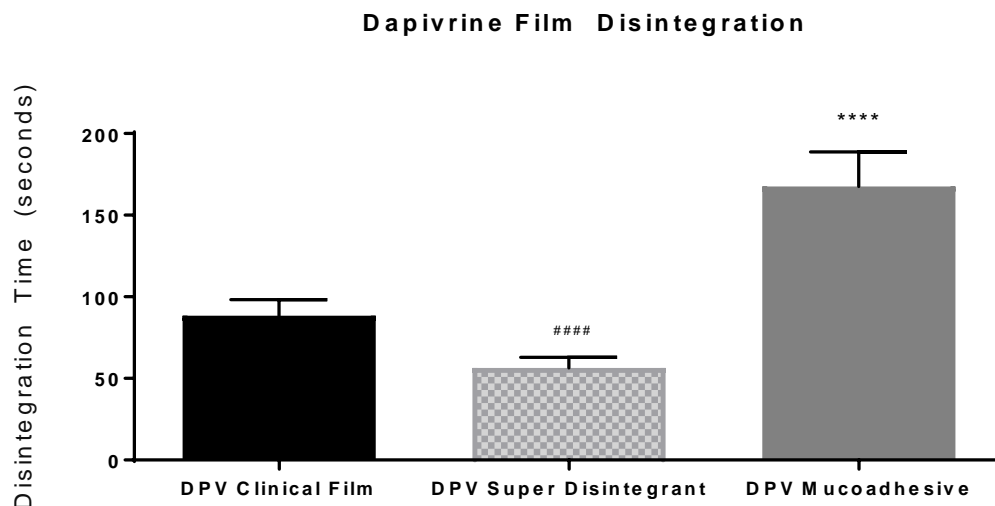


Figure 8: Texture Analyzer Disintegration for Modified Dapivirine Films
 Disintegration times obtained using the TA.XTPlus Texture Analyzer disintegration method for dapivirine (DPV) films which have formulation modifications hypothesized to impact release profile. Data shown is the mean \pm standard deviation of four to eighteen replicates.

2.4.5 *In Vivo* Characterization

Dapivirine and tenofovir films were formulated as described above. They were scaled to a 1" x 1" size for macaque studies. They were formulated to include a water soluble blue dye for disintegration and retention studies or a MRI contrast agent, gadolinium for MRI studies. To characterize the *in vivo* distribution in the macaque vagina, gadolinium loaded TFV and DPV films were vaginally inserted into (4) pigtailed macaques. MRI was conducted at four and twenty-four hours post film insertion. Representative images for TFV and DPV films can be seen in Figs. 9 and 10. Contrast was clearly seen throughout the vagina and ectocervix at four and twenty four hours after film placement. No evidence of contrast was noted in other areas of the genitorectal tract at either time-point.

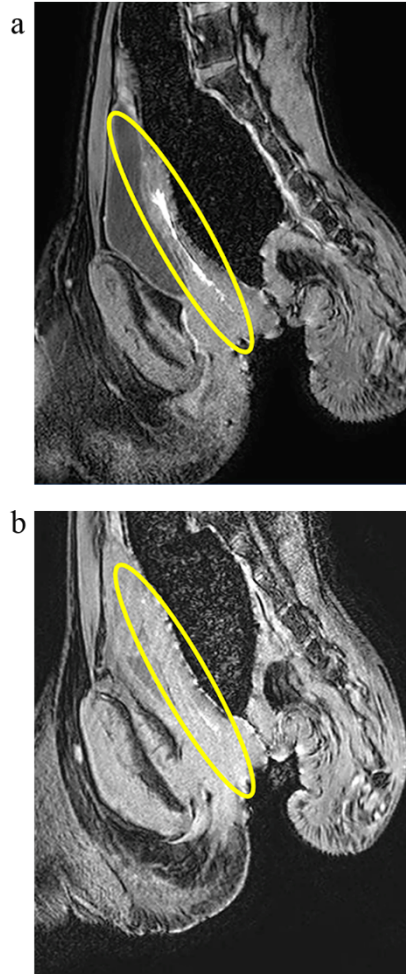


Figure 9: The Tenofovir Clinical Film Distributes throughout the Vagina
Characterization of *in vivo* distribution in the vagina of gadolinium loaded tenofovir (TFV) films which were vaginally inserted into pigtailed macaques. Representative sagittal images for TFV MRI at (a) four hours and (b) twenty-four hours post film insertion. Contrast highlighted in yellow.

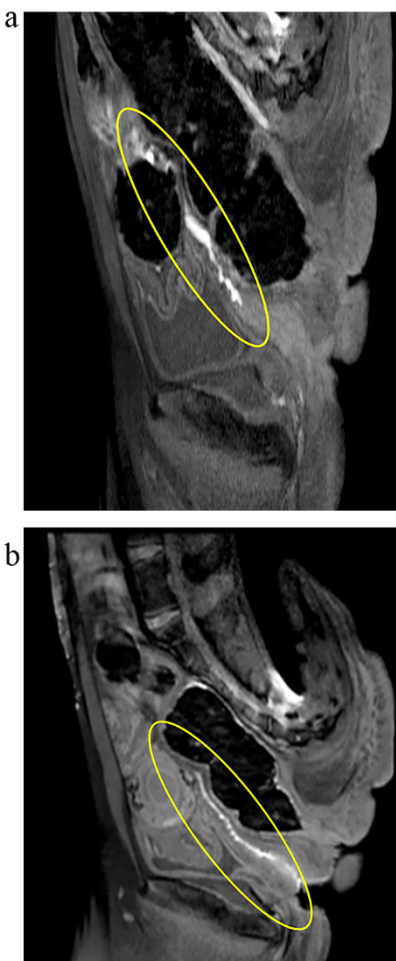


Figure 10: The Dapivirine Clinical Film Distributes throughout the Vagina

Characterization of *in vivo* distribution in the vagina of gadolinium loaded dapivirine (DPV) films which were vaginally inserted into pigtailed macaques. Representative sagittal images for DPV MRI at (a) four hours and (b) twenty-four hours post film insertion. Contrast highlighted in yellow.

Colposcopy images were taken on days pre- and post- film insertion on day 1, and on days 2-5 and day 8 for film visualization. A blue dye was incorporated into the films to aid in this visualization. Vaginal swabs were taken to analyze drug content in vaginal fluid on days 1-5 and day 8. Drug levels are reported in ng/swab and menses animals are denoted by ^M. TFV and DPV images can be seen in Figs. 11 and 12, respectively. TFV film presence was noted in the vaginal compartment up to day 2, while the DPV film was retained for a longer period through

day 3. It should be noted that the number of animals varied for different colpophotography imaging experiments due to the limited availability of the animals, however, a minimum of three was used per experiment.

Day1 Pre-Film	Day1 Post-Film	Day2	Day3	Day4	Day5	Day8
						
absent	present	present	absent	na	na	na
		66375.00 ng/swab	7625.00 ng/swab	1512.50 ng/swab	240.00 ng/swab	7.70 ng/swab
						
absent	present	present	absent	na	na	na
		88500.00 ng/swab	14875.00 ng/swab	3725.00 ng/swab	422.50 ng/swab	17.45 ng/swab
						
absent ^M	present ^M	absent ^M	na	na	na	na
		47.75 ng/swab	24.58 ng/swab	17.43 ng/swab	27.75 ng/swab	5.85 ng/swab
						
absent	present	present	absent	na	na	na
		32500.00 ng/swab	4862.50 ng/swab	1166.25 ng/swab	635.00 ng/swab	2.70 ng/swab

Figure 11: Tenofovir Clinical Film *In Vivo* Retention and Release

Colpophotography images taken on days pre- and post- insertion of a tenofovir (TFV) clinical film formulation for film visualization in pigtailed macaques (n = 4). Blue dye was incorporated into the films to aid in this visualization. Vaginal swabs were taken to analyze drug content in vaginal fluids on days 1-5 and day 8. Drug levels are reported in ng/swab and mensing animals are denoted by ^M. The TFV film was retained up to day 2, while drug was detected in vagina fluids through day 8, suggesting film presence was not indicative of drug presence in the vaginal fluid.





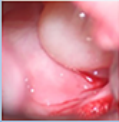
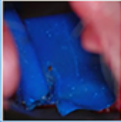


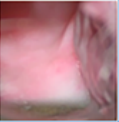



Day1 Pre-Film	Day1 Post-Film	Day2	Day3	Day4	Day5	Day8
						
absent	present ^M	present	absent	na	na	na
		225.25 ng/swab	42.75 ng/swab	56.00 ng/swab	40.75 ng/swab	0.65 ng/swab
						
absent	present	present	present	absent	na ^M	na ^M
		91.00 ng/swab	6.18 ng/swab	6.03 ng/swab	BLQ ng/swab	BLQ ng/swab
						
absent	present ^M	absent ^M	na ^M	na ^M	na	na
		4.78 ng/swab	BLQ ng/swab	0.27 ng/swab	BLQ ng/swab	BLQ ng/swab

Figure 12: Dapivirine Clinical Film *In Vivo* Retention and Release

Colpophotography images taken on days pre- and post- insertion of a dapivirine (DPV) clinical film formulation for film visualization in pigtailed macaques (n = 3). Blue dye was incorporated into the films to aid in this visualization. Vaginal swabs were taken to analyze drug content in vaginal fluids on days 1-5 and day 8. Drug levels are reported in ng/swab and menses animals are denoted by ^M. The DPV clinical film was retained up to day 3, while drug was detected in vagina fluids through day 8, suggesting film presence was not indicative of drug presence in the vaginal fluid. BLQ= below limit of quantitation (LOQ = 0.2ng/mL)

In addition, DPV was incorporated into the cellulose-base to evaluate impact of polymeric base on retention and release in the vaginal compartment. *In vitro* and *in vivo* characterizations can be seen in Table 1 and Fig. 13. When incorporated into a cellulose-based formulation, the film was retained up to day 5 (Fig. 13). Detection of DPV in vaginal fluid was consistent with findings from the TFV films, with detectable drug levels present after complete film dissolution.

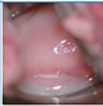






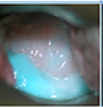

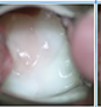




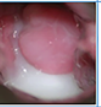



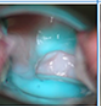
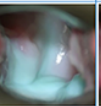
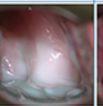
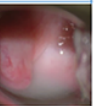
Day 1 Pre-Film	Day 1 Post-Film	Day 2	Day 3	Day 4	Day 5	Day 8
						
absent	present	present	present	absent	na	na
		4175.00 ng/swab	582.5 ng/swab	21.73 ng/swab	62.00 ng/swab	2.20 ng/swab
						
absent	present	present	present	present	absent	na
		807.50 ng/swab	213.00 ng/swab	95.25 ng/swab	50.50 ng/swab	3.48 ng/swab
						
absent	present	present	absent	na	na	na
		865.00 ng/swab	66.25 ng/swab	2.63 ng/swab	1.68 ng/swab	0.25 ng/swab
						
absent	present	present	present	present	present	absent
		15.48 ng/swab	4.00 ng/swab	1.69 ng/swab	0.46 ng/swab	0.28 ng/swab

Figure 13: Altered DPV Film *In Vivo* Retention and Release

Colpophotography images taken on days pre- and post- film insertion of a dapivirine (DPV) cellulose film for film visualization in pigtailed macaques (n = 4). Blue dye was incorporated into the films to aid in this visualization. Vaginal swabs were taken to analyze drug content in vaginal fluids on days 1-5 and day 8. Drug levels are reported in ng/swab and mensing animals are denoted by ^M. The DPV cellulose film was retained up to day 5, while drug was detected in vagina fluids through day 8, suggesting film presence was not indicative of drug presence in the vaginal fluid.

2.5 DISCUSSION AND CONCLUSIONS

Topical microbicides are promising strategies to combat new sexually transmitted HIV infections, especially in women. Gels, films, and rings are the most clinically advanced dosage forms under investigation as topical microbicides [26, 31, 89, 91, 108]. Discrete, female controlled methods of prevention which are cost effective to manufacture are especially attractive because of the socioeconomic and cultural norms in sub-Saharan Africa where the HIV

pandemic is most severe. The polymeric film is a versatile dosage form which has been shown to be favored over other vaginal microbicide dosage forms [35, 108]. The main goals of this chapter was to establish methods which could be used evaluate films *in vitro* and *in vivo* as part of microbicide development and evaluation. We aimed to develop two methods to examine film quality and performance and even correlate these two. There are not standard, film-specific methods which have been used across labs developing these products and here we aimed to develop such standards. Current *in vitro* disintegration methods are based off methods developed for other dosage forms such as tablets or on methods with user-controlled endpoints. Here we wanted to develop a film specific, quantitative disintegration method. There is a clear need for a standard, quantitative method to measure disintegration of the versatile film dosing platform. The Texture Analyzer disintegration method described here provides such a test. Using a focused set of experiments, this test was developed to provide a reproducible and robust method which can distinguish between products and product attribute alterations. Utilization of a Texture Analyzer instrument enabled the design of a method that provided the sensitivity, selectivity and precision set forth before establishing this method. Control definitions (force and volume) were set and the reproducibility using the marketed product VCF[®] was measured. Forces of 5, 10, and 15 grams can be used based on film-specific indication if needed, though the 10 gram force was most suitable for these tests. Fluid amount applied to films can impact disintegration time, and at higher volumes of fluid used, the variation of the test increased. This shows the need to keep volume low ($\leq 30 \mu\text{L}$). The repeatability and reproducibility of the method was confirmed through 7 different trials done by two different users, confirming minimal user bias introduced. Specificity of the method was evaluated using a range of clinically advanced antiretroviral drug containing films, with alterations in base polymer, excipient, and

volume. Specific parameters of this test, such as force applied, volume and type of fluid applied, can be tailored to fit the exact application for oral, vaginal, or other topical films. This quantitative test is essential to understand ultimate *in vivo* functionality and efficacy and it could be included as a product target specification for film products.

To date, there is little data regarding the *in vivo* vaginal behavior of films and how these behaviors relate to film functionality and potential efficacy. The polymeric vaginal microbicide film is designed as quick dissolve, coitally-dependent dosage form intended to deliver HIV preventative compounds to the vaginal tract, but there is little data supporting this. *In vitro* data has been used extensively to characterize the drug release from these films, but the correlation with *in vivo* data has yet to be established. Here, we explore the potential of these films here through use of the well-established and commonly used non-human primate model. The use of the non-human primate has been pivotal in the development of HIV prevention strategies [66, 68-71]. Macaques are the most commonly used model and the rhesus, pigtailed and cynomolgus species are the three most common species used due to anatomical and reproductive similarities to humans, and their ability to be infected with simian immunodeficiency viruses (SIV) SIV/HIV hybrids [54, 64, 65]. We utilize for the first time the non-human primate model to track film distribution throughout the vaginal tract using of magnetic resonance imaging (MRI). Previously formulated tenofovir and dapivirine films [49, 91, 141], were used throughout these experiments. As a solid dosage form, little is known about the behavior of the film after exposure to the vaginal fluids and subsequent disintegration. These films were characterized *in vitro* and *in vitro* dissolution profiles and complete drug release occurred within thirty minutes (Table 3 and Fig. 2). Films tagged with a MRI contrast agent aimed to show both the time frame and extent product spreading and retention after dosage form

disintegration by the vaginal fluid. MRI was used to track film distribution throughout the vaginal tract at 4 and 24 hours post insertion. Four hours was the first time point in which a proper signal could be read without saturation. For both films, MRI contrast was clearly seen throughout the vagina and ectocervix at four hours after placement of the vaginal film, thus confirming film spreading and retention (Figs. 9 and 10). No evidence of contrast was noted in other areas of the genitorectal tract at either time-point. These data suggest that the film provides an effective targeted delivery system since the microbicide film is contained to the vagina and does not spread to other tissues. Images confirm that films disintegrated and spread throughout the vagina, as hypothesized. DPV films appeared to show a stronger signal compared to TFV films at 24 hours post insertion seen by greater contrast (Figs. 9b and 10b). Imaging studies showed evidence of film product at 24 hours post dose, while no film product remained following *in vitro* dissolution. This is an indication that this test is not predictive of *in vivo* behavior. The goal of these studies was to establish methods to track film product *in vivo*, and MRI was able to accomplish this although MRI as a standard characterization technique is not feasible due to high costs, instrument availability, and sedation needed for animals.

As a lower cost alternative to MRI, we attempted to develop a method which could be used to evaluate films *in vivo* without the burdensome cost of MRI in the non-human primate model. To aid in visualization, we incorporated a water-soluble blue dye into the TFV and DPV film platforms. Visualization in the presence of vaginal fluids of the clear films was a potential hurdle, so a colorful agent was hypothesized to aid in visualization. Colpophotography images provided clear evidence of film disintegration and retention over time. The water soluble dye aided in early time points, but is not a clear indicator of film presence as the vaginal fluids diluted its coloring over time and the dye was not retained well in the film product as it lost

structural integrity. Swabs of vaginal fluid were taken and the drug content was tracked throughout the imaging. Clearly seen in the Figs. 11-13, film presence was not indicative of drug presence. Drug was retained in the vaginal fluid without noted film presence, confirming blue dye alone was not sufficient to track film presence. A combination colpophotography with fluid analysis provided the most accurate representation of film retention and drug release. The TFV film was retained in the vagina through day 2, while the DPV film was retained in the vagina through day 3. While the scale of disintegration *in vitro* and *in vivo* differs by magnitudes, the rank order of disintegration is consistent. The DPV film had a longer disintegration time *in vitro* and was retained in the vagina for a longer period of time, thus showing some correlation between the two tests. The MRI signaling with the DPV film at 24 hours was also greater when compared to the TFV film, thus showing another correlation with the disintegration time measured using the novel method described here and film retention.

To provide more comprehensive data for *in vivo* drug release, the amount of fluid collected on each swab should be controlled for in future experiments by weighing the swab pre and post insertion. The swab weight (and therefore fluid amount) was not controlled for in these studies, and therefore a real concentration in amount/volume was not reported. Controlling of this would allow the normalization to the amount of fluid collected. These *in vivo* colposcopy and swab studies show clearly how the product disintegrates and how the drug is released in the vagina and are crucial for the future development of extended release films. As they are not as invasive or expensive as MRI studies, they can be used throughout the development of an extended release platform. The disintegration method developed is also supported by these studies as the rank order of the retention and disintegration patterns for the clinically advanced DPV and TFV films follow that of which was found *in vivo*.

Here we provide two novel methods for the evaluation of vaginal films which will be used throughout this dissertation for the development of novel film platforms. These two methods will be extremely valuable assessment tools for the development of extended release films in this project, but they can also be utilized throughout other areas of microbicide product development and pharmaceutical film development. These methods can provide crucial data for a film characterization parameters, *in vitro* disintegration and *in vivo* functionality. The development and standardization of these tests will help unite the field of microbicide development by providing methods that can be used across labs so that products can be compared.

2.6 ACKNOWLEDGMENT

The current work was funded through the National Institute of Allergy and Infectious Diseases (grant number: 5U19AI082639) and the Bill & Melinda Gates Foundation. Tenofovir was graciously provided by CONRAD (Arlington, Virginia, USA) and dapivirine was graciously provided by the International Partnership for Microbicides (IPM, Silver Spring, MD, USA). I would also like to acknowledge the following people for their assistance in completing these projects. From the University of Washington, Dr. Dorothy Patton and Yvonne Cosgrove Sweeney for all of the work done with the non-human primates. From the University of Pittsburgh, Phillip Graebing for his work on the mass-spectrometry analysis of samples and Lin Wang for general input and suggestions regarding formulation and testing.

3.0 TO ESTABLISH THE USE OF THE VAGINAL POLYMERIC FILM AS AN EXTENDED RELEASE DOSAGE FORM FOR DELIVERING POTENT ANTIRETROVIRALS THROUGH GEOMETRIC MANIPULATION (IE. INCREASED DOSAGE FORM VOLUME).

3.1 INTRODUCTION

Polymeric films have been used in various applications within the pharmaceutical industry to deliver pharmaceutically active agents to the oral, ocular, skin and vaginal mucosa [50, 143-146]. Originally developed to help overcome issues with swallowing of traditional tablets or capsules, these films have now gained use in other fields. Films have indications ranging from use in pain management to opioid dependence to over-the-counter breath fresheners like Listerine® strips. Films are solid dosage forms made up of excipients which create a matrix into which an active pharmaceutical ingredient can be dispersed, dissolved or suspended. Upon introduction into the body, this dosage form comes in contact with bodily fluids like saliva which allows the film to disintegrate and the active agent to be released [28]. Several film attributes make them an attractive platform for pharmaceutical products. Films are non-invasive to administer through oral, ocular and vaginal routes [95]. They can offer targeted drug delivery and therefore enhanced bioavailability. For example, oral films can deliver APIs directly to the oral mucosa. The permeability and vasculature of the mucosa allows for “rapid transmucosal

absorption, high bioavailability and almost immediate onset of action” [147]. Films are associated with good patient compliance [95], as a solid dosage form they are stable and easy to handle during manufacturing and transport and they are cost effective in large scale manufacturing [95]. In addition, films are discrete, they do not require an applicator for usage, can accommodate drugs that could be degraded in an aqueous environment, can be used around the time of sex for immediate release of the active agent, there is no leakage of the product, and there is low side-effect potential [148].

Given these advantages, films have been applied for the delivery of microbicide drug candidates intended to prevent HIV infection. There are several microbicide vaginal films in preclinical development [52, 92, 149-152] and two films that have entered into the clinica, a TFV and DPV containing film [35, 91]. This delivery platform has been able to accommodate hydrophobic, hydrophilic, bacterial and biomolecular microbicide candidates [28] thus demonstrating its versatility as a platform. The two most clinically advanced films, the DPV and TFV, were developed as coitally dependent products for use at or around the time of sex for protection from sexual HIV acquisition. Acceptability and compliance data from recent clinical trials indicates that daily or pericoital use may be a potential barrier to product adherence [22, 26, 89, 153]. For this reason, the field is shifting towards development of longer acting and sustained release products [26, 89]. Combining a highly advantageous dosage form which has favorable characteristics for women [97, 98], with a more acceptable, extended release dosing platform, is a promising strategy to explore for the development of effective microbicide products.

Drug release from a dosage form is broadly classified by the USP into two categories; immediate or modified release [154]. Here, the coitally dependent films evaluated in the clinic to

date, would be an example of immediate release products. Modified release products are dosage forms that alter drug release from a product based on “time, course, and/or location that are designed to accomplish therapeutic or convenience objectives not offered by conventional or immediately release forms” [155-157] and alter when the drug is released from the product or the release rate of the drug from the product [158]. This category encompasses delayed and extended release dosage forms [159]. According to guidelines from USP General Chapter <1121> Nomenclature, delayed release products “are deliberately modified to delay release of the drug substance for some period of time after initial administration” [121]. Extended release products prolong to release of a compound thereby allowing the dosing frequency to be reduced [159]. Throughout this dissertation work, films will be referred to as ‘extended release’ as they are being designed to extend the release rate of the API from the dosage form and to reduce the dosing frequency of current microbicide film products.

As a polymeric solid dosage form, there are physical aspects of the film which can be manipulated to extend the dosing interval. Classified as a swellable dosage form, release of the API from a polymeric film is governed by the imbibement of fluid into the polymeric matrix, swelling of dosage form, resultant polymer chain relaxation and eventual creation of an erosion front [160, 161]. The created erosion front will allow the dispersed or dissolved drug to be release from the film. Using this theory, if the volume of the dosage form is augmented in terms of thickness, then the imbibement of fluid into the dosage form will be slower compared to a thinner film. The amount of fluid in the vagina is limited, with 0.5–0.75 mL of fluid present in the vagina at a time [81], therefore there is not abundant fluid which can keep the imbibement rate constant as polymer concentration in the dosage form increases. Therefore, the relaxation of the polymer chains, creation of an erosion front and drug release will also be retarded. Further, it

is an accepted principal that increased polymer concentration can slow drug release [162]. To slow release of drug from polymeric films and to slow the degradation of the film product, the dosage form can be manipulated by increasing film thickness. Film hydration analysis will differ based on this theory, which has been confirmed this in previous studies [163]. Reinhart et al., using a novel method quantitative imaging refractometry technique, studied film hydration of microbicide containing films after exposure to fluid in the publication *Analysis of Vaginal Microbicide Film Hydration Kinetics by Quantitative Imaging Refractometry*. These studies used two cellulose based microbicide containing films with identical polymer makeup which differed only in thickness. The T1A film had a thickness of 120 μm while T1B had a thickness of 240 μm .

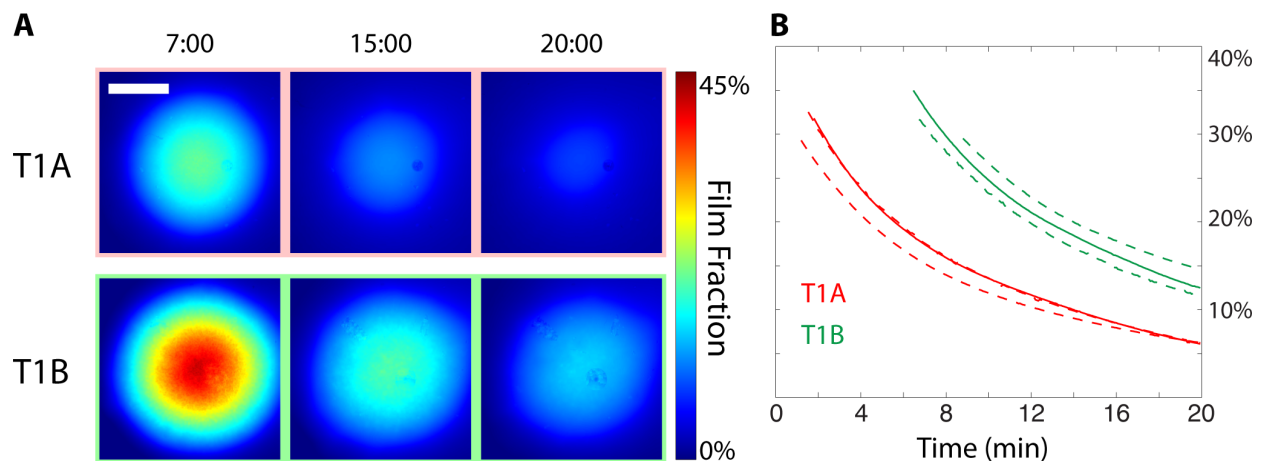


Figure 14: Comparison of two films with different thicknesses, T1A (120 μm) and T1B (240 μm).

(A) Calculated film fraction at 7, 15, and 20 minutes. (B) Film fraction remaining within the initial circular film area during hydration. The three repeats of T1A (red) show a distinctly faster hydration rate than the repeated measurements of samples from the T1B film (blue). Solid lines indicate experimental data depicted in (A), while dashed lines are data from repeated experiments with identical conditions. Scale bar: 0.5 mm. Reproduced from *Analysis of Vaginal Microbicide Film Hydration Kinetics by Quantitative Imaging Refractometry* by Rinehart et al, from *PLOS ONE* [163]

In Fig. 14 [163], as the films are hydrated, film fractions remaining over time are greater for the thicker film, T1B, than for the thinner film, T1A. Rinehart et al. confirmed hydration differences based on thickness because the thicker films consistently dissolved slower than their thin film counterparts after twenty minutes of hydration [163]. Based on these hydration data, we hypothesize that the physical change of increased thickness can lead to a slower release profile which can prolong the residence time of microbicide containing films and lead to a prolonged dosing interval.

To evaluate the impact of film thickness of drug release, both *in vitro* and *in vivo*, films were compared to currently developed TFV and DPV coitally dependent, quick dissolve films [89, 91]. The TFV and DPV platforms will be stressed to test the feasibility of the solvent cast method of manufacture for producing extended release films. These two compounds, which both work to inhibit viral reverse transcription [164], and their formulations will be used throughout this chapter and have been described previously (Chapter 2.3.1).

3.2 MATERIALS

Tenofovir and dapivirine were graciously provided by CONRAD (Arlington, Virginia, USA) and the International Partnership for Microbicides (IPM, Silver Spring, MD, USA), respectively. Film excipients and suppliers are described in Chapter 2.2. Manufacturing materials for films are also found in Chapter 2.2.

3.3 METHODS

3.3.1 Film manufacture for DPV and TFV Films

The PVA based DPV film formulation and the cellulose based TFV film formulation are described in Chapter 2.3.1. Film manufacture for all films followed the solvent casting method, also described in Chapter 2.3.1. Doctor Blade gap setting for films was determined by experimenting with gap size to see resulting film thickness. Film thickness plateaued at a certain Doctor Blade gap. For the TFV film casting, the thickest films that could be manufactured resulted from a Doctor Blade thickness of 400 μm . For DPV film casting, the thickest films that could be manufactured resulted from a Doctor Blade thickness of 430 μm . For tracking and visualization, films were made with the incorporation of a water soluble blue dye (composition listed in the Materials Chapter 2.2) for visual retention studies. The thick, extended release films were used for the visualization studies.

3.3.2 *In Vitro* Film Characterization

Films were characterized *in vitro* for mass (mg), thickness (μm), puncture strength (g/mm), water content (% w/w) and disintegration time (sec.). Mass was measured using an analytical balance and thickness was measured using a Mitutoyo Absolute electronic thickness gauge. Puncture strength and disintegration time were determined using a TA.XTPlus Texture Analyzer. Residual film water content was measured using a Karl Fischer (Titrand®) titration. For further details, see Chapter 2.3.2. Drug content analysis was done using reverse phase UPLC as described in Chapter 2.3.2.

Drug release from the films was determined for each film using a USP 4 flow through SOTAX CE 7 dissolution system followed by UPLC analysis as previously described for individual API quantification (Chapter 2.3.3). Dissolution media was chosen to maintain sink conditions for each compound. Specifically, for the hydrophilic TFV, 1X Phosphate-buffered saline was used and for the hydrophobic DPV, 50% acetonitrile in water was utilized. These methods were used as quality controls for each of the films as neither of the media used were biologically relevant. Aliquots were taken at the following times for TFV: 0, 3, 6, 10, 15, 30, 45, 60, 75, 90, 110, 130, and 150 minutes and for DPV: 0, 3, 6, 10, 15, 30, 45, 60 minutes. Aliquots were analyzed using UPLC methods as described (Chapter 2.3.2).

3.3.3 *In Vivo* (NHP) Film Retention and Drug Release

Methods of *in vivo* swab collection and retention in the NHP, as well as quantification for TFV and DPV on vaginal swabs are found in Chapter 2.3.6. Dr. Dorothy Patton and Yvonne Cosgrove-Sweeny at the University of Washington conducted the studies with the NHP at the Washington National Primate Research Center (WaNPRC).

3.3.4 Statistical Analysis

Data is represented as average (mean) and variability is represented as the standard deviation (SD). Statistical analysis was performed using unpaired t-tests and one-way ANOVA using one (GraphPad Prism 6.07). P values less than 0.05 were considered statistically significant.

3.4 RESULTS

3.4.1 Film manufacture and characterization for DPV and TFV Films

The TFV cellulose-based formulation which is currently in development as a coitally dependent product [165] was manufactured as previously described. The excipients included in the formulation of this film and the uses of each excipient can be found in Table 7. The % solids in the formulation is 14%. Film characterization parameters are summarized in Table 8. The extended release film resulted in a film that was approximately five times the thickness of the film that was used clinically (100 μm) [165], with an average thickness of $462.15 \pm 50.88 \mu\text{m}$ and an average weight of $325.95 \pm 29.23 \text{ mg}$. The films were scaled to a 1" x 1" size as were the films in Chapter 2. TFV drug loading in the clinically applied film was 10 mg and 40 mg per 2" x 2" inch film [165]. For macaque studies, films are scaled to $\frac{1}{4}$ of the human 2" x 2" size. Due to increased film thickness, drug content was targeted to be $\frac{1}{2}$ of the 40 mg dose (20mg/ film), which was also targeted in Chapter 2. TFV is hydrophilic and dissolves in this formulation. This was an initial target loading dose for this feasibility study. Drug content was $18.70 \pm 0.69 \text{ mg/film}$. Weight and thickness for the clinical and extended release profiles show differences, which is expected when manipulating the geometry of the film. Water content of extended release formulation, when compared to the clinically formulated TFV film, was nearly double ($p = 0.0042$), while puncture strength showed an inverse correlation. Puncture strength for the extended release formulation was 2.5 times lower than that of the clinical formulation ($p = 0.0004$). Comparisons of the clinical and extended release TFV films in terms of disintegration and *in vitro* release can be found in Figs. 15 and 16. Disintegration times between the two TFV films show that the extended release has a significantly longer disintegration time compared to

the clinical film ($p < 0.001$). The *in vitro* release profiles also show differences between the two film products as seen through a burst release profile with the thin film and a more sustained profile with the extended release film.

Table 7: Formulation for Tenofovir Films

Excipient	
Hydroxyethyl cellulose (Medium Viscosity)	Film Forming Polymer
Hydroxypropyl methylcellulose (HPMC) E5	Film Forming Polymer
Carboxymethylcellulose Sodium (Low Viscosity)	Film Forming Polymer
Tenofovir	API
Glycerin	Plasticizer
Sodium Hydroxide	Base
MilliQ Water	Solvent

Table 8: Tenofovir Clinical and Extended Release Film Characterizations

	TFV Clinical Film	TFV Extended Release Film
Weight (mg)	88.92 ± 3.64	325.95 ± 29.23
Thickness (μm)	87.67 ± 5.37	462.15 ± 50.88
Drug Content (mg/film)	18.17 ± 1.18	18.70 ± 0.69
Puncture Strength (g/mm)	8.58 ± 0.83	3.37 ± 0.18
Water Content % (w/w)	5.94 ± 0.28	10.02 ± 1.17
Disintegration Time (sec)	41.28 ± 3.35	156.20 ± 20.00
Size	1"x1"	1"x1"

Data shown is the mean \pm standard deviation

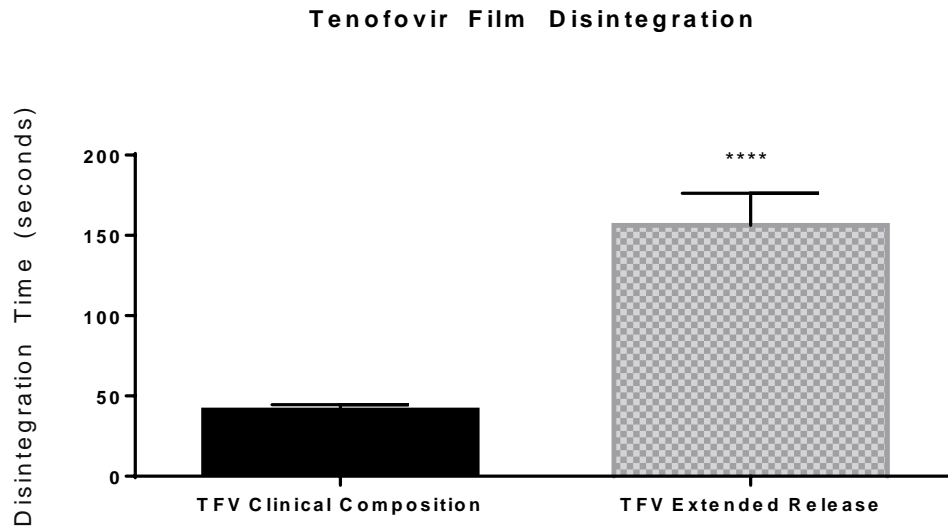


Figure 15: Disintegration Time for Tenofovir Clinical and Extended Release Films
 The tenofovir (TFV) clinical film had a statistically significant shorter disintegration time compared to the extended release film ($p < 0.0001$) evaluated using the TA.XTPlus Texture Analyzer quantitative disintegration method with 15 μL of fluid. Data shown is the mean \pm standard deviation of five replicates.

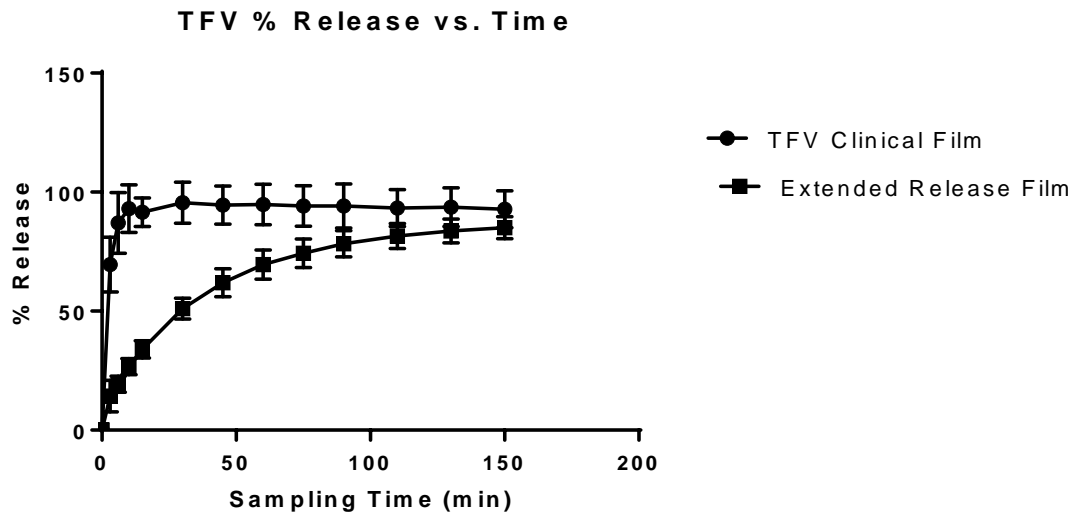


Figure 16: *In Vitro* Tenofovir Release for Clinical and Extended Release Films
 The tenofovir (TFV) clinical film had a characteristic burst release profile compared to the TFV extended release profile evaluated using the USP 4 SOTAX *in vitro* release method using 1X phosphate buffered saline as the release media. Data shown is the mean \pm standard deviation of three replicates.

Similar to TFV, DPV had previously been formulated into a coitally dependent film [35, 49]. The current DPV clinical PVA-based formulation was also used for the extended release formulation of this film and the formulation for this film can be found in Table 9. The % solids in the formulation is 11%. The extended release film was cast approximately three times the thickness of the clinical film (100 μm), with an average thickness of $268.48 \pm 37.95 \mu\text{m}$. This film was also to be used in a macaque for retention studies so it was made in a 1" x 1" size according to procedures in Chapter 2. Drug loading was meant to reflect what was loaded in the clinical films made in Chapter 2, and was reduced so that the increased mass would not increase drug content. The drug loading for the clinical product was 1.25 mg/ film [35] in a 1" x 2" film. This was the initial target for the 1" x 1" macaque thick film. Drug loading was not increased as with the TFV film, because DPV does not readily dissolve in the water based formulation. 1.25 mg/film was the initial target for this feasibility study. Average drug content was 1.47 ± 0.12 mg/film. This was greater than the clinical dose, but this dose was an initial target. Other characterizations for the extended release film can be found in Table 10 and *in vitro* release can be found in Fig. 18. Puncture strength and water content for the extended release and clinical DPV films were not statistically different ($p > 0.05$). Comparisons of release profiles and disintegration times for the extended release and clinical films are seen in Figs. 17 and 18. Disintegration times for the two different DPV films are significantly different ($p < 0.001$) though release profiles between films do not show evidence of different release profiles.

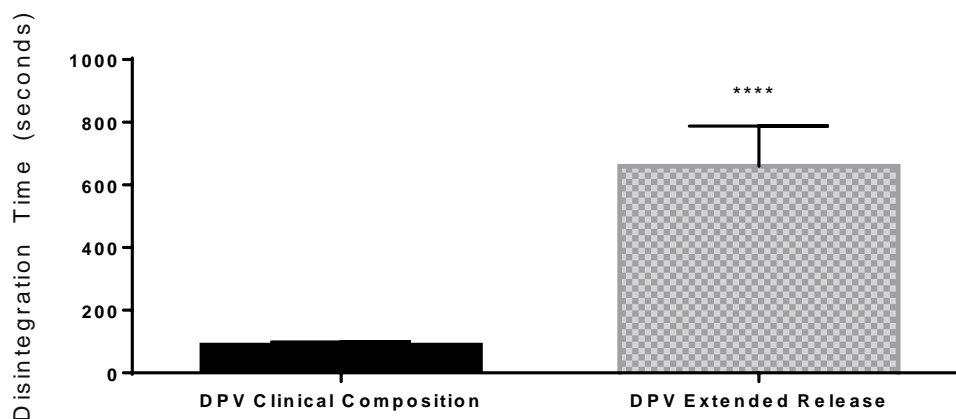
Table 9: Formulation for Dapivirine Films

Excipient	
Polyvinyl alcohol	Film Forming Polymer
Polyethylene glycol 8000	Disintegrant
Hydroxypropyl methylcellulose (HPMC) E5	Film Forming Polymer
Dapivirine	API
Propylene Glycol	Plasticizer
Glycerin	Plasticizer
MilliQ Water	Solvent

Table 10: Dapivirine Clinical and Extended Release Film Characterizations

	DPV Clinical Film	DPV Extended Release Film
Weight (mg)	76.68 ± 3.44	177.03 ± 17.62
Thickness (µm)	98.91 ± 6.67	268.48 ± 37.95
Drug Content (mg/film)	1.76 ± 0.13	1.47 ± 0.12
Puncture Strength (g/mm)	12.01 ± 0.50	11.95 ± 1.75
Water Content % (w/w)	3.52 ± 0.42	3.58 ± 0.46
Disintegration Time (sec)	88.36 ± 10.61	659.63 ± 128.12
Size	1"x1"	1"x1"

Data shown is the mean ± standard deviation

Dapivirine Film Disintegration**Figure 17: Disintegration Time for Dapivirine Clinical and Extended Release Films**

The dapivirine (DPV) clinical film had a statistically significant shorter disintegration time compared to the extended release film ($p < 0.0001$) evaluated using the TA.XTPlus Texture Analyzer quantitative disintegration method with 15 µL of fluid. Data shown is the mean ± standard deviation of five replicates.

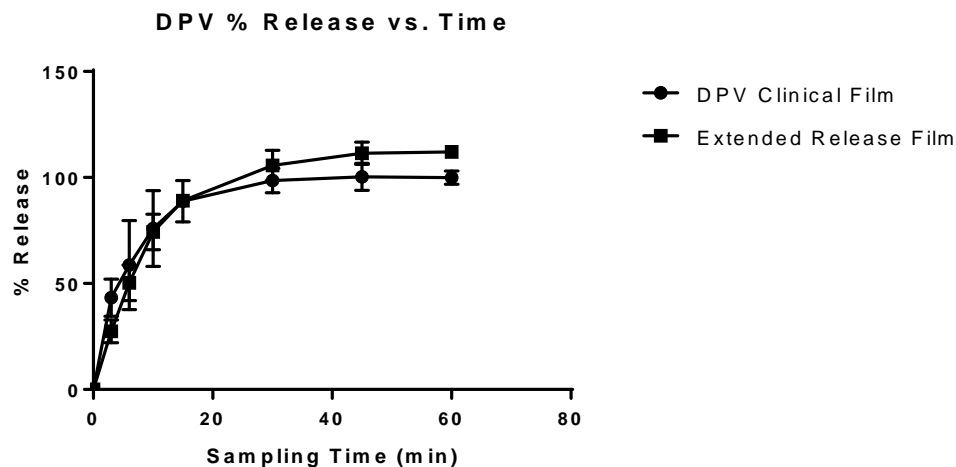


Figure 18: *In Vitro* Dapivirine Release for Clinical and Extended Release Films

In vitro release profiles for the clinical and extended release films show similar release profiles with characteristic burst release, both evaluated using the USP 4 SOTAX *in vitro* release method using 50% acetonitrile as the release media. Data shown is the mean \pm standard deviation of three replicates.

3.4.2 *In Vivo* (NHP) Film Retention and Drug Release

Both TFV and DPV could be formulated into thick, extended release films that contained the exact formulation as the thin film counterparts (Tables 7 and 9). To test the ability of these films to extend drug release and film retention *in vivo*, the TFV and DPV films were selected to be tested in the NHP model at the Washington National Primate Research Center (WaNPRC) led by the Patton Lab. These two films could be compared to the coitally developed product which is under current clinical investigation. Films were administered intravaginally to macaques for film tracking through measurement of retention times and drug release into vaginal fluid. Blue dye was added to the TFV and DPV films as these films were clear to translucent thereby making visual observation difficult. Colpophotography images taken on days pre- and post- insertion of a TFV or DPV extended release film for film visualization. Vaginal swabs were taken to analyze drug content in vaginal fluids on days 1-5 and day 8. Drug levels are reported in ng/swab. The

cellulose based TFV film was retained for up to 4 days in the NHP (Fig. 19). The PVA based DPV films were retained up to 5 days (Fig. 20). Drug was detected in vagina fluids through day 8, suggesting film presence was not indicative of drug presence in the vaginal fluid which is consistent with what was observed in previous studied (Chapter 2). Clinical films, when tested in the NHP (Chapter 2) showed that the TFV film could be retained through day 2, while the DPV film was retained through day 3.

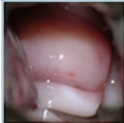


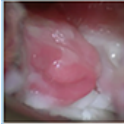
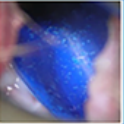
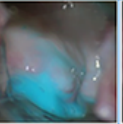
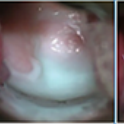
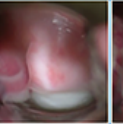
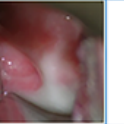







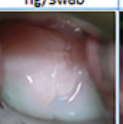

Day1 Pre-Film	Day1 Post-Film	Day2	Day3	Day4	Day5	Day8
						
absent	present	absent	na	na	na	na
		627.50 ng/swab	39.25 ng/swab	51.75 ng/swab	15.45 ng/swab	3.20 ng/swab
						
absent	present	present	present	present	absent	na
		55250.00 ng/swab	14125.00 ng/swab	2400.00 ng/swab	37.25 ng/swab	6.73 ng/swab
						
absent	present	present	present	absent	na	na
		132500.00 ng/swab	20500.00 ng/swab	7662.50 ng/swab	1312.50 ng/swab	9.15 ng/swab
						
absent	present	present	absent	na	na	na
		BLQ ng/swab	BLQ ng/swab	BLQ ng/swab	BLQ ng/swab	BLQ ng/swab

Figure 19: Extended Release Tenofovir Film *In Vivo* Retention and Release
Colpophotography images taken on days pre- and post- insertion of a tenofovir (TFV) extended release film for film visualization in pigtailed macaques (n = 4). Blue dye was incorporated into the films to aid in this visualization. The TFV film was retained up to day 4, while drug was detected in vagina fluids through day 8, suggesting film presence was not indicative of drug presence in the vaginal fluid. BLQ= below limit of quantitation (LOQ = 0.2ng/mL)

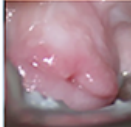

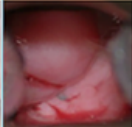











Day1 Pre-Film	Day1 Post-Film	Day2	Day3	Day4	Day5	Day8
						
absent	present ^M	present ^M	absent	na	na	na
		7.30 ng/swab	10.40 ng/swab	0.35 ng/swab	0.28 ng/swab	BLQ ng/swab
						
absent	present	present	present	absent	na	na
		13425.00 ng/swab	440.00 ng/swab	1.97 ng/swab	1.07 ng/swab	0.33 ng/swab
						
absent	present	present	present	present	absent	na
		1492.50 ng/swab	90.75 ng/swab	29.00 ng/swab	1.63 ng/swab	0.54 ng/swab

Figure 20: Extended Release Dapivirine Film *In Vivo* Retention and Release

Colpophotography images taken on days pre- and post- insertion of a dapivirine (DPV) extended release film for film visualization in pigtailed macaques (n = 3). Blue dye was incorporated into the films to aid in this visualization. Vaginal swabs were taken to analyze drug content in vaginal fluids on days 1-5 and day 8. Drug levels are reported in ng/swab and menses animals are denoted by ^M. The DPV clinical film was retained up to day 3, while drug was detected in vagina fluids through day 8, suggesting film presence was not indicative of drug presence in the vaginal fluid. BLQ= below limit of quantitation (LOQ = 0.2ng/mL)

3.5 DISCUSSION AND CONCLUSIONS

Traditionally manufactured as thin dosage forms for pharmaceutical applications, data presented here demonstrates that polymeric films are versatile and parameters during manufacturing can be altered to prolong film retention and drug release. Two clinically advanced formulations, the TFV and DPV formulations [35, 91] were altered during manufacturing to increase the thickness of the final film product. These manufacturing alterations produced films that were up to five times the thickness of the clinically available films, showing that solvent cast film process can be

manipulated to change physical film dimensions and geometry. Changing the physical film attribute of thickness could further be utilized to increase the amount of the active agents which can be loaded into films, as this is a current limitation to thin film delivery [28], and thicker films could potentially accommodate greater quantities API.

The cellulose based TFV film had 14% solids content and produced a film that was nearly five times the thickness of the coital product. *In vitro* release was conducted for all films using a SOTAX USP 4 as a quality control method. Calculated release constants for the TFV films did show differences, and highlighted in Fig. 16, two characteristically different profiles for the TFV can be observed. Using first order modeling, the release rate constants for the TFV clinical and extended release films were 0.1524 min^{-1} and 0.0118 min^{-1} , respectively. Disintegration times between the increased thickness films and the thin films were significantly different, with the extended release films having increased disintegration times for both the TFV and DPV Films. The disintegration method developed in Chapter 2 was useful in providing quantitative disintegration times to evaluate this characterization parameters in films. The TFV films did show significant differences between water content and mechanical strength (as evaluated through puncture strength). The increased water content in the extended release TFV film resulted in decreased mechanical strength (Table 8). Water has been shown to have a plasticizing effect in certain films [166] which can impact mechanical properties such as tensile strength, Young's modulus and elongation [167]. The decreased puncture strength could be a direct result of the increased water content in these films.

Thickness differences between the PVA based DPV clinical and extended release films did not differ as greatly as what was seen for the cellulose based films. The percent of solids in the polymer melt (11% solids) caused the PVA based films to plateau at a thickness of

approximately 270 μm . Increasing the gap of the Doctor Blade was unable to make a film that was similar in thickness to the TFV film. DPV films did show disintegration times that were significantly different (Fig. 17) although DPV film characterizations did not show significant differences in the mechanical strength (puncture strength) or water content properties.

In vivo assessments of the extended release films showed interesting results. Compared to the clinical films (results seen in Chapter 2), the extended release cellulose films were retained for 2 days longer and the PVA films were retained for 1 day longer the NHP model. The cellulose films had greater differences in terms of thickness between the clinical and extended release films than the PVA based films (Tables 8 and 10). HPMC and PVA have both been used as mucoadhesive polymers [145, 168], and when incorporated in buccal patches they were both found to exhibit mucoadhesive properties [169] so the increased retention *in vivo* could be a combination of polymer type, film thickness and chemical entity. TFV and DPV could still be quantified in vaginal fluid on day 8, which again demonstrated that film presence was not indicative of drug presence. Therefore the increased thickness and polymer content of the films can allow for extended film dosing intervals, but there is only an added day or two extension. This period may not be sufficient for the development of a truly coitally independent product, but there is evidence that thickness of the dosage form impacts retention. The extended release film studies further demonstrated that the SOTAX dissolution method did not accurately predict *in vivo* release. *In vitro* release showed >50% release of TFV and DPV within minutes while film presence was noted for days after placement. These data further support the use of SOTAX dissolution only as a quality control parameter to evaluate film reproducibility and stability over time, rather than to predict any *in vivo* behavior.

There were limitations to these studies. Firstly, there were difficulties during the manufacture of the thick film products. Pouring solvent cast films at 400-450 μm , as opposed to 100-120 μm , was problematic. Keeping the polymer solution pouring consistently as the Doctor Blade moved was challenging and this was reflected in larger deviations in mass and thickness for the extended release films than for the clinical films (Tables 8 and 10). This can affect reproducibility and characterization parameters. Another limitation is the feasibility and acceptability in delivering films that are three to five times thicker than current clinical products. While these films were soft and flexible and could be inserted intravaginally into the NHP, there could be issues with vaginal insertion and female acceptability. The dosage form could be seen as too bulky or cumbersome as it has lost some of the thin film attributes. For the *in vivo* macaque imaging studies, the weight of the fluid sample was not collected, so drug concentrations as measured per swab, not as a true concentration. This can skew release data since fluid amount impacts concentration. This should be controlled for in future studies. It should also be noted that the number of animals varied for different imaging experiments due to the limited availability of the animals, however, a minimum of three was used per experiment.

These studies show the feasibility of altering the solvent cast manufacturing process to create thick films to decrease drug dissolution over time. The polymeric film is a solid dosage form that disintegrates when exposed to environmental fluid in the vagina. It was hypothesized that this disintegration and downstream dissolution could be altered by increasing the polymer content in the films. Disintegration times for the increased thickness films for the TFV and DPV films were greater than what was seen for thin, coitally dependent films (Figs. 15 and 17). Using these two model thick films, *in vivo* retention was tested and also showed the feasibility of these films to extend retention and release *in vivo*. Changing a physical film parameter such as

thickness is one such avenue to explore to extend release, however there is a limit to which thickness can be increased from both a manufacturing standpoint and from a product acceptability standpoint. Offering a day or two of increased retention may not be sufficient to provide the increased dosing window needed for a longer acting product and it is not plausible to continually increase film thickness to get longer film retention.

The studies presented in this chapter provide significant contributions to the field of film study and microbicide research. These are some of the first proof-of-concept studies with microbicide films that evaluated the impact of changing a physical parameter (thickness) on release profile. While an extra day or two of *in vivo* retention time may not be classified as extended release, these results are still promising as thick films manufactured were retained in the NHP for longer than their thin film counterparts, showing that thickness is an important product attribute capable of impact release profile. These studies provide support of the developed disintegration technique discussed in Chapter 2 and utilize the visualization technique for *in vivo* retention. The solvent casting manufacturing process has traditionally been used to manufacture thin films [170]. Here we provide evidence that this manufacturing method can be used to cast thick films (250 μm), though there may be limitations in reproducibility and residual solvent impact with this method.

3.6 ACKNOWLEDGMENT

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4.0 APPLYING GEOMETRICAL THICKNESS MANIPULATION FOR THE FORMULATION OF AN EXTENDED RELEASE VAGINAL FILM FOR THE DELIVERY OF A COMBINATION OF MK-2048 AND DAPIVIRINE

4.1 INTRODUCTION

HIV treatment has evolved since the approval of the first nucleoside reverse transcriptase inhibitor (NRTI), azidothymidine (AZT), by the FDA in 1987. The International Antiviral Society-USA (IAS-USA), the US Department of Health and Human Services (HHS), and the World Health Organization (WHO) now all recommend a three drug combination for the treatment of HIV [41-43]. Following this, prevention strategies also use combinations to provide enhanced protection against HIV. Currently, the only FDA approved product for prevention is a combination product of emtricitabine and tenofovir. Combination film products in development are also following treatment strategies which use multiple compounds to target HIV at different points of the lifecycle. Combination regimens can lead to increased potency through additive or synergistic effects against HIV replication [171, 172]. Combination products also provide a bigger challenge to drug resistant viral strains [171, 172] because targeting HIV at different points in the lifecycle limits viral adaptation [138]. Some combination films that were developed include combinations of EFdA, a nucleoside reverse transcriptase inhibitor (NRTI) and CSIC a non-nucleoside reverse transcriptase inhibitor (NNRTI) [173], tenofovir (TFV) (NRTI) and

dapivirine (DPV) (NNRTI), TFV and maraviroc (MVC), an entry inhibitor, and DPV and MVC [44].

DPV has already been shown to be highly potent against HIV and has been used in conjunction with other entities to provide enhanced protection in the form of combination microbicides [44, 49, 164]. Another model compound, MK-2048, will also be utilized in this chapter. This compound targets viral integrase, compared to TFV and DPV, which work by inhibiting viral reverse transcriptase. MK-2048 prevents the integration of viral genetic material into the host genetic material. It is a hydrophobic molecule (logP of 2.3), with a molecular mass of 461.884 and a molecular formula of $C_{21}H_{21}ClFN_5O_4$ [174]. It is a potent molecule shown to inhibit HIV with an IC_{50} of 3.58 nM (unpublished data). Although no longer under investigation for treatment, MK-2048 is an attractive candidate for development in this field as it is a second generation integrase inhibitor because of its favorable resistance profile [175]. MK-2048 is under investigation for formulation in long-acting intravaginal rings (IVR) (MTN-027 Phase 1 Safety and Pharmacokinetics Study of MK-2048/Vicriviroc (MK-4176)/MK-2048A Intravaginal Rings)) [102]. Prior to work with IVRs, MK-2048 was investigated for safety after administered orally in non-clinical animal models. It was found that there was no renal, respiratory or cardiovascular toxicity in the sheep, therefore supporting further clinical microbicide development [176]. In addition, a rabbit penile irritation study, which is commonly used to evaluate vaginal products [60, 177, 178], found no issues with toxicity [176]. Therefore, MK-2048 was selected to be formulated with DPV and here we present formulation work for vaginal films. Formulation of these two compounds in the same film platform will provide significant challenges in terms of manufacturing as they have varying degrees of hydrophobicity and the solvent casting process is an aqueous based manufacturing process.

4.2 MATERIALS

MK-2048 and DPV were graciously provided by Merck & Co., Inc. (Kenilworth, NJ, USA) and the International Partnership for Microbicides (IPM, Silver Spring, MD, USA), respectively. Film excipients and suppliers are described in Chapter 2.2. Manufacturing materials for films are also found in Chapter 2.2. Sodium chloride (Fisher Scientific), potassium hydroxide (Spectrum Chemical), calcium hydroxide (Spectrum Chemical), bovine serum albumin (Spectrum Chemical), lactic acid (Spectrum Chemical), acetic acid glacial, glycerol (Spectrum Chemical), urea (Spectrum Chemical), glucose (Sigma-Aldrich) and hydrochloric acid (Fisher Scientific) were purchased for vaginal fluid simulant manufacture.

4.3 METHODS

4.3.1 Vaginal Fluid Simulant Manufacture

Vaginal fluid simulant was made as previously described [81]. Briefly, sodium chloride (Fisher Scientific), potassium hydroxide (Spectrum Chemical), calcium hydroxide (Spectrum Chemical), bovine serum albumin (Spectrum), lactic acid (Spectrum Chemical), acetic acid glacial, glycerol (Spectrum Chemical), urea (Spectrum Chemical), glucose (Sigma-Aldrich) were added to Milli-Q water and stirred until all the constituents dissolved. pH was then adjusted to 4.2 using 10% hydrochloric acid.

4.3.2 Formulation for Determination of a Suitable Film Base for the Combination Film

To find an appropriate base to formulate a combination film, MK-2048 was first formulated as a single entity product. Initially, MK-2048 was formulated into two commonly used cellulose and PVA based films which have been developed in the lab previously. Excipients for these formulations can be found in Tables 12 and 13. For MK-2048, Doctor Blade thickness was set at 300 μm for single entity films.

4.3.3 Film manufacture of a combination of DPV and MK-2048

Combination films were formulated with excipients from the cellulose based film formulation as described in Chapter 2.3.1 using the same film forming polymers of HEC, HPMC E5 and NaCMC. Plasticizer amounts/ratios were increased in the formulation to support the dispersion of two APIs and to increase film softness and flexibility. These films were manufactured using the solvent casting method described in Chapter 3.3.1. Doctor Blade thickness was set at 350 μm for MK-2048/DPV combination films.

4.3.4 *In Vitro* Film Characterization

Films were characterized *in vitro* for mass (mg), thickness (μm), puncture strength (g/mm), water content (% w/w) and disintegration time (sec.). Mass was measured using an analytical balance and thickness was measured using a Mitutoyo Absolute electronic thickness gauge. Puncture strength and disintegration time were determined using a TA.XTPlus Texture Analyzer.

Residual film water content was measured using Karl Fischer titration and a Metrohm Titrando® instrument. For further details, see Chapter 2.3.2.

Drug content analysis was done using reverse phase high-performance liquid chromatography (HPLC). MK-2048 was extracted from films using 80% acetonitrile in water solution. This dispersion was then diluted 5 times using 70% 10 nM potassium phosphate buffer/30% acetonitrile. HPLC analysis was conducted using Dionex UltiMate3000 HPLC system equipped with Photodiode array detector and Chromeleon data acquisition software. A Waters XBridge C18 5 μ m, 2.1 x 50 mm column was used and 10 nM potassium phosphate buffer and 100% acetonitrile were used as mobile phases A and B at an initial ratio of 70%/30%. MK-2048 retention time was 1.397 ± 0.018 min at a wavelength of 344 nm.

Drug release from the films was determined for each film using a USP 4 flow through SOTAX CE 7 dissolution system followed by HPLC analysis as described above. To select an appropriate film base for combination films, a biologically relevant dissolution media was used. 500 mL of vaginal fluid simulant was used as dissolution media and 0.5 mL aliquots were taken at the following time points; 5, 10, 20, 30, 45, 60, 120, 240, 360, 480, 600, and 720 minutes. Initial testing showed maximum release occurred prior to 720 minutes and subsequent testing was shortened.

In vitro release of combination MK-2048/DPV film products was conducted as a quality control parameter using a dissolution media of 50% acetonitrile. 0.5 mL aliquots were taken at the following time points; 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 270, and 330 minutes. Following sample collection, DPV samples were analyzed as described in Chapter 2.3.2. A stability indicating HPLC assay was developed and used to analyze all MK-2048 samples for stability. This assay was conducted using Dionex UltiMate3000 HPLC system Photodiode array

detector and Chromeleon data acquisition software. A Waters XBridge C18 5 μm , 2.1 x 50 mm column was used and 0.1% formic acid in water and 100% acetonitrile were used as mobile phases A and B at an initial ratio of 90%/10%. MK-2048 retention time was 4.86 ± 0.25 min at a wavelength of 344 nm.

4.3.5 Stability Assessment

Stability assessments were done according to ICH Guidelines Q1E [179]. The long term and accelerated temperatures/relative humidity (RH) conditions were used as specified in these guidelines for long-term ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}/60\%$ RH) and accelerated ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\%$ RH) stability. The intermediate condition, $30^{\circ}\text{C} \pm 2^{\circ}\text{C}/65\%$ RH $\pm 5\%$ RH, will be tested if “significant change occurs at any time during 6 months’ testing at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria” according to ICH guidelines [179]. The following properties were assessed; appearance, weight, thickness, disintegration, water content, puncture strength, drug content, dissolution, bioactivity and cellular and *Lactobacilli* toxicity. Sample pull times were at 3, 6, 9, and 12 months. At 3 months: appearance, weight, thickness, disintegration, puncture strength, water content and drug content were assessed at both storage conditions. At 6 months, appearance, weight, thickness, disintegration, puncture strength, water content, drug content, dissolution, bioactivity, cellular toxicity and *Lactobacilli* toxicity were assessed at both conditions. At 9 months, appearance, weight, thickness, disintegration, puncture strength, water content and drug content were assessed at $25^{\circ}\text{C}/60\%$ RH. At 12 months, appearance, weight, thickness, disintegration, puncture strength, water content, drug content, dissolution, bioactivity, cellular toxicity and *Lactobacilli* toxicity were assessed at $25^{\circ}\text{C}/60\%$ RH. Stability guidelines

for acceptable characterization parameters are seen in Table 11. Disintegration and puncture strength were monitored over the course of stability for significant changes from time 0.

Table 11: Stability Acceptability Criteria

Acceptability criteria defined for stability testing

Characterization Parameter	Acceptable Range
Drug Content, Mass, Thickness	85-115% of Time 0
Water Content	<10%
% Dissolution	85-115% Release @ 330 min.
<i>Lactobacilli</i> toxicity	Reduction in viability was < log ₁₀

4.3.6 *In Vitro* Toxicity and Efficacy

Films were assessed for their *Lactobacilli* toxicity *in vitro* in Dr. Bernard Moncla's Lab at Magee-Womens Research Institute as previously described [49]. *Lactobacilli* toxicity was evaluated by dissolution of the film in ACES buffer (N-(2-Acetamido)-2-aminoethanesulfonic acid). Three strains of *Lactobacilli* (*L. jensenii* ATCC 25258, *L. jensenii* LBP 28AB, *L. crispatus* ATCC 33197) were each suspended in 1X PBS. For control samples, 1 mL of inoculum was mixed with 1 mL of ACES buffer. T₀ was immediately plated while T₃₀ incubated for 30 minutes at 37°C and then subsequently plated. For film testing, films were dissolved in ACES. The amount of buffer used was determined by the film size (1" x 1" film films dissolved in 1 mL ACES buffer, 2" x 2" films dissolved in 2 mL ACES buffer). Film and ACES buffer solution was then mixed with matching amounts of inoculum (bacteria and 1X PBS). Film solutions were incubated for 30 mins at 37°C and then were plated. All plates were incubated for 48 hours at 37°C (6% CO₂) and then plate count was measured. Plate count between T₀ and T₃₀ controls were compared with film incubations. Films were considered to be non-toxic to the *Lactobacilli*

strain if the decrease in viability was $<\log_{10}$ [49]. Films were considered toxic to *Lactobacilli* in general if they were harmful to 2 or more strains tested.

Cellular toxicity and *in vitro* efficacy studies were conducted in Dr. Charlene Dezzutti's lab at Magee-Womens Research Institute as previously described [180]. TZM-bl cells were plated and films were dissolved in saline solution. Film dilutions were further diluted 2 times with TZM-bl media. Film solutions (100 μ L) were added to TZM-bl plates. For toxicity assays, TZM-bl media was then added to plates (100 μ L) and allowed to incubate overnight. The following day media was removed and 100 μ L of Celltiter-Glo was added, allowed to rest for 10 minutes in the dark and luminescence was then read. Luminescence of the cells treated with the film dilutions was compared to controls which were only treated with media. For efficacy, film dilutions were added to TZM-bl plates. TZM-bl media containing HIV-1 was then added to plates (100 μ L). Cells were allowed to incubate for 48 hours and following this period, media was removed and replaced with 100 μ L Bright-Glo. Plates were allowed to rest for 2 minutes and then luminescence was read. Luminescence of the cells treated with the film dilutions was compared to controls which were only treated with media.

4.3.7 Statistical Analysis

Data is represented as average (mean) and variability is represented as the standard deviation (SD). Statistical analysis was performed using one way ANOVA with multiple comparisons (GraphPad Prism 6.07). P values less than 0.05 were considered statistically significant.

4.4 RESULTS

To determine a formulation for the combination of MK-2048 and DPV, a suitable base had to be selected. MK-2048 had not previously been formulated into a film, therefore it was formulated into two common bases to determine which would be better suited for combination film development. MK-2048 was incorporated into the commonly used cellulose and PVA formulations. These films were cast at an increased thickness to extend drug release from this film product. MK-2048 was formulated into formulations seen in Tables 12-13. Both of these formulations were able to make films with acceptable film properties (soft, flexible) which could be poured at an increased Doctor Blade gap which resulted in a thicker film. The base polymers and percentages were consistent with the cellulose formulation (Table 12) but there were some formulation changes because of the substitution of MK-2048 for TFV. NaOH was not included and propylene glycol was used as the plasticizer because of the MK-2048 solubility in this plasticizer (Merck Research Laboratories MK-2048 Preformulation Report). MK-2048 was also formulated into the PVA-based formulation, which was used for the DPV film (Table 13). Base polymer percentages and plasticizers were unchanged. Films were poured at a thickness of 300 μm thickness at 71 °C.

Table 12: MK-2048 Formulation A- Cellulose-Based Formulation

Component	Percent (w/w)
Hydroxyethyl cellulose (Medium Viscosity)	6.00
Hydroxypropyl methylcellulose- E5	6.00
Carboxymethylcellulose Sodium (Low Viscosity)	2.00
MK-2048	0.17
Propylene Glycol	3.00
MilliQ Water	82.83

Table 13: MK-2048 Formulation B- PVA- Based Formulation

Component	Percent (w/w)
Polyvinyl alcohol	7.02
Polyethylene glycol (PEG) 8000	2.34
Hydroxypropyl methylcellulose (HPMC) E5	1.75
MK-2048	0.10
Propylene Glycol	0.73
Glycerin	0.73
MilliQ Water	87.26

In vitro dissolution testing was conducted using films from each of the different formulations to determine which formulation had the greatest impact on extending the release of MK-2048. This parameter was used as the main selection method for an appropriate base for the combination formulation of MK-2048 and DPV.

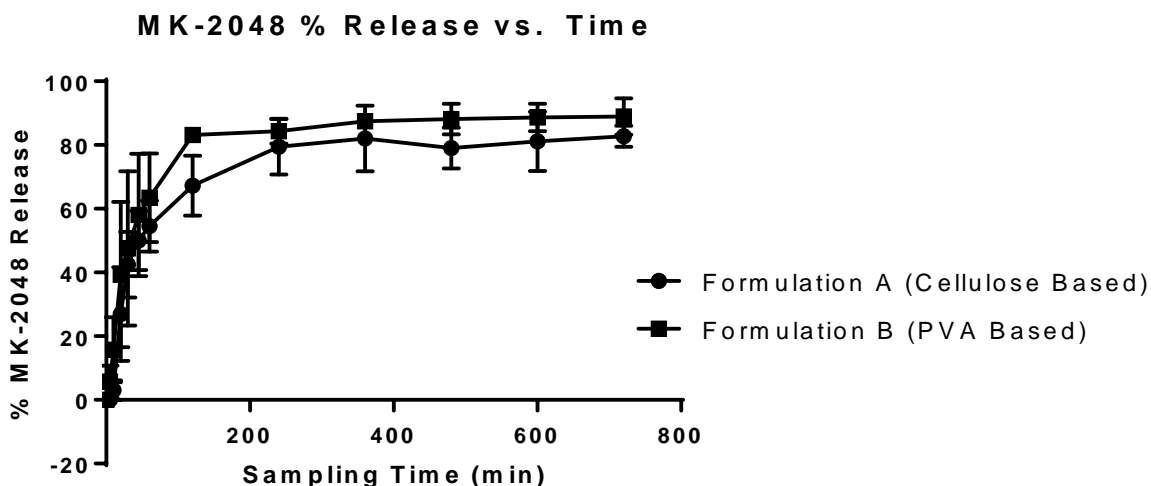


Figure 21: MK-2048 *In Vitro* Release Profiles for Experimental Formulations
In vitro release profiles for the experimental MK-2048 formulations compared using the SOTAX USP 4 flow through system using vaginal fluid simulant as the release media. Data shown is the mean \pm standard deviation of two to three replicates.

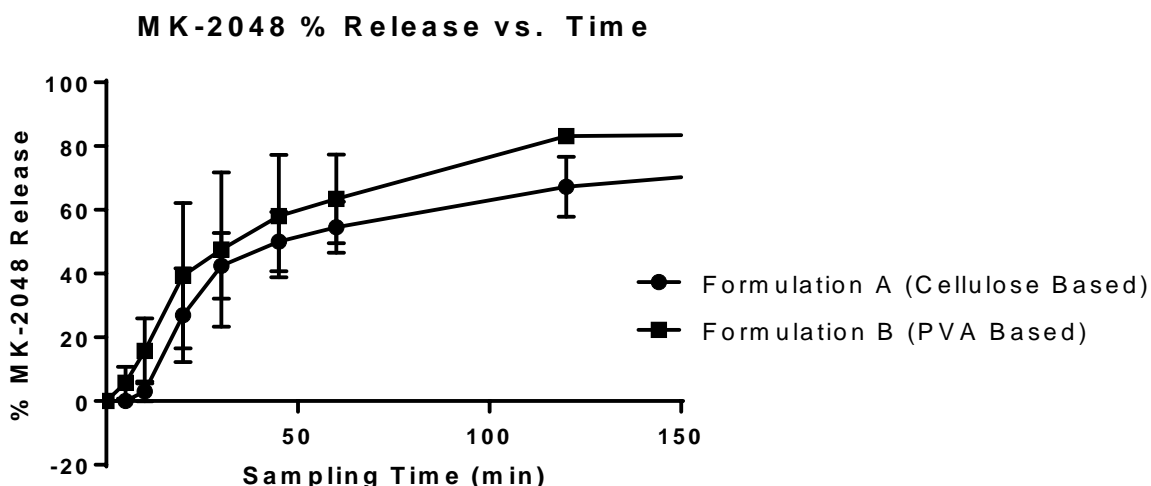


Figure 22: MK-2048 Truncated *In Vitro* Release

Highlighted section of Figure 21 showing initial release (up to 120 minutes) between experimental MK-2048 formulations using SOTAX USP 4 flow through system using vaginal fluid simulant as the release media. Data shown is the mean \pm standard deviation of two to three replicates.

Initial dissolution results showed that the cellulose-based formulation had the slower release profile compared to the other formulation (Figs. 21 and 22). First order release rate constants calculated for the release profiles [181] showed that the cellulose base formulation had a slower release rate constant (0.0035 min^{-1}) compared to the PVA based formulations (0.0041 min^{-1}).

MK-2048 in cellulose-based formulation was selected as the lead formulation for the extended release based off data from release profiles and favorable film properties such as smoothness and flexibility. This formulation was cast at an increased thickness of $350 \mu\text{m}$ to further slow release. An initial goal for an extended release film was a retention time of 1 week. Based off of daily *in vitro* drug release from the MK-2048 ring, 3 mg was selected as a dose in the 1" x 1" film because the target in the 2" x 2" film was 9.5-15 mg [176]. This film was characterized *in vitro* for physical film properties. Average film mass and thickness were 277.13

± 16.75 mg and 376.20 ± 54.87 μm , respectively. Drug content was 2.94 ± 0.14 mg per film.

Other characterization parameters are summarized in Table 14 and Fig. 23.

Table 14: MK-2048 Extended Release Film Characterizations

MK-2048 Film	Extended Release Film
Weight (mg)	277.13 ± 30.84
Thickness (μm)	376.20 ± 54.87
Drug Content (mg)	2.94 ± 0.14
Puncture Strength (g/mm)	3.31 ± 1.11
Water Content %(w/w)	6.47 ± 0.37
Disintegration Time (sec)	606.85 ± 115.41

Data shown is the mean \pm standard deviation

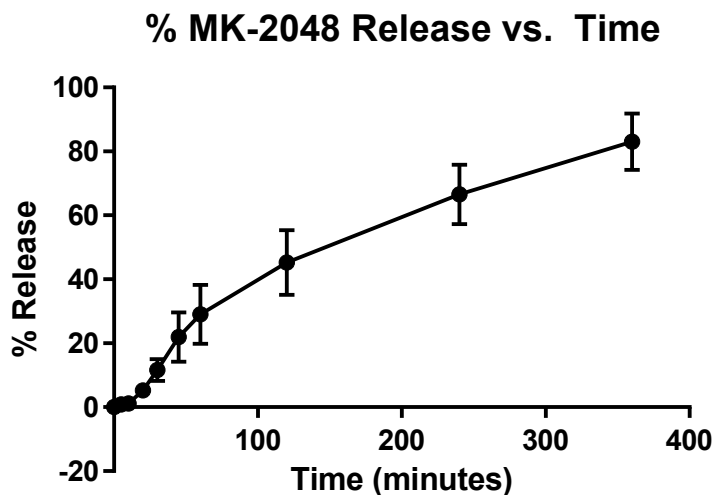


Figure 23: MK-2048 *In Vitro* Release for Extended Release Film

In vitro release profile for the cellulose-based (modified) film selected as lead extended release film formulation evaluated using the USP 4 SOTAX *in vitro* release method using vaginal fluid simulant as the release media. Data shown is the mean \pm standard deviation of three replicates.

4.4.1 Single Entity Extended Release Film Summary

DPV was formulated into this cellulose base to test the feasibility of the formulation. It was able to be formulated into the cellulose based formulation and had an average mass of 307.53 ± 21 mg and thickness of 399.58 ± 32.90 μm . Using the increased thickness platform, two single

entity extended release films were manufactured (DPV and MK-2048) into cellulose based polymeric films.

4.4.2 Film manufacture of a combination of DPV and MK-2048 Film

The goal of these studies was to assess the versatility of the extended release platform by formulating a combination film. DPV and MK-2048 were selected for a combination product because they target HIV at different points of the lifecycle (reverse transcription and integration) and since they are both hydrophobic agents, their combination presents a greater challenge using the solvent cast method of film manufacture. The combination of MK-2048 and DPV was formulated into the same cellulose base film that MK-2048 was formulated into. This film had acceptable properties in terms of physical characteristics (softness and flexibility) and characterization parameters, and was capable of loading 2.94 mg of MK-2048 and had the slowest release profile *in vitro* when MK-2048 was incorporated alone. This formulation has a high solids content which was favorable from a manufacturing standpoint and when considering the formulation of two dispersed, hydrophobic agents. From previous studies in Chapter 3, it was also found that compared to the PVA formulation, this cellulose formulation can produce thicker films than the PVA based formulation. When formulating both DPV and MK-2048 into this formulation, plasticizer amounts and ratios were to support two solid drug dispersions. The propylene glycol in the original formulation was increased 1.5 times to support solid dispersions of MK-2048 and DPV, and to keep the film soft and flexible. The final film formulation for the combination product is seen in Table 15. DPV drug loading was targeted to match that of MK-2048.

Table 15: DPV/ MK-2048 Combination Film Formulation

Component	%w/w
Hydroxyethyl cellulose (Medium Viscosity)	6.00
Hydroxypropyl methylcellulose	6.00
Carboxymethylcellulose Sodium (Low Viscosity)	2.00
Glycerin	3.00
Propylene Glycol-DPV	1.09
Propylene Glycol-MK-2048	1.09
MK-2048	0.17
DPV	0.17
MilliQ Water	80.65
Total	100

After the successful manufacture of the combination film product, the film was put under stability assessment to support further development of the combination product. Films were held for a 12 month stability study up as described in section 4.3.5.

To date, the combination films have completed 9 months of stability testing and the results for the characterization parameters are shown in the following figures and tables. Data from each time point was compared to data from time zero. All values for film weight and thickness were within range and there were no significant changes to weight during the stability assessments to date. MK-2048 drug had 1 value that fell outside of the 15% range. Water content, dissolution and *Lactobacillus* toxicity were within limits set for stability. *In vitro* efficacy for time zero and 6 months (25°C and 40°C) showed IC₅₀ values for DPV and MK-2048 which were similar to IC₅₀ values of the API in the TZM-bl cellular model (Table 18).

Weight of the combination film over time was consistent with no significant differences between time points (Fig. 24).

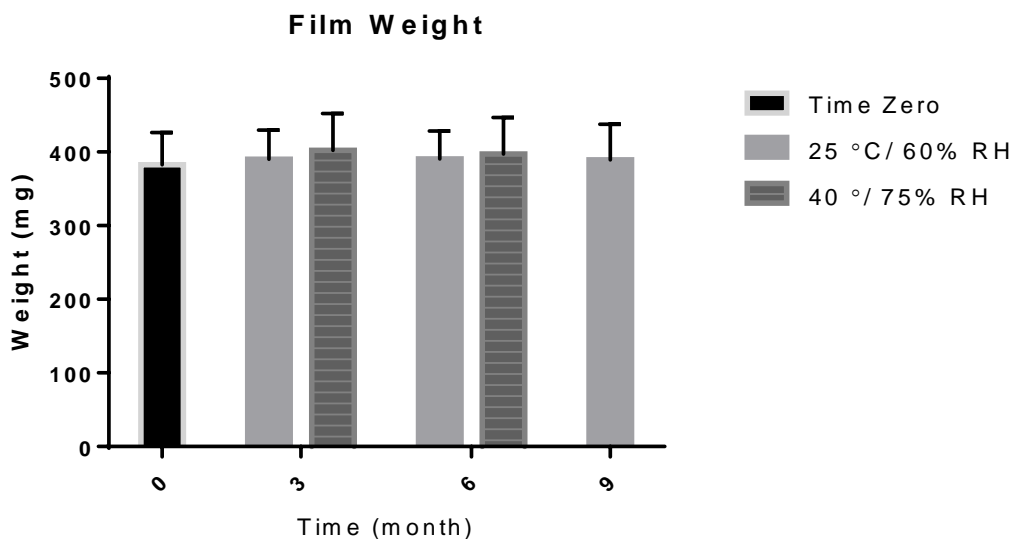


Figure 24: MK-2048/Dapivirine Combination Film Stability Assessment- Weight
 Film weight of combination dapivirine (DPV)/MK-2048 films over time at 25 °C/ 60% RH and 40 °C/ 75% RH conditions measured using an analytical balance. Data shown is the mean \pm standard deviation of twenty replicates.

Release profiles for MK-2048 and DPV at both time points show plateaus in release at approximately 150 mins. % of MK-2048 vs. % DPV released at each point was consistently greater for all studies (Figs. 25-26).

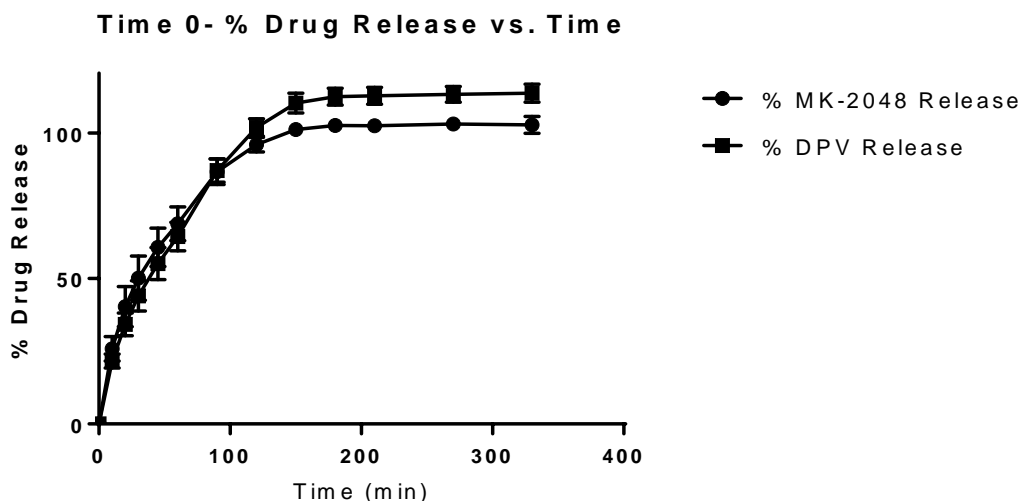


Figure 25: Time Zero MK-2048/Dapivirine Combination Film Stability Assessment- *In Vitro* Release

In vitro release profiles of combination dapivirine (DPV)/MK-2048 films at time zero evaluated using USP 4 SOTAX *in vitro* release method. Data shown is the mean \pm standard deviation of three replicates.

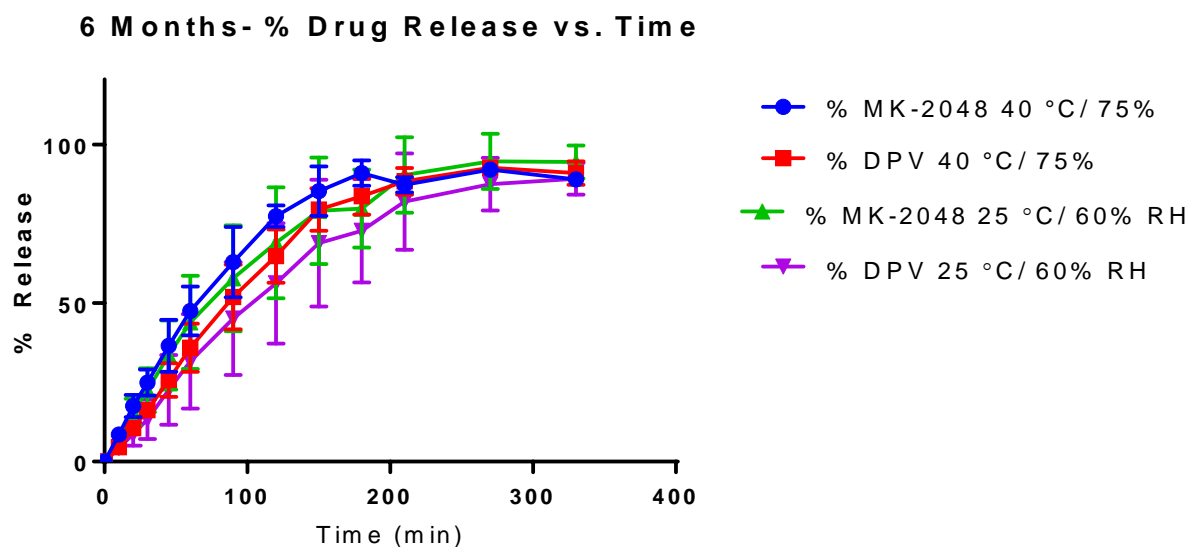


Figure 26: 6 Months MK-2048/Dapivirine Combination Film Stability Assessment- *In Vitro* Release

In vitro release profiles of combination dapivirine (DPV)/MK-2048 films at time 6 months at 25 °C / 60% RH and 40 °C / 75% RH conditions evaluated using USP 4 SOTAX *in vitro* release method using 50% acetonitrile as the release media. Data shown is the mean \pm standard deviation of three replicates.

Residual water content showed that there were no significant differences between any of the values from time zero and all values were less than the 10% residual water content limit (Fig. 27).

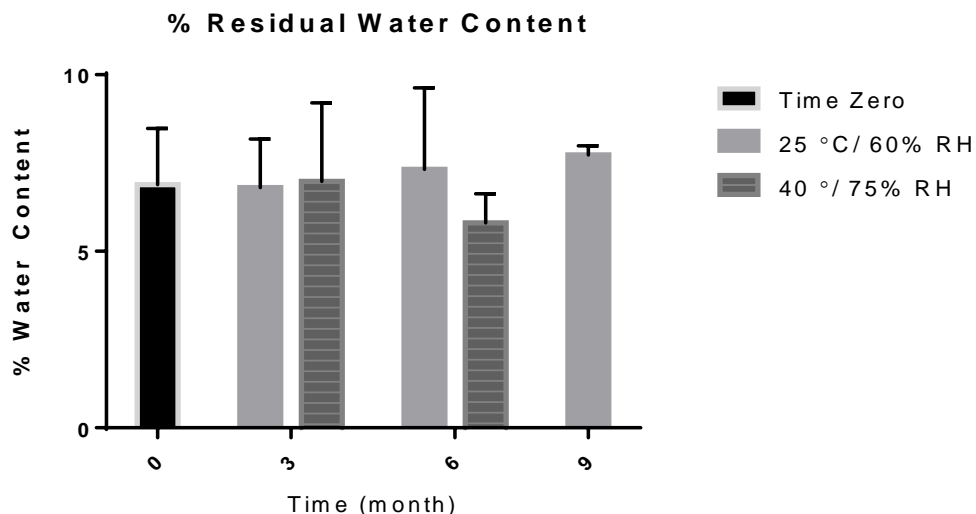


Figure 27: MK-2048/Dapivirine Combination Film Stability- Water Content
Residual water content analysis of combination dapivirine (DPV)/MK-2048 films over time at 25 °C/ 60% RH and 40 °C/ 75% RH conditions analyzed by a Karl Fischer apparatus (890 Titrando, Metrohm®). Data shown is the mean \pm standard deviation of three replicates.

Disintegration time had one value, 40 °C/ 75% RH 6 months, that differed significantly from time 0 (Fig. 28) when analyzed using ANOVA.

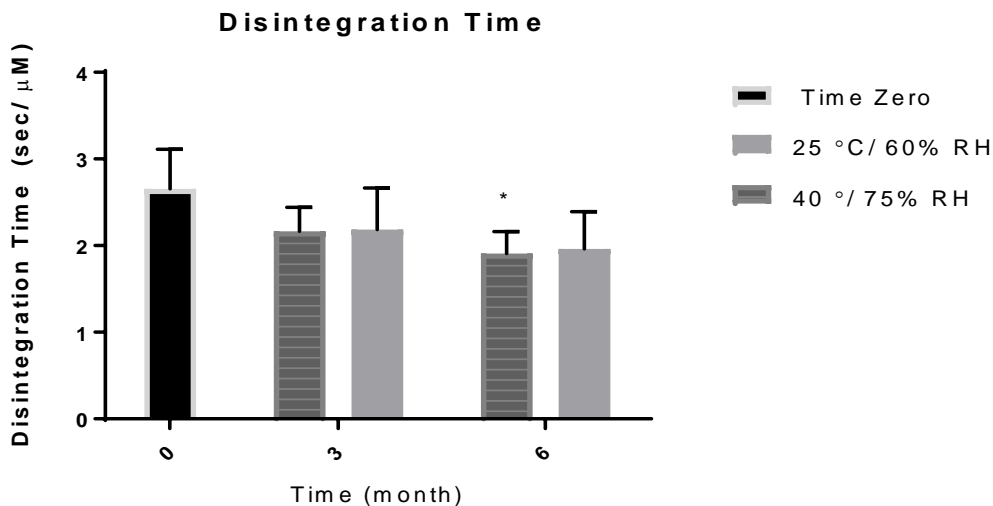


Figure 28: MK-2048/Dapivirine Combination Film Stability- Disintegration Time
 Disintegration time analysis of combination dapivirine (DPV)/MK-2048 films over time at 25 °C/ 60% RH and 40 °C/ 75% RH conditions analyzed by a TA.XTPlus Texture Analyzer and probe force of 10g. Data shown is the mean \pm standard deviation of four replicates.
 * indicates time 0 vs. 6 months 40 °C/ 75% RH

Drug content analysis of DPV over time in the combination films showed that the 9 month 25 °C/ 60 % RH time point was significantly different from 3 months DPV content at that same condition (Fig. 29). However, all the time points showed an acceptable drug content within the range 85-115%.

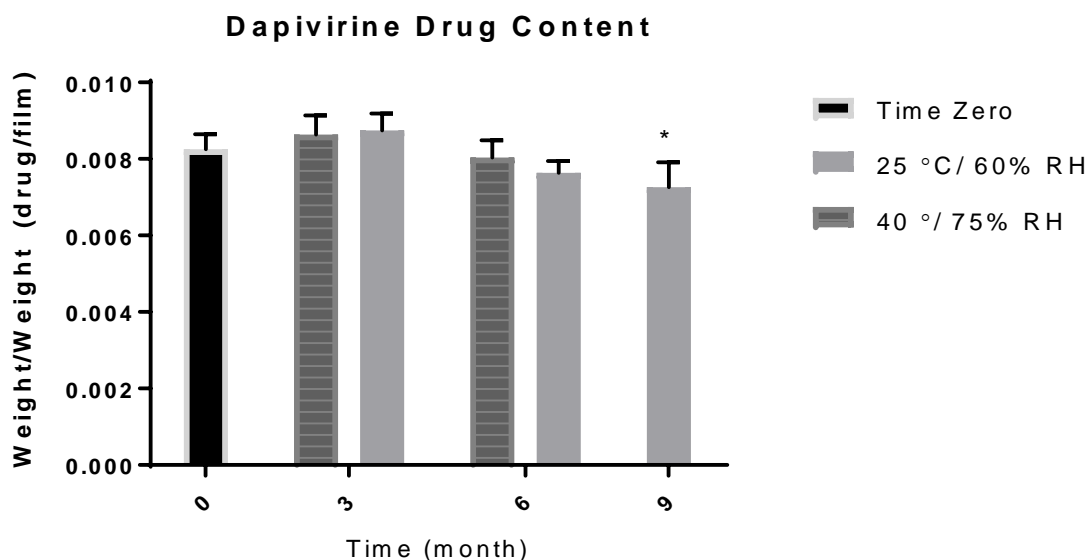


Figure 29: MK-2048/Dapivirine Combination Film Stability- Dapivirine Drug Content
Drug content analysis for w/w of dapivirine (DPV) in combination DPV/MK-2048 films over time at 25 °C/ 60% RH and 40 °C/ 75% RH conditions analyzed at indicated time points. Data shown is the mean \pm standard deviation of three replicates. *Indicates 25 °C/ 60% RH 3 months vs. 9 months

Analysis of MK-2048 content (Fig. 30) in combination films showed no significant differences between values over time, but the 6 month 40 °C/ 75% RH condition was not within the 15% of time zero specification set for stability.

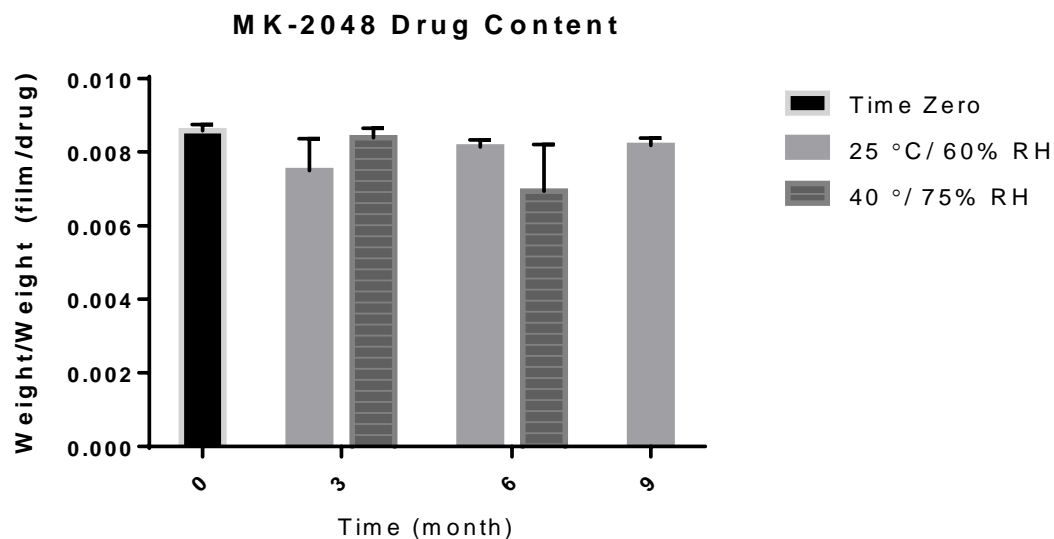


Figure 30: MK-2048/ Dapivirine Combination Film Stability- MK-2048 Drug Content
Drug content analysis for w/w of MK-2048 in combination dapivirine (DPV)/MK-2048 films over time at 25 °C/ 60% RH and 40 °C/ 75% RH conditions analyzed at indicated time points. Data shown is the mean \pm standard deviation of three replicates.

Puncture strength analyses found no significant differences found between comparisons of these mechanical tests over time at different temperatures (Fig. 31).

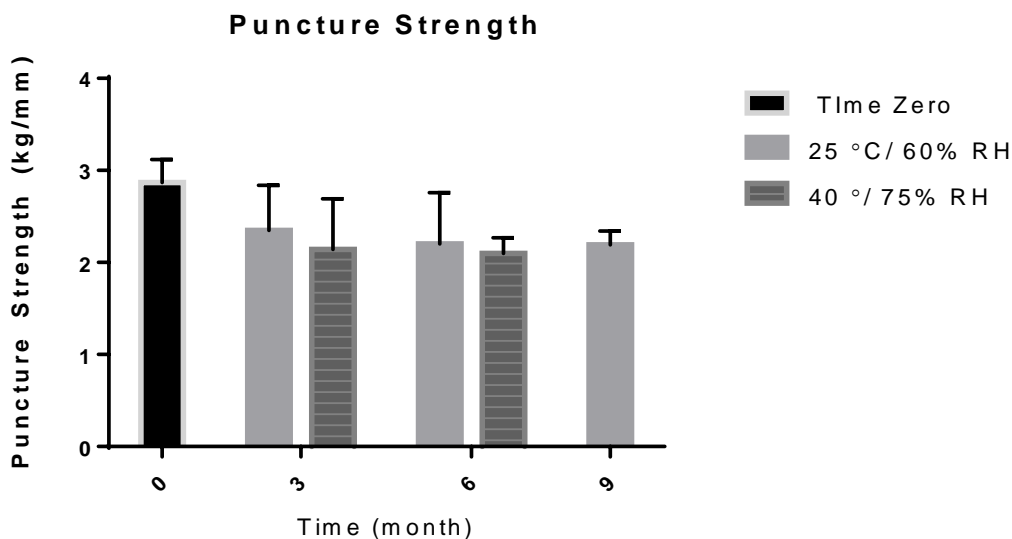


Figure 31: MK-2048/Dapivirine Combination Film Stability- Puncture Strength

Puncture strength analysis combination dapivirine (DPV)/MK-2048 films over time at 25 °C/ 60% RH and 40 °C/ 75% RH conditions analyzed by a TA.XTPlus Texture Analyzer and force required to break film/cross-sectional area. Data shown is the mean \pm standard deviation of three replicates.

Compatibility with *Lactobacilli* (*L. jensenii* ATCC 25258, *L. jensenii* LBP 28AB, *L. crispatus* ATCC 33197) indicated that none of the films at time 0 or 6 months, had any toxicity to these species as there was not a decrease in bacterial viability between control plates and plates exposed to films $> 1 \log_{10}$.

Table 16: MK-2048/DPV Combination Film Stability- *Lactobacilli* Compatibility Time Zero

	Log Difference
<i>Lactobacillus</i> Strain	Time Zero
<i>L. jensenii</i> ATCC 25258	-0.039
<i>L. jensenii</i> LBP 28AB	-0.189
<i>L. crispatus</i> ATCC 33197	-0.076

Viability difference between control and film treated bacterial plates. A difference $> 1 \log_{10}$ indicates a significant change in bacterial viability. Bacterial strains were tested in triplicate.

Table 17: MK-2048/DPV Combination Film Stability- *Lactobacilli* Compatibility 6 Months

<i>Lactobacillus</i> Strain	Log Difference	
	25°C Film	40°C Film
<i>L. jensenii</i> ATCC 25258	0.011	0.117
<i>L. jensenii</i> LBP 28AB	-0.009	-0.110
<i>L. crispatus</i> ATCC 33197	-0.003	-0.174

Viability difference between control and film treated bacterial plates. A difference > 1 log10 indicates a significant change in bacterial viability. Bacterial strains were tested in triplicate.

Anti-HIV activity was measured in the cellular TZM-bl model. Curves for time 0 and 6 months (at both long term and accelerated temperatures) can be found in Fig. 32. Curves are overlaid for all time points and temperatures. IC₅₀ values from these curves can be found in Table 18.

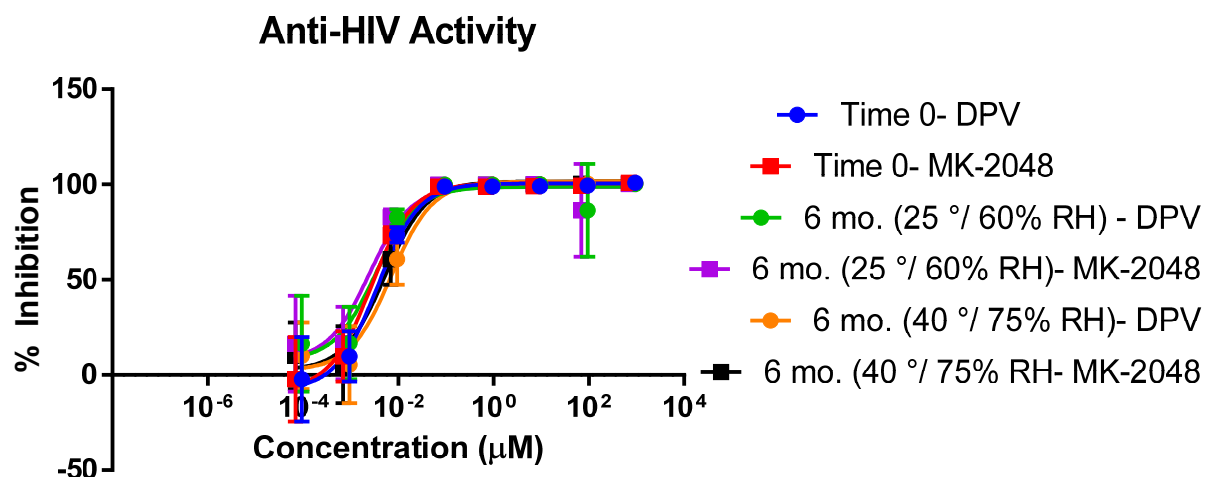


Figure 32: MK-2048/DPV Combination Film Stability Assessment- *In Vitro* Efficacy
Time zero and 6 months *in vitro* anti-HIV activity in TZM-bl cellular assay at 6 months (25 °C/ 60% RH, 40 °C/ 75% RH) for DPV and MK-2048 in triplicate.

Table 18: Half Maximal Inhibitory Concentration (IC₅₀) Values for Dapivirine and MK-2048

	DPV IC ₅₀ (nM)	MK-2048 IC ₅₀ (nM)
API	7.90 [49]	3.58 (unpublished data)
Time 0	3.65	2.70
6 months- 25°C	3.35	2.48
6 months- 40°C	7.15	5.29

IC₅₀ values of individual APIs as measured in the TZM-bl cellular assay and calculated using nonlinear regression from the plots created in Figure 32.

4.5 DISCUSSION

To formulate a combination extended release product, suitable agents first had to be selected. DPV is a highly potent reverse transcriptase inhibitor which has successfully been incorporated into vaginal films, gels, and rings and has been shown to have efficacy against HIV when tested in these products clinically [31, 32, 35, 49]. MK-2048, an integrase inhibitor, targets HIV at a different point of the HIV lifecycle than DPV and has been shown to have favorable resistance and safety profiles [176, 182]. These two agents were selected for formulation in a geometrically manipulated vaginal film to extend release in this platform. The manufacturing method used for current clinical TFV and DPV microbicide films, as well as the films developed in this work, is solvent casting [35, 49, 89, 91]. The polymers used here, HEC, HPMC, and NaCMC are water-soluble, film forming polymers [95]. Aqueous based systems are preferred for pharmaceutical film manufacturing because there are strict regulations regarding residual organic solvents in dosage forms [145, 183] including a USP guidance (USP 476). DPV and MK-2048, with limited aqueous solubilities, provided formulation challenges in this aqueous based system. Using two entities with limited aqueous solubility would further validate the feasibility and versatility of this extended release film platform.

MK-2048 had not been previously formulated into the thick film platform so a polymer based formulation that could accommodate this agent first had to be selected. Two initial formulations were tested to see which resulted in the most prolonged release of this compound. The HEC based TFV and PVA based DPV formulations were used as platforms for MK-2048 formulation. These bases are commonly used in the laboratory and cellulose polymers and PVA are film forming polymers [184, 185]. In preliminary dissolution testing, the HEC based formulation of MK-2048 showed the slower release profile and release rate of the two tested formulations. Both first order and Higuchi transformations were tested to see which model was better suited for the data. R^2 values for the first order transformations were higher for both the profiles tested so this transformation was used. The limitations to this model are recognized [186] and release rate is not the only justification for formulation selection. In addition, it was known that high solids content of this formulation can allow for thicker films (as seen in Chapter 3) and the solids content can support the dispersion of two hydrophobic entities in the formulation. Disintegration time for this MK-2048 single entity film (Table 3) was greater than what was seen for the coitally dependent TFV and DPV thin films from Chapter 3 (Tables 8 and 10). DPV was also able to be incorporated into this cellulose platform.

When formulating the combination film, the thickness of the lead formulation was increased to 350 μm to further slow drug release, since thickness can impact disintegration time and drug release from this cellulose formulation (Table 8 and Fig. 16). As this was a prototype film, initial drug loading was based on a current extended release product in development, the MK-2048 IVR ring. A target for drug delivery from the extended release film was 1 week. Based data release data from the MK-2048 IVR with initial burst release followed by approximately 0.4 - 1mg release per day [176], a 9.5-15 mg per 2" x 2" inch film (3 mg per 1" x 1" film) was

targeted. This loading level could be adjusted for future development but it was an initial loading dose for film development. DPV drug loading was targeted to match the MK-2048 loading. The high percent of solids in this formulation was able to support the use of two APIs with limited aqueous solubility. The plasticizers, glycerin and propylene glycol, were adjusted to accommodate the two compounds and to improve the softness and flexibility of the film. The combination film was placed on stability assessment and to date 9 months stability has been completed. Analysis of stability assessments was conducted by comparing results to time zero assessments and statistical analysis was done to determine which values differed significantly between groups. Significant differences were found in DPV drug content at 25 °C/ 60% RH and disintegration at 40 °C/ 75%. DPV content at 9 months was significantly less than what was seen at 3 months. The stability indicating assay for this compound showed no evidence of degradation peaks. Analysis of the 12 month data could show if this value was an outlier or if there is a trend towards decreasing DPV drug content over time.

Mechanical testing of films over time, as measured by disintegration time and puncture strength, had only 1 significant difference. Disintegration at 6 months (40 °C/ 75%) differed from time 0. As previously stated in Chapter 3, reproducibility, as assessed by deviations in mass and thickness, is greater for the hand poured solvent cast thick films than what is typically seen with thin films. Furthermore, residual water content analysis was higher than what is typical of coitally dependent microbicide films (approximately $\leq 5\%$) [49, 187, 188]. With solvent casting, as the solvent evaporates, a polymer density gradient is produced which can be a barrier for further solvent evaporation [189]. While this phenomenon may not be of a large concern with thin films, because of the increased thickness at which the extended release films are poured, it is hypothesized that more water is trapped in these films. This increased water is not necessarily

uniformly distributed, hence the large deviations in water content ($RSD > 30\%$), and this can lead to mechanical changes in films reflected in puncture strength and disintegration time. Residual water can also have a plasticizing effect which can change mechanical properties such as puncture strength [166, 167]. This excess solvent could also account for drug w/w% discrepancies because films have varying residual solvents. While some discrepancies seen with hand casted films can be overcome with batch scale-up, future studies should take into account the possibility of excess film solvent which could lead to larger stability issues. Residual solvent in films may be a potential barrier to producing a completely uniform and stable product, and should be kept in mind when using this method.

None of the films tests were found to have any toxicity to the *Lactobacilli* strains tested proving that these films are compatible with the common innate bacterial species commonly found in the vagina [190]. IC_{50} values for the DPV and MK-2048 combination films were comparable to the IC_{50} values for the APIs themselves (Table 18) proving that the manufacturing process and film excipients do not alter the ability of the API to halt viral replication. The assessments showed there were no toxicity and efficacy issues over time for the combination film product, but mechanical tests showed variability, which could be a result of the casting process.

The work done here show the feasibility of formulating two compounds into an aqueous based, solvent casted film. The thick, extended release platform was used for the formulation of this combination product. Future studies can be done with these extended release platforms to stress the levels of API that they can accommodate, but these preliminary studies did not try to stress the platform to the highest extent. These studies do offer further support for the

development of combination vaginal film products and further utilization of thickness as a mechanical parameter to decrease release rate over time.

There were limitations to the studies done here. First, only two bases were tested to accommodate the combination film. While it is hypothesized that these two bases were the best suited, they could be altered to increase solids content to produce even thicker films or an alternative base could be developed. The limitations to the models used to compare release profiles are also recognized. These studies also show that reproducibility is a limitation when manufacturing thick films, consistent with what was seen in Chapter 3. Also consistent with what was discussed in Chapter 3 is the practicality of delivering films that have lost their thin film qualities and are 3.5 times the thickness of the coitally dependent films. There could be potential issues with insertion and acceptability in target populations.

4.6 ACKNOWLEDGMENT

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Dezzutti's lab at Magee-Womens Research Institute for performing the efficacy and cellular toxicity assays, Bryony Brown from Dr. Bernard Moncla's lab at Magee-Womens Research Institute for performing the compatibility with *Lactobacilli* assays.

5.0 DEVELOPMENT OF AN EXTENDED RELEASE VAGINAL FILM PLATFORM THROUGH EXCIPIENT BASED FORMULATION STRATEGIES

5.1 INTRODUCTION

Worldwide, over 2 million new infections of HIV occur annually and women now account for over half of those living with HIV [78]. Physiological and behavioral factors increase women's susceptibility for HIV infection when compared to male counterparts [191] and therefore many prevention strategies are focused on women. Various dosage forms are in development for the delivery of pre-exposure prophylactics (PrEP) or microbicides for the prevention of the sexual acquisition of HIV in women. These dosage forms include IVR, gels and creams, films, and inserts [79, 178]. One dosage form which was found to be acceptable to women was the thin film [97], and two films have been evaluated in clinical trials [89, 90, 192, 193]. These films were developed for use prior to intercourse to protect the user from HIV acquisition if exposed to virus. Other clinical trials evaluating various daily (oral, vaginal gel) or extended release (vaginal ring) microbicide products showed that adherence to product regimen impacted product efficacy [26, 31, 32]. In the studies using DPV containing IVRs, it was found that there was lower adherence in groups of younger women which resulted in lower levels of protection [31, 32] and taken from the ASPIRE study by Baeten et al., "Greater HIV-1 protection was observed among subgroups of women who had evidence of higher rates of adherence than among those with

lower rates of adherence” [31]. Similar results tying product efficacy and adherence were found in with oral and topical TFV products. From the VOICE study by Marrazzo et al., it was found that “daily adherence to study products — oral or vaginal TFV-based formulations — was low, and no regimen significantly reduced the risk of HIV-1 acquisition in a modified intention-to-treat analysis” [26]. These results were consistent with other studies which tied efficacy to adherence like the studies FEM-PrEP trial [22] and the CAPRISA 004 trial [30]. Based off these data, an accepted dosing platform which offers a longer window of protection would be highly beneficial. This would overcome pitfalls seen with inconsistent microbicide use which is insufficient to establish effective concentrations at the site of action. Therefore, we hypothesize that the dosing interval of the vaginal film can be extended through formulation strategies that will increase the disintegration time of the product and dissolution time of the active pharmaceutical ingredient (API).

To extend the dosing interval of the vaginal film, formulation strategies using specific excipients will be employed. Polymers can impact disintegration and dissolution of solid dosage forms based off of molecular weight and erosion, mucoadhesive interactions, and solubility in various media. To that end, polymers with functional groups that can interact with the glycoproteins which constitute the majority of mucus would increase mucoadhesion in the vagina. Such functional groups are hydroxyl, carboxyl or amine groups [194] therefore, polymers like hydroxypropyl methylcellulose (HPMC) can favor these interactions [195]. Other groups that favor mucoadhesive interactions include thiol groups, polymers grafted with PEG, polymers crosslinked with poly(acrylic acid) and cationic polymers like chitosan [194, 196, 197]. Polymers range in molecular weight and higher molecular polymers may favor a slower drug release profile since polymer erosion over time and release rate are both inversely correlated with

molecular weight [198-201]. Hydrophilicity can also impact drug release from a polymer matrix with greater hydrophilicity increasing drug release rate since the solvent can penetrate hydrophilic matrices more readily than hydrophobic matrices [201, 202]. Solvent penetration will impact polymer chain relaxation and drug dissolution from the dosage form. Therefore, to slow drug release over time, a more hydrophobic matrix would be favorable. Another strategy which can be used to slow overall drug dissolution rate is through the inclusion of polymers with limited aqueous solubility. Due to their limited solubility, the rate of which they imbibe fluid which in turn allows for the relaxation of polymer chains, is limited. Further insoluble inclusions in the film can limit fluid transport, swelling kinetics and therefore film disintegration [203]. Therefore, drug release from these systems is also limited [204, 205]. Mucoadhesion, molecular weight and solubility factors are three different mechanisms which were targeted to increase the physical film residence time in the vagina. Three polymers were chosen to employ these mechanisms: high molecular weight HPMC to decrease release rate and increase mucoadhesion; high molecular weight hydroxypropyl cellulose (HPC) for its hydrophobicity and increased molecular weight; and ammonio methacrylate copolymer, an insoluble polymer to limit fluid imbibement.

Not only does the film need to be retained in the vagina, but a potent API must also be used to keep the user protected from HIV acquisition. Ideally, this agent would penetrate the mucosa while limiting systemic exposure. MK-2048 is a second-generation integrase inhibitor which targets the HIV enzyme responsible for allowing HIV to incorporate its viral genetic material into the DNA of host cells. HIV can mutate and confer resistance to certain anti-HIV compounds. Second generation compounds can retain activity against the mutated HIV due to structural changes of these compounds [182, 206]. MK-2048 has been shown to have efficacy

against raltegravir and elvitegravir resistant HIV viruses, and it also has EC_{95} values in the nanomolar range [207]. Combining this agent with a film which can be retained in the vagina with a slow drug release rate could extend the dosing interval of the traditionally quick dissolve film; thus increasing the period of protection offered by a polymeric vaginal film. The overall goal of this project was to design a vaginal film product that can be applied on a weekly basis and deliver sufficient drug over that weeklong period.

To efficiently formulate films, a design of experiments (DOE) approach was used. This is a statistical approach to formulation development where multiple factors are varied simultaneously, and the interactions between input parameters can be studied. This is in contrast to other formulation approaches such as ‘best guess’ or ‘one factor at a time’ approaches which are not as efficient and do not take into account interactions between factors [208]. A statistical approach to formulation allowed the development of statistical models so that ideal film parameters could be targeted through formulation variation. In this chapter, data generated through this DOE informed the development of a lead extended release film containing MK-2048 for the prevention of the HIV in women.

5.2 MATERIALS

MK-2048 drug substance was graciously supplied by Merck Sharp & Dohme Corp. (2000 Galloping Hill Road Kenilworth, NJ 07033 U.S.A.). Hydroxypropyl methylcellulose (HPMC) K4M (Colorcon), hydroxypropyl methylcellulose (HPMC) E5 (Dow Chemical Co.), hydroxypropyl cellulose (HPC) (Ashland), hydroxyethyl cellulose (HEC) 250 L Pharm

(Ashland), Eudragit RS 30D (Evonik Industries), and polyethylene glycol (PEG) 400 and propylene glycol (Spectrum Chemical) were used in varying percentages to formulate films.

5.3 METHODS

5.3.1 Vaginal Fluid Simulant Manufacture

Vaginal Fluid simulant was prepared as previously described [81], and the process is described in Chapter 4.3.1.

5.3.2 DOE Formulation and Assessment

5.3.2.1 Formulation

A film base of hydroxypropyl methylcellulose (HPMC) E5 (Dow Chemical Co.) and hydroxyethyl cellulose (HEC) 250 L Pharm was developed so that regardless of ratios of input polymers, a film could be produced. Two plasticizers were used, propylene glycol and PEG 400, in all formulations. Excipients percentages of extended release components HPMC K4M, HPC JXF and Eudragit RS 30D were generated by JMP[®] software.

5.3.2.2 DOE Design

Using JMP[®] software, a design of experiments using a central composite design (CCD) was constructed. An orthogonal design as used with three input variables at four levels. Input parameters were the three extended release polymers selected: HPMC K4M, HPC JXF and

Eudragit RS 30D. Levels of input polymers were determined based off the JMP[®] software's orthogonal response surface design. The grade of Eudragit used in these formulations, RS30D, is an aqueous suspension containing only 30% of the ammonio methacrylate. When notated in the tables and figures, the percentage used is the entire suspension not only the solid polymer. Therefore, for example, where 15% Eudragit RS 30D is used, only 4.5% solid ammonio methacrylate is included in the formulation. All films were manufactured using the solvent cast method of manufacture using a Caframo Ultra Torque overhead mixer. After mixing the polymer melt was cast on an Elcometer[®] vacuum table heated to 71 °C using an Elcometer[®] Doctor Blade. Films were cut into 2" x 2" sizes for all analyses. Placebo and drug loaded formulations were manufactured for all formulations.

5.3.2.3 Model Assessment and Statistical Analysis

Data of inputs were fit to effect screening models and assessed for which input variables significantly impacted effects. JMP[®] software allows for interaction effects which were also used to construct models.). P values less than 0.05 were considered statistically significant. For each model, p values were evaluated as well as r squared values and the root-mean-square error (RMSE). These values taken together evaluated the strength of the model.

5.3.3 *In Vitro* Characterization

5.3.3.1 Film Characterization

Viscosity of the polymer melt prior to casting was assessed using a Brookfield rheometer and Rheocalc DVIII software. Viscosity (cgs) was assessed at 37°C, at 1 RPM, using a CPE- 51 Brookfield spindle and 0.5 mL of polymer melt. Mass (mg), thickness (µm), water content (%)

w/w), puncture strength (g/mm) and disintegration (sec) were measured as previously described (Chapters 2 and 3). Drug was extracted using 80% acetonitrile. Solutions were then diluted using a 70 %/30% mixture of mobile phases A and B (A: 0.1% Formic Acid in Water, B: 100% Acetonitrile). Drug content was measured using a Dionex reverse phase HPLC system with Chromeleon software. Dissolution testing was conducted using a SOTAX CE 7 USP 4 flow through system at a wavelength of 344 nm. 250 mL of dissolution media was used (vagina fluid simulant [81]) at a flow rate of 10 mL/min with sampling at every 2 minutes for 12 hours. A novel, modified dissolution method was also developed. This used a Distek USP 1 basket apparatus using 5 mL of vaginal fluid simulant as a biologically relevant dissolution test method. The film dosage form was placed in a cylindrical mesh basket and submerged in 5 mL of vaginal fluid simulant. A paddle was used as the stirring element and the speed was maintained at 6 rotations per minute. Temperature of the experiment was done at 37 °C for the entirety of the test. Samples were collected daily by removing all the media and replacing with fresh media. MK-2048 analysis in samples was done by HPLC as described above. A curve of drug release over time was constructed. Contact Angle was measured on a Biolin Scientific® instrument using OneAttension® software. An 8 µL droplet of water was placed on the film sample and images were taken at 15FPS for 5-10 seconds. Mucoadhesion studies were conducted using a TA.XTPlus Texture Analyzer. Porcine intestinal tissue, which was collected from animals that had been sacrificed, was secured using the TA-96HDC2 2 Clamp hold-down fixture and a piece of film was affixed to the TA-24 1/4" diameter cylinder probe. 10 µL of vaginal fluid simulant was applied to the tissue and the film/probe applied a constant force for 60 seconds. A force vs. time graph was made and force of mucoadhesion (peak force) as well work of mucoadhesion (area under the curve) was measured.

5.3.3.2 *In Vitro* Imaging

Scanning electron microscopy was performed on a JEOL JSM-6510LV/LGS Scanning Electron Microscope (SEM) with Oxford Inca at a magnification of 5000 times and an aperture size was 30 μm . Samples were not coated before SEM was run. Atomic Force Microscopy was performed using a Nanoscope V Scanning Electron Probe Microscope with Nanoscope V7.20 software. Probe used was a cone-shaped silicon probe with a radius of curvature of <10nm, full tip cone angle of 40 degree, and tip height of 12 – 18 μm .

5.3.3.3 *In Vitro* Toxicity and Efficacy

In vitro Lactobacillus toxicity and efficacy were conducted as described in Chapter 4.3.6. Compatibility with *Lactobacillus* was done in the Moncla lab at Magee-Womens Research Institute and efficacy testing was performed in the Dezzutti lab at Magee-Womens Research Institute.

5.3.4 *In Vivo* Safety and Pharmacokinetic Assessment

The macaque non-human primate (NHP) model was used to assess film retention and drug release of the lead film formulation. Studies were conducted at the Washington National Primate Research Center (WaNPRC) by the Patton Lab. In accordance to IACUC, films were scaled for the macaque to a 1”x 1” film size. A film retention and drug release study in which a single film (placebo or drug loaded) was administered (n=3 placebo film, n=3 drug loaded film) was performed in this model. Colpophotography of the cervix was used to visualize film presence for retention studies and vaginal fluids were swabbed for drug release using Dacron swabs. Drug quantification from vaginal swabs was quantified by the Anderson lab at the University of

Colorado using an ultra-performance liquid chromatographic tandem mass spectrometry (UPLC-MS/MS) assay for the determination of MK-2048 in cervical vaginal fluid (CVF). The CVF collected on vaginal Dacron swabs was extracted and further diluted with blank human plasma. The resulting plasma extract is subjected to the validated plasma methodology and the final results are corrected for dilution and the volume of CVF contained on the swab and results are reported as ng/mL of CVF fluid.

5.4 RESULTS

5.4.1 DOE Design and Formulation

HPMC K4M, HPC and Eudragit RS 30D were selected as input polymers, however grade of HPC had not been determined. To assess the grade of HPC to be used in these experiments, 4 different grades were initially tested with molecular weights ranging from 80,000- 850,000 Daltons. These different grades were incorporated into the film base, HEC and HPMC E5, and tested to determine which level the film platform could accommodate. Grades and film observations are in Table 19. HPMC JXF was the grade selected for use in the DOE.

Table 19: Hydroxypropyl Cellulose (HPC) Grade Characteristics

Grade	Molecular Weight (Daltons)	Highest % In Single Film	Film Observations
HPC MXF Pharm	850,000	<3 %	Polymer melt was extremely viscous. Left undissolved chunks after overnight stirring. Could not make a film.
HPC JXF Pharm	140,000	Up to 7%	Started to have difficulty dissolving, but made a film.
HPC LXF Pharm	95,000	Up to 10%	Started to have difficulty dissolving, but made a film.
HPC EXF Pharm	80,000	>9%	Still no difficulty dissolving. Could keep adding polymer to solution.

HPC characteristics and observations after maximum levels were incorporated into solvent cast films.

A cellulose based film formulation was used for all films (Table 20). HPMC K4M, HPC JXF Pharm and Eudragit RS 30D percentages were varied based off the design of experiments (DOE) generated from the JMP[®] software. HPMC E5 and HEC remained at constant percentages, PEG 400 and propylene glycol varied based off total percent solids in the formulation. Percentages of PEG 400 and propylene glycol were determined based on total percent solids. PEG 400 and propylene glycol constituted 11% and 8% of total solids, respectively, and these percentages were based off previously manufactured solvent casted films.

Table 20: General Film Formulation for Design of Experiments MK-2048 Formulations

Components	
Hydroxypropyl Methylcellulose E5	Base Polymer
Hydroxyethyl Cellulose	Base Polymer
Hydroxypropyl Methylcellulose K4M	DOE Input Polymer
Hydroxypropyl Cellulose JXF Pharm	DOE Input Polymer
Eudragit RS 30D	DOE Input Polymer
Polyethylene Glycol 400	Plasticizer
Propylene Glycol	Plasticizer

The original orthogonal design created using JMP[®] software produced 15 distinct formulations, 4 of which were center points and 6 of which were axial points (Table 21). Replicates (3) were also included. An additional formulation was created and added to the matrix to try to stress all polymer inputs in a single formulation. In total, 19 formulations were manufactured to include replicates that were generated from the JMP[®] software program. All formulations produced a film, but film characteristics varied. A response surface orthogonal design was used so that axial points of the input parameters stressed the formulation limits.

Table 21: Formulation Patterns and Percentages from JMP®¹

Pattern	HPMC K4M	HPC JXF	Eudragit RS 30D
----	0.65	0.97	2.50
---+	0.65	0.97	10.00
--+-	0.65	4.00	2.50
-++-	0.65	4.00	10.00
+---	2.50	0.97	2.50
++--	2.50	0.97	10.00
+++-	2.50	4.00	2.50
++++	2.50	4.00	10.00
a00	0.00	2.49	6.25
A00	3.18	2.49	6.25
0a0	1.58	0.00	6.25
0A0	1.58	5.01	6.25
^b 00a	1.58	2.49	0.00
00A	1.58	2.49	12.51
^c 000	1.58	2.49	6.25
^a ++A [*]	2.50	4.00	15.00

Formulations generated from the JMP® program using a central composite design (CCD) orthogonal design.

Formulation coding for levels; high (+), low (-), high axial (A), low axial (a) and midrange (0).

^{*}Exceeded axial, ^{a,b,c} Indicate film formulation used in SEM and AFM imaging studies.

5.4.2 *In Vitro* Characterization and Model Assessments

5.4.2.1 Model Assessments

Parameter model assessments were conducted for mass, water content, thickness, puncture strength, viscosity of the polymer melt, disintegration, contact angle, dissolution and mucoadhesion. All parameters had statistically significant p values ($p < 0.05$) except for the model fitting used for contact angle.

¹ Percentages in total formulation

Each specific parameter was modeled using standard least squares to construct linear models and screens for which inputs were active. A plot of actual vs. predicted data was produced for each input. The summary of the fit of the model, analyses of variance, and parameter estimates were generated. Fit of the model is evaluated by RSquare (Rsq) and Root Mean Square Error (RMSE). The RSq value is the sum of squares of the model/sum of squares total. The closer the values to 1, the better the fit of the model. The RMSE estimates the standard deviation of the random error. Parameter Estimates report shows the estimates of the model parameters and, for each parameter, gives a *t* test for the hypothesis that it equals zero. The usefulness of this program and a DOE is that it can look at interactions between polymer inputs and incorporates this into the parameter estimates. Each of the following outputs are listed in the figures and tables below. These measures together analyzed the overall fit of the model and which inputs impacted the outcome parameter. A summary table (Table 31) was configured with values for: Estimate, Std Error, *t* Ratio, and Prob>|*t*| for each factor and combination factors.

Three parameters; disintegration, viscosity of the polymer melt and dissolution were defined as the most crucial parameters for manufacturability (polymer melt viscosity) and overall product residence time (disintegration and dissolution). These three parameters were prioritized because viscosity is directly related to manufacturability, crucial to initial and scale up production of solvent cast film products. Disintegration of the film product is closely tied with drug dissolution. Slower film disintegration can prolong drug release as the dosage form remains intact, limiting release and dissolution of the API. Developing an extended release product is highly dependent on disintegration and dissolution rates.

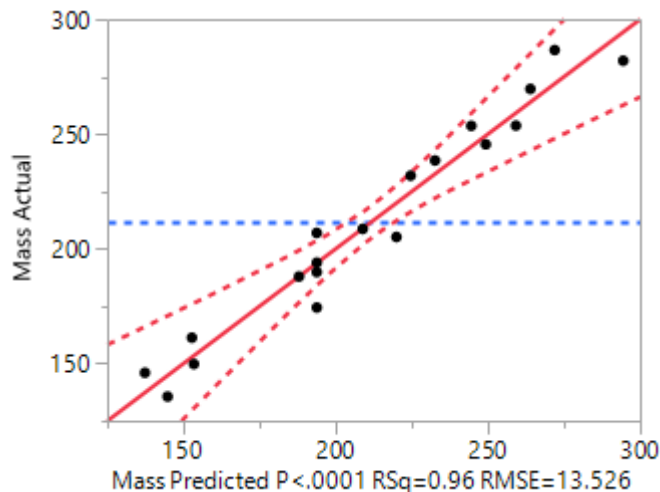


Figure 33: Mass Actual (mg) vs. Mass Predicted (mg) of MK-2048 Films

Film mass of DOE MK-2048 films measured using an analytical balance plotted versus least fit of squares model generated from the JMP® program. Data presented are the average of the number of films produced ranging from 38-73 films.

Analysis of the model from the generated from the JMP® program for mass showed a tight-fitting model with an R^2 value of 0.96 and a significant p value. Therefore, film mass was directly impacted by polymer input and the rank order of the inputs can be seen below in Table 22.

Table 22: Sorted Parameter Estimates for Mass by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
HPC(0.97,4)	38.115918	3.645022	10.46		<.0001*
Eudragit(2.5,10)	23.945378	3.520615	6.80		<.0001*
K4M(0.65,2.5)	-16.50023	3.645022	-4.53		0.0014*
K4M*K4M	15.320287	3.797767	4.03		0.0030*
HPC*Eudragit	-6.508898	4.441658	-1.47		0.1768
HPC*HPC	5.2316202	3.797767	1.38		0.2016
K4M*Eudragit	-5.138898	4.441658	-1.16		0.2771
K4M*HPC	-3.314171	4.721362	-0.70		0.5005
Eudragit*Eudragit	-0.38937	3.042452	-0.13		0.9010

HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

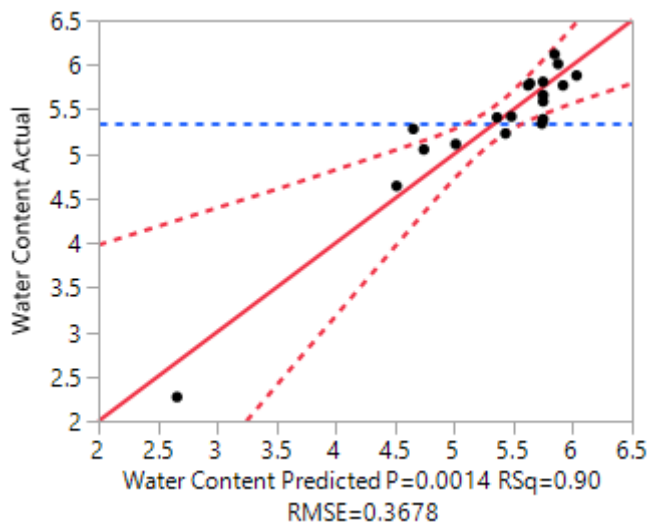


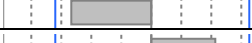





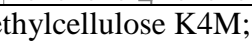


Figure 34: Water Content Actual (%) vs. Water Content Predicted (%) for MK-2048 Films

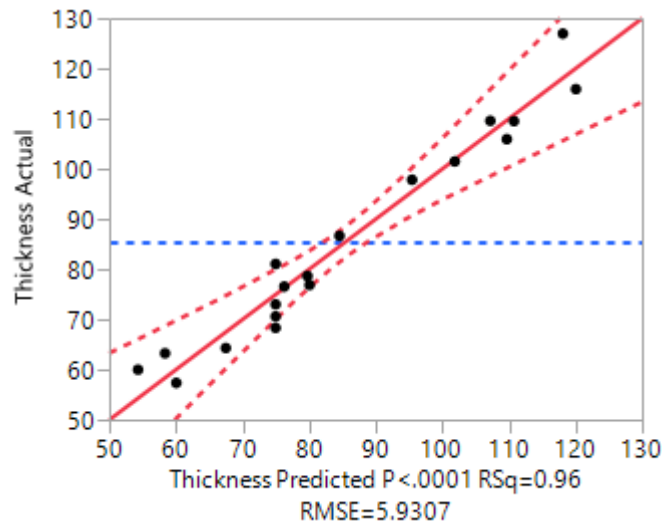
Residual water content of DOE MK-2048 films analyzed by a Karl Fischer apparatus (890 Titrando, Metrohm[®]) plotted versus least fit of squares model generated from the JMP[®] program. Data presented are the average of the three films.

There appeared to be a clustering of water content data as opposed to the spread seen with the mass data in Fig.33. Similarities in water content could be the result of similarities across all formulations which include the same polymeric base, as well as the same manufacturing process for all films which include dry times for solvent evaporation between 9-11 minutes. Similar dry times in manufacturing can impact residual water content. Analysis of fit of the model showed that there was a significant p value (<0.05) and that the R^2 value of 0.90.

Table 23: Sorted Parameter Estimates for Water Content by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
Eudragit(2.5,10)	-0.337154	0.095746	-3.52		0.0065*
Eudragit*Eudragit	-0.241987	0.082742	-2.92		0.0169*
HPC*Eudragit	-0.226892	0.120794	-1.88		0.0931
K4M*HPC	0.1934747	0.128401	1.51		0.1661
HPC(0.97,4)	-0.145447	0.099129	-1.47		0.1764
K4M(0.65,2.5)	-0.113194	0.099129	-1.14		0.2830
K4M*Eudragit	-0.124392	0.120794	-1.03		0.3300
K4M*K4M	-0.071896	0.103283	-0.70		0.5039
HPC*HPC	-0.026969	0.103283	-0.26		0.7999

HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

**Figure 35: Actual Thickness (µm) vs. Predicted Thickness (µm) for MK-2048 Films**

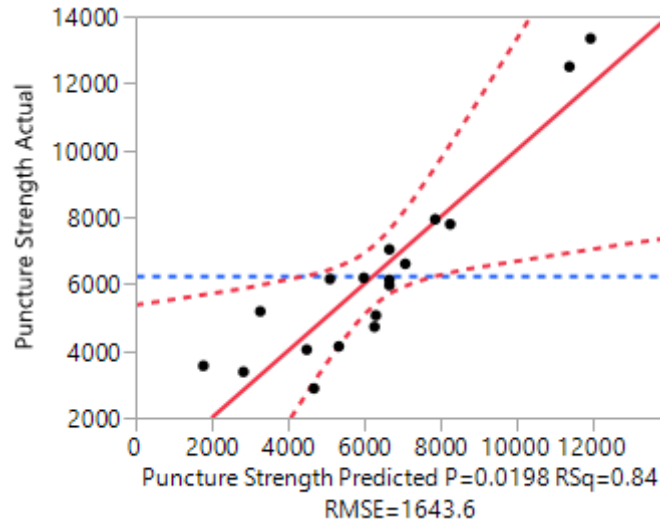
Thickness of DOE MK-2048 films measured by a Mitutoyo Absolute electronic thickness gauge plotted versus least fit of squares model generated from the JMP® program. Data presented are the average of the number of films produced ranging from 38-73 films.

Thickness modeling and parameter estimates were reflective of results seen with mass predictions in Fig. 33, with an identical fit (R^2 value of 0.96). The input parameter that most significantly impacted this output was HPC for both mass and thickness (Tables 22 and 24).

Table 24: Sorted Parameter Estimates for Thickness by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
HPC(0.97,4)	15.378819	1.598257	9.62		<.0001*
K4M*K4M	10.110351	1.665232	6.07		0.0002*
Eudragit(2.5,10)	9.0616613	1.543707	5.87		0.0002*
K4M(0.65,2.5)	4.5814386	1.598257	2.87		0.0186*
HPC*HPC	3.2522975	1.665232	1.95		0.0826
K4M*HPC	3.6740616	2.070206	1.77		0.1097
Eudragit*Eudragit	-1.99287	1.334044	-1.49		0.1694
K4M*Eudragit	-2.804898	1.947563	-1.44		0.1837
HPC*Eudragit	1.8618591	1.947563	0.96		0.3640









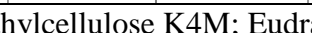
HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

**Figure 36: Actual Puncture Strength (g/mm) vs. Predicted Puncture Strength (g/mm) for MK-2048 Films**

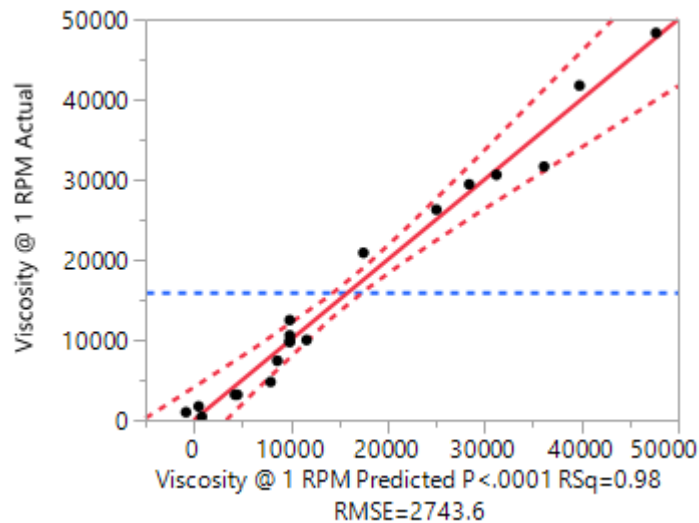
Puncture strength of DOE MK-2048 films measured by a TA.XTPlus Texture Analyzer and force required to break film/cross-sectional area plotted versus least fit of squares model generated from the JMP[®] program. Data presented are the average of three films.

Puncture strength analysis showed a significant p value and an R^2 value of 0.84. The Eudragit RS 30D most impacted the puncture strength of the film by decreasing this value, as did interactions between the HPMC K4M and HPC.

Table 25: Sorted Parameter Estimates for Puncture Strength by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
Eudragit(2.5,10)	-1617.646	428.5473	-3.77		0.0054*
K4M*HPC	-1608.811	574.0169	-2.80		0.0231*
K4M(0.65,2.5)	1065.0406	443.0645	2.40		0.0429*
K4M*K4M	-1115.295	483.5438	-2.31		0.0500*
HPC(0.97,4)	-945.5337	443.0645	-2.13		0.0654
Eudragit*Eudragit	729.20455	383.6899	1.90		0.0939
HPC*Eudragit	260.25979	541.4029	0.48		0.6436
K4M*Eudragit	-159.8352	541.4029	-0.30		0.7753
HPC*HPC	7.2734734	483.5438	0.02		0.9884

HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

**Figure 37: Actual Viscosity @ 1RPM vs. Predicted Viscosity at 1RPM**

Viscosity of the polymer melt of the DOE MK-2048 films measured by a Brookfield rheometer plotted versus least fit of squares model generated from the JMP® program. Data presented are the average of two viscosity measurements from 0.5 mL sample.

Viscosity of the polymer melt had a nearly perfect linear correlation with the modeling with an R^2 value of 0.98 and a highly significant p value. The HPMC K4M polymer most impacted the viscosity of the polymer melt which was evident visually when experimenting with single polymer films. The Eudragit RS 30D did not significantly impact the viscosity of the polymer

melt (Table 26), which was also hypothesized during the manufacture of single entity films during preliminary testing.

Table 26: Sorted Parameter Estimates for Viscosity by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
K4M(0.65,2.5)	14058.816	739.38	19.01		<.0001*
K4M*K4M	5161.6214	770.3638	6.70		<.0001*
HPC(0.97,4)	2873.5537	739.38	3.89		0.0037*
HPC*HPC	1011.5241	770.3638	1.31		0.2217
K4M*Eudragit	1173.9648	900.975	1.30		0.2249
Eudragit(2.5,10)	903.8496	714.1446	1.27		0.2374
K4M*HPC	608.53705	957.7118	0.64		0.5410
HPC*Eudragit	384.31978	900.975	0.43		0.6797
Eudragit*Eudragit	82.249093	617.1508	0.13		0.8969

HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

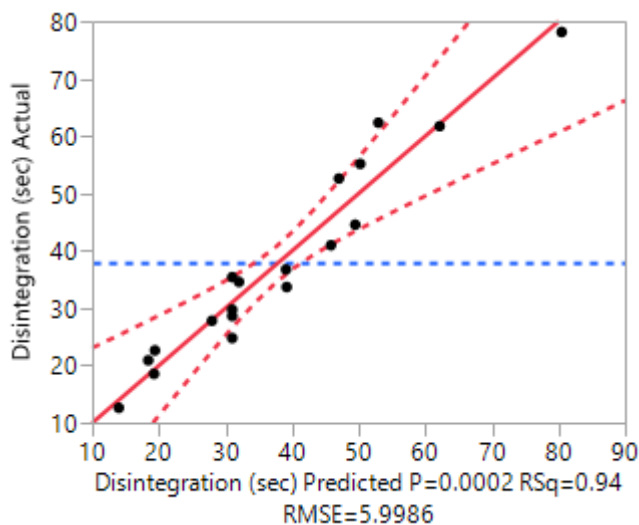


Figure 38: Actual Disintegration Time (sec) vs. Predicted Disintegration Time (sec) for MK-2048 Films

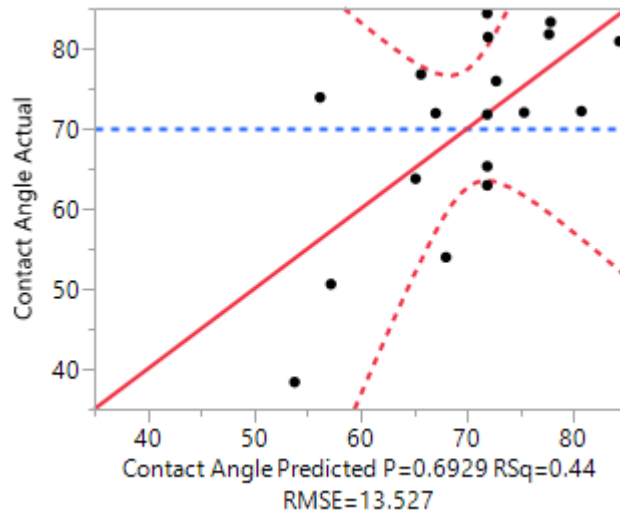
Disintegration time of DOE MK-2048 films measured by a TA.XTPlus Texture Analyzer plotted versus least fit of squares model generated from the JMP[®] program. Data presented are the average of four films.

Disintegration time was another parameter, similar to thickness and mass, whose model fit tightly. The insoluble Eudragit RS 30D most significantly increased disintegration time.

Table 27: Sorted Parameter Estimates for Disintegration Time by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
Eudragit(2.5,10)	10.354518	1.561395	6.63		<.0001*
HPC(0.97,4)	9.2381325	1.61657	5.71		0.0003*
K4M(0.65,2.5)	4.4932903	1.61657	2.78		0.0214*
K4M*K4M	3.0577305	1.684312	1.82		0.1028
K4M*HPC	3.1423325	2.093927	1.50		0.1677
Eudragit*Eudragit	1.6789394	1.349329	1.24		0.2448
HPC*HPC	1.3680195	1.684312	0.81		0.4376
K4M*Eudragit	-1.304391	1.969878	-0.66		0.5245
HPC*Eudragit	-0.898891	1.969878	-0.46		0.6590

HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

**Figure 39: Actual Contact Angle vs. Predicted Contact Angle for MK-2048**

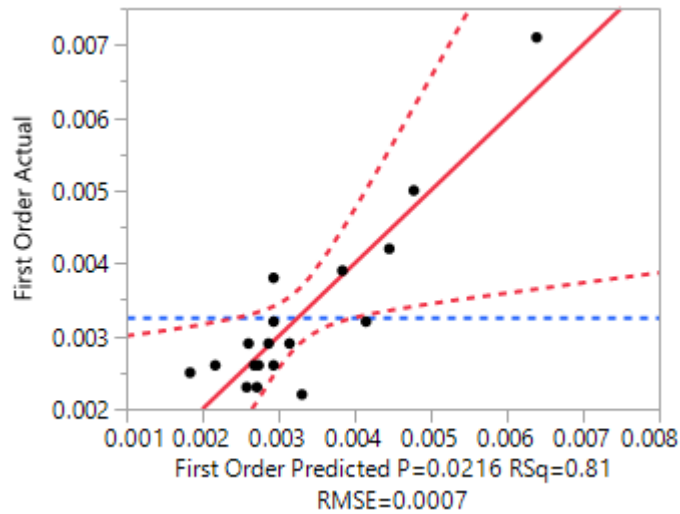
Contact angle of DOE MK-2048 films measured by a Biolin Scientific® instrument plotted versus least fit of squares model generated from the JMP® program. Data presented are the average of three films.

Contact angle was one of the few parameters measured whose model did not have a significant p value and the R^2 value was closer to 0 than the 1. A value closer to 0 shows that this model does not fit this data. Further, none of the inputs were found to significantly impact contact angle.

Table 28: Sorted Parameter Estimates for Contact Angle by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
Eudragit(2.5,10)	-8.422159	3.67276	-2.29		0.0510
K4M*Eudragit	3.4215625	4.782467	0.72		0.4947
K4M(0.65,2.5)	2.1152158	3.67276	0.58		0.5805
HPC(0.97,4)	-1.470235	3.67276	-0.40		0.6994
K4M*K4M	-0.963617	3.846687	-0.25		0.8085
HPC*HPC	-0.850553	3.846687	-0.22		0.8305
Eudragit*Eudragit	-0.587584	3.846687	-0.15		0.8824
K4M*HPC	0.1396875	4.782467	0.03		0.9774
HPC*Eudragit	-0.093438	4.782467	-0.02		0.9849









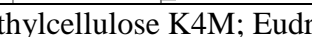
HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

**Figure 40: Actual First Order Release Constant vs. Predicted First Order Release Constant for MK-2048 Films**

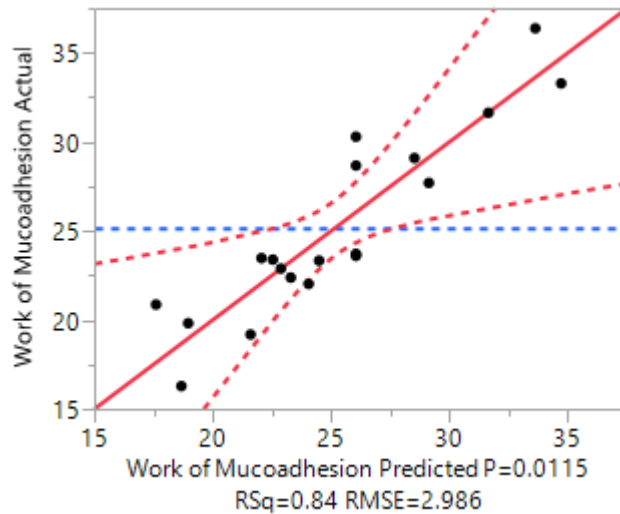
First order release constants of DOE MK-2048 films measured after SOTAX USP 4 *in vitro* release plotted versus least fit of squares model generated from the JMP® program. Data presented are the average of three films.

The release rate constant model had a significant p value (0.0216) and a R^2 of 0.81, showing that input parameters significantly impacted the dissolution rate constant and that the model fit the data for this output parameter.

Table 29: Sorted Parameter Estimates for First Order Release Constants by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
K4M*HPC	0.0009577	0.000255	3.76		0.0045*
K4M(0.65,2.5)	-0.00049	0.000197	-2.49		0.0345*
HPC*Eudragit	-0.000532	0.00024	-2.22		0.0536
K4M*Eudragit	-0.000457	0.00024	-1.91		0.0890
K4M*K4M	0.0003685	0.000205	1.80		0.1058
HPC(0.97,4)	-0.000347	0.000197	-1.76		0.1116
Eudragit(2.5,10)	0.0001309	0.00019	0.69		0.5085
HPC*HPC	0.000117	0.000205	0.57		0.5823
Eudragit*Eudragit	5.518e-5	0.000164	0.34		0.7446

HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

**Figure 41: Actual Work of Mucoadhesion vs. Predicted Work of Mucoadhesion for MK-2048 Films**

Work of mucoadhesion of DOE MK-2048 films measured by a TA.XTPlus Texture Analyzer plotted versus least fit of squares model generated from the JMP® program. Data presented are the average of three films.

Mucoadhesion analysis showed a significant p value and an R^2 value of 0.84, which was similar to what was seen with puncture strength. The Eudragit RS 30D most impacted this parameter, as it did with puncture strength, but decreased this value. The Eudragit RS 30D is not commonly used as a mucoadhesive polymer.

Table 30: Sorted Parameter Estimates for Work of Mucoadhesion by Rank Order

Term	Estimate	Std Error	t Ratio		Prob> t
Eudragit(2.5,10)	-3.54817	0.777228	-4.57		0.0014*
HPC(0.97,4)	2.7297988	0.804693	3.39		0.0080*
K4M*K4M	-2.315807	0.838413	-2.76		0.0220*
K4M(0.65,2.5)	1.1964603	0.804693	1.49		0.1712
Eudragit*Eudragit	0.9873261	0.671667	1.47		0.1756
K4M*Eudragit	-1.233274	0.980562	-1.26		0.2401
HPC*HPC	0.3749232	0.838413	0.45		0.6653
K4M*HPC	-0.181225	1.042311	-0.17		0.8658
HPC*Eudragit	-0.005274	0.980562	-0.01		0.9958

HPC, hydroxypropyl cellulose; K4M, hydroxypropyl methylcellulose K4M; Eudragit, Eudragit RS 30D

A summary for all parameter assessments for all characterization parameters can be found in Table 31. As highlighted by preceding tables and figures, the contact angle characterization parameter was the only characterization parameter whose model did not have a significant p value.

Table 31: Summary of Parameter Model Statistics

Test Parameter	P Value	RMSE	R Squared
Thickness (μm) Predicted	<.0001	5.93	0.96
Mass (mg) Predicted	<.0001	13.89	0.96
Water Content (% w/w) Predicted	0.0014	0.37	0.90
Viscosity @ 1 RPM (cgs) Predicted	<.0001	2755.50	0.98
Disintegration (sec) Predicted	0.0002	6.11	0.94
Puncture Strength (g/mm) Predicted	0.020	1643.60	0.84
Contact Angle ($^{\circ}$) Predicted	0.70	13.53	0.44
First Order Dissolution Rate Constant Predicted (min^{-1})	0.022	0.0007	0.81
Work of Mucoadhesion (g*sec) Predicted	0.0115	2.986	0.84

5.4.2.2 In Vitro Imaging

Imaging studies were conducted to assess the impact of formulation input on pore formation and surface topography. Three formulations were selected for imaging studies as these formulations represented axial and center points (Table 21 denoted ^{ABC}). These formulations differed from each other greatly based on amount of polymer present in the formulation and therefore provided

a range of polymer inputs. None of the tested DOE formulations showed evidence of pore formation using SEM but there was evidence of differences in surface topography (Fig. 42) with pits or craters observed on film surfaces (Fig. 42b). AFM was used to quantify surface roughness, as quantified by measurements of average roughness (S_a) and root mean square roughness (S_q), between formulations (Fig. 43). Measurements of these parameters showed that differences between formulations (Fig. 43) based on polymer levels with a rank order of roughness of: medium > low > and high polymer levels.

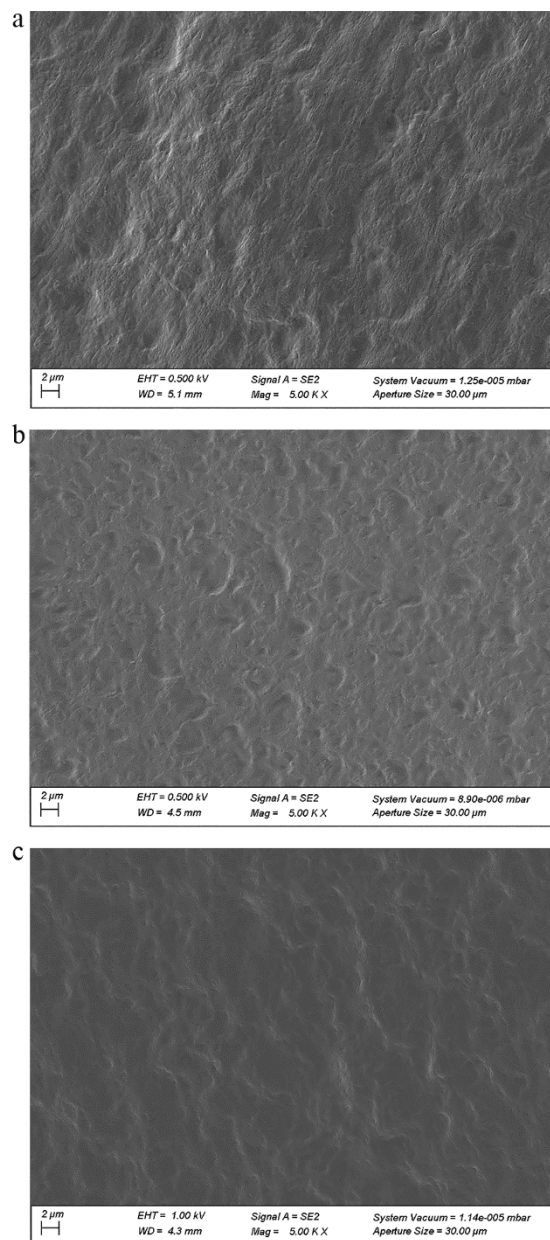


Figure 42: Scanning Electron Microscopy Imaging of Film Surface

The scanning electron microscopy images of three different formulations are shown. Formulations were selected based off input levels and are of the highest (a), lowest (b) and medium (c) polymer levels as noted in Table 21. None of the formulations showed any evidence of pore formation but there were differences in the surfaces of the films depending on formulation.

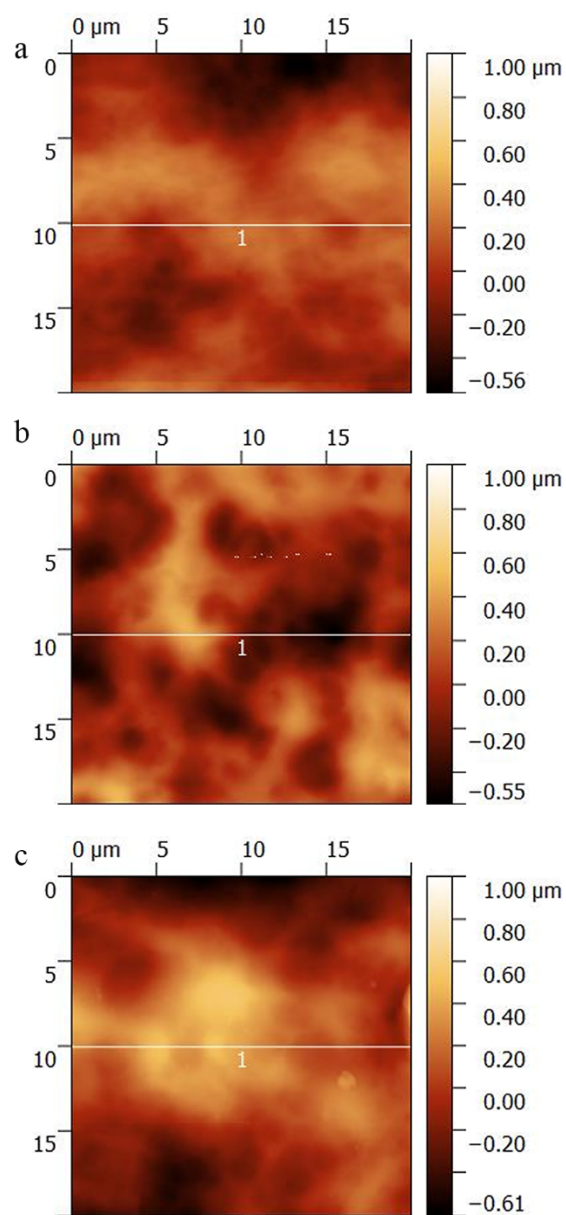


Figure 43: Atomic Force Microscopy Images of Films

Atomic force microscopy images for three formulations. Formulations were selected based off input levels and are of the highest (a), lowest (b) and medium (c) polymer levels as noted in Table 21. Surface topography scales (from light to dark) are present on each image. Topography was also quantitated for Sa (average roughness) and Sq (root mean square roughness) and (a) Sa: 146.080 nm, Sq: 180.272 nm, (b) Sa: 168.474 nm, Sq: 205.010 nm, (c) Sa: 203.6 nm, Sq: 246.0 nm. Values indicate differences in surface topography based off formulation which was also reflected in scanning electron microscopy images.

5.4.3 Lead Film Selection and Assessment

5.4.3.1 *In Vitro* Assessment and Characterization

Based off of analysis of the data generated from the DOE, a lead formulation was selected for further development for clinical delivery of the HIV preventative agent MK-2048. This film contained the highest level of Eudragit RS 30D, had the longest disintegration time and one of the slowest release rates.

Two initial doses were proposed for an MK-2048 extended release film. Based off of release from the MK-2048 IVR as discussed in Chapter 4, a 12 mg dose was targeted from a 2" x 2" film. In efforts to prove the utility of the film to deliver high levels of MK-2048 to the vagina, a second dose was also proposed for an extended release MK-2048 film which doubled the initial 12 mg dose to 24 mg. Drug loading was increased to target 24 mg per 2" x 2" film, with hypothetical loading of 6 mg per 1" x 1" film. 1" x 1" films were manufactured and characterized for mass, thickness, puncture strength, drug content, water content and disintegration. The film was soft, flexible and yellow in color. Characterizations are found in Table 32. Actual drug loading exceeded the theoretical value and was $7.47 \text{ mg} \pm 0.11$ per 1" x 1" film. Dissolution was measured using the novel method described in the method section 5.3.3.1 using the small volume Distek setup and vaginal fluid simulant (VFS) as the dissolution media. The solubility of MK-2048 in VFS is approximately 12 $\mu\text{g/mL}$ (unpublished data). This dissolution is solubility limited because of the low solubility of MK-2048 in the dissolution media, and it is possible that there was undissolved MK-2048 in the samples which was not accounted for. Therefore, dissolution is represented as amount of MK-2048 released over time instead of % released over time because of the small percentage of total drug released (<10%).

Further characterizations were done to test film compatibility with three strains of *Lactobacillus* (*L. jensenii* ATCC 25258, *L. jensenii* LBP 28AB and *L. crispatus* ATCC 33197). The placebo film and the MK-2048 containing film were compatible with all strains (Table 33), proving that the films were not harmful to the innate bacterial species present in the vagina. Anti-HIV activity measured in TZM-bl cells showed that the film had an IC₅₀ of 3.574 nM which is nearly equivalent the IC₅₀ of the pure drug substance, 3.58 nM (unpublished data).

Table 32: Characterization of MK-2048 Containing Films

Film Size	1"x 1"
Weight (mg)	87.8 ± 4.56
Thickness (mm)	0.111 ± 0.006
Drug Content (mg)	7.47 ± 0.11
Puncture Strength (g/mm)	5.73 ± 0.75
Water Content % (w/w)	4.61 ± 0.51
Disintegration Time (sec.)	142.98 ± 36.91

Data shown is the mean ± standard deviation

Table 33: MK-2048 Film Compatibility with *Lactobacillus*

<i>Lactobacillus</i> Strain	Log Difference	
	Placebo Film	Drug Loaded MK-2048 Film
<i>L. jensenii</i> ATCC 25258	-0.179	-0.091
<i>L. jensenii</i> LBP 28AB	-0.982	-0.543
<i>L. crispatus</i> ATCC 33197	0.271	-0.238

Viability difference between control and film treated bacterial plates. A difference > 1 log₁₀ indicates a significant change in bacterial viability. Bacterial strains were tested in triplicate.

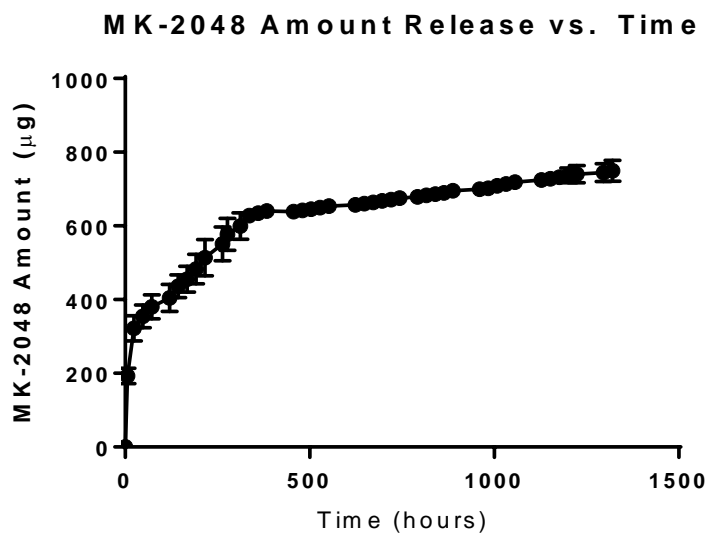


Figure 44: MK-2048 Amount Release vs. Time

In vitro release of MK-2048 lead formulation assessed through a modified Distek USP 1 Basket apparatus using vaginal fluid simulant as the release media. n= 3

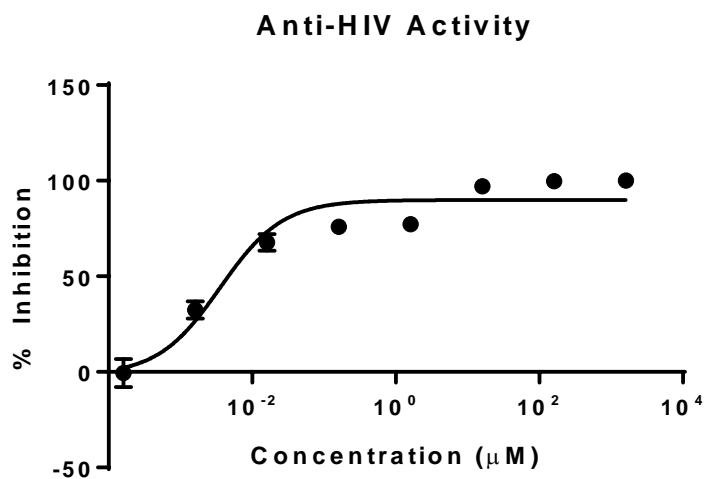


Figure 45: Anti-HIV Activity of MK-2048 Film

In vitro anti-HIV activity in TZM-bl cellular assay of MK-2048 lead formulation in triplicate.

This lead formulation was also tested to see if could accommodate a higher drug amount of MK-2048. Intended drug loading was 60 mg per 2" x 2" film. However, this amount was exceeded at approximately 100 mg/film. Characterizations are found in Table 34 and compatibility with

Lactobacillus can be found in Table 35. The high drug loaded film was not compatible with the *L. jensenii* LBP 28AB strain of *Lactobacillus*, as noted by the decrease in viability was $> 1 \log_{10}$. Cellular anti-HIV activity was measured in the TZM-bl model and the IC_{50} for the film product was found to be 11.13 nM. This is higher than what is found for the IC_{50} of the pure drug substance, 3.58 nM (unpublished data).

Table 34: Characterization of MK-2048 Containing Films

Film Size	2" x 2"
Weight (mg)	503.6 \pm 15.32
Thickness (mm)	0.187 \pm 0.007
Drug Content (mg)	96.03 \pm 3.39

Data shown is the mean \pm standard deviation

Table 35: MK-2048 Containing Film Compatibility with *Lactobacillus*

<i>Lactobacillus</i> Strain	Log Difference
<i>L. jensenii</i> ATCC 25258	-0.290
<i>L. jensenii</i> LBP 28AB	-2.215
<i>L. crispatus</i> ATCC 33197	-0.365

Viability difference between control and film treated bacterial plates. A difference $> 1 \log_{10}$ indicates a significant change in bacterial viability. Bacterial strains were tested in triplicate.

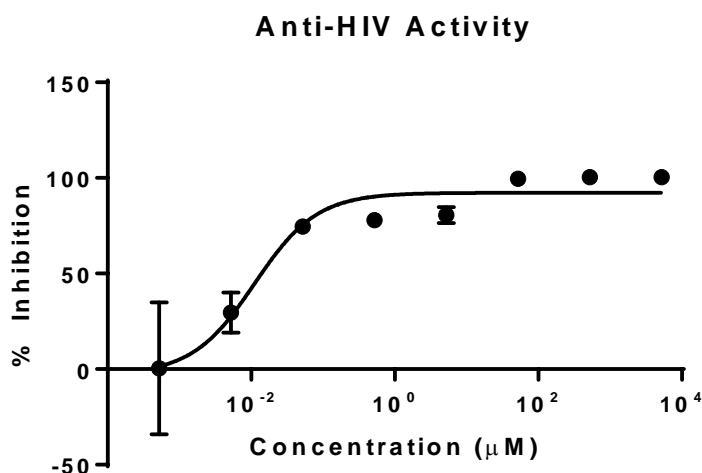


Figure 46: Anti-HIV Activity for MK-2048 Film

In vitro anti-HIV activity in TZM-bl cellular assay of MK-2048 formulation with increased drug loading in triplicate.

5.4.3.2 *In Vivo* Assessment and Characterization

This lead film, target drug content of 24 mg per 2" x 2" inch film, was selected for testing in the NHP model to assess safety, film retention and drug release. As previously discussed, this animal model is commonly used for vaginal microbicide product evaluation due to its anatomical similarities to humans as well as biological similarities between bacterial species in the vaginal tract [64, 65, 69, 71, 74, 209]. This is the film that is characterized in Tables 32 and 33. An *in vivo* study was conducted with this lead film platform using a placebo or drug loaded films placed in the macaque model. Visualization of the film product was confirmed up to day 36 (n=1) and 24 (n=2) for the placebo and drug loaded films (Fig. 47a), respectively. All animals menses at least once throughout the study, and film was retained through this menstruation (Fig. 47). MK-2048 was detected vaginal fluid in two of the three animals who received the drug loaded film above the IC₅₀ for the compound up to day 30 (Fig.47b). It was found that the third

animal physically removed the film which resulted in MK-2048 levels below the IC_{50} for this animal for the duration of the experiment.

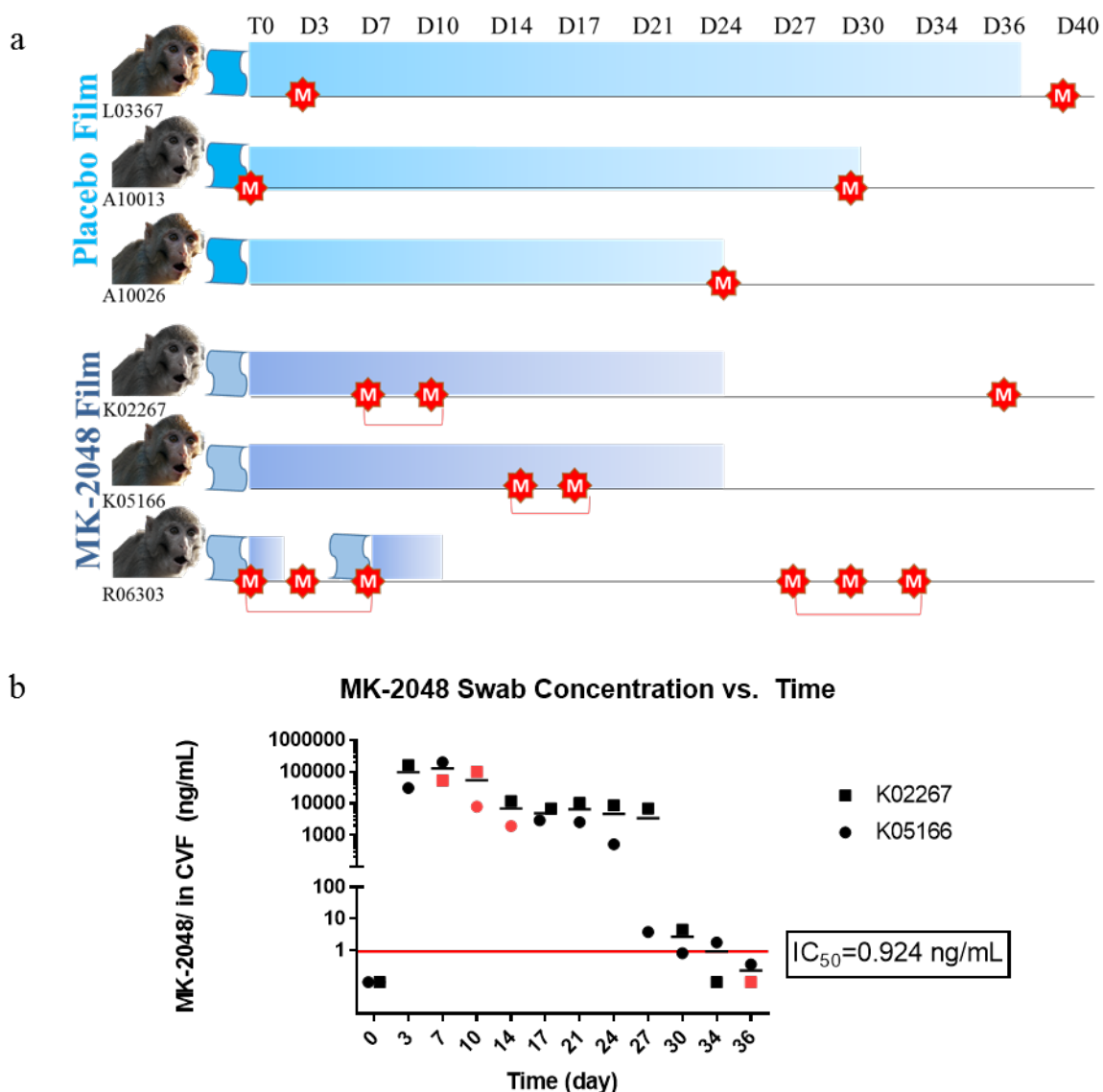


Figure 47: *In Vivo* Film Retention and Drug Release in the Non-Human Primate for MK-2048 Films

(a) Film retention for placebo and MK-2048 loaded films showing length of retention for each pigtailed macaque based on colpophotography after films were intravaginally placed in each animal at time zero (T0) (b) MK-2048 concentration in cervicovaginal fluid (CVF) over study duration. IC_{50} for MK-2048 notated on (b).

and red symbols indicated animal was menstruating

5.5 DISCUSSION AND CONCLUSIONS

In an effort to overcome barriers seen with coitally dependent microbicide products, longer acting methods are in development. A preferred dosage form, the thin polymeric film, has been traditionally used for rapid release of an active agent. Here we used a systematic approach to formulate a novel film, which can extend the dosing interval of a microbicide candidate while keeping the ‘thin film’ characteristic which is a coveted parameter of the dosage form. Drug release kinetics is governed by the matrix of the dosage form, the API itself and the release medium [160]. In theory, these individual categories have specific parameters which can be manipulated and which can in turn impact drug release. However, since the release media is a biological media (vaginal fluid) this parameter cannot be altered. Therefore, to develop and extended release film platform, strategies were employed to control the matrix of the dosage form and API incorporated into this dosage form. The active agent used, MK-2048, is a hydrophobic agent which will not readily solubilize or diffuse through aqueous environments. The film can be classified as a swellable dosage form which will imbibe fluid and cause an erosion front. This imbibement of fluid allows the relaxation of the polymer chains of the polymeric film matrix to allow the dispersed drug to be released from the film. High molecular weight polymers, hydrophobic polymers and polymers with limited solubility can slow fluid imbibement and further polymer chain relaxation. Drug is released from the matrix as the dosage form swells and polymer chain relaxation occurs. The selected polymers used in these formulations, hydroxypropyl methylcellulose K4M, hydroxypropyl cellulose JXF and Eudragit RS 30D all had properties which can slow polymer erosion and downstream drug release from the swellable film matrix.

A design of experiments allowed for a controlled set of formulations to be manufactured and tested. A response surface design was used to model three polymer inputs and their effects on film functionality outputs. One of the most common response surface designs, the central composite design (CCD), was used as it efficient for fitting second order models which was predicted for these polymer inputs and interactions. These input parameters, and the interactions between them, were then used in models of the response variables which measured film functionality and *in vitro* behaviors such as disintegration, viscosity, water content, dissolution rate, mucoadhesion, and contact angle. Formulations were generated from the design of experiments program, JMP[®] Pro 12.1.0 program, and provided 15 distinct formulations with replicates built into the design of experiments (Table 21). An additional formulation, designed to stress the upper limits of selected polymers was generated based off initial data collected and the well-accepted principal that increased polymer concentrations in dosage forms can slow drug release [162]. Increased polymer levels have been shown to decrease release rates for drugs because of decrease initial porosity (which was not applicable to these films) or porosity that forms as during fluid imbibement [201, 210]. In this formulation, the Eudragit RS 30D exceeded the axial point generated from the initial DOE because as a liquid suspension, this polymeric suspension had little to no impact on the viscosity of the polymer melt. The HPMC K4M and HPC JXF could not be stressed to the same level as the Eudragit RS 30D because these solid polymers impacted the already highly viscous polymer melt. Input parameters significantly impacted all responses tested (p value < 0.05) apart from contact angle (Table 31). Contact angle is used to study the wetting, adhesion, and hydrophobicity and hydrophilicity of surface materials [211]. The majority of the formulation inputs were hydrophilic polymers (HPMC E5, HEC, and HPMC K4M). The fraction of the insoluble Eudragit and hydrophobic HPC on the

film surface may not have significantly impacted contact angle, thus results from these tests had relatively similar contact angles across formulation.

Three responses, viscosity of polymeric melt, product disintegration, and drug dissolution were prioritized as the most crucial response variables for the development of an extended release film. Viscosity of the polymer melt is vital in the manufacturing process of polymeric films. Viscosities that are either too high or too low will create a polymer melt which cannot be cast and/or cannot produce a solvent cast film. Viscosity was highly correlated with HPMC K4M and HPC JXF inputs. These are high molecular weight, solid polymers, and the correlation of molecular weight and viscosity is well documented [212]. During film casting, differences in polymer melt viscosity were noticeable and impacted the overall ease of the casting process. The lead formulation had one of the highest viscosities, but if this would be an issue during manufacturing and scale up, results from the DOE could aid in the reformulation or formulation modifications. Small decreases in HPMC K4M or HPC JXF percentages could significantly impact the overall viscosity of the polymer melt.

Disintegration and dissolution were the two other characterization parameters which were prioritized over the large range of physiochemical tests. Disintegration and dissolution are two properties which will govern the extended release nature of the API from films. Disintegration time was significantly impacted by all three input parameters in a rank order of Eudragit RS 30D, HPC JXF and then HPMC K4M (Table 27). Eudragit RS 30D is a highly insoluble, synthetic polymer which will clearly impact disintegration in an aqueous solvent. HPC JXF, while a hydrophilic polymer, has a lower polarity and therefore hydrophilicity of other cellulose polymers. This will also aid in the prolonged disintegration time [202, 213]. As a swellable matrix system, the increased molecular weight of the HPMC K4M can also impact the

disintegration time because of the erosion of the polymer matrix of high molecular weight polymers, such as HPMC K4M, is slowed compared to lower molecular weight systems [198, 201]. As seen with the *in vivo* NHP data, the film was retained in the vagina for 24-36 days. If the disintegration time of the film needs to be altered to decrease this disintegration time, the Eudragit percentage in the film could be decreased. Increased disintegration time was correlated with increased retention time in the NHP with the TFV and DPV films from Chapter 3.

Modeling dissolution was not as straightforward as other parameters, as the goal was to capture the entire release profile of each formulation. Instead of a single data point of the dissolution curve, the data was transformed using first order release kinetics to obtain a release rate. Dissolution data was also modeled using the Weibull and Higuchi models, but ultimately first order modeling was selected to study these dissolution curves. Data was first plotted to confirm that each profile followed first order release kinetics before obtaining rates. Interestingly, the interaction between the HPC JXF and HPMC K4M inputs most significantly increased the rate of drug release (Table 29). Interactions between the two swellable polymers can impact matrix formation or viscosity of the gel layer which is reflective in the model generated [162]. This interaction parameter (HPMC K4M and HPC) was also seen to decrease puncture strength (Table 25) providing additional support that matrix formation could be impacted by the interaction of these two polymers. There was a trend towards increased water content for this interaction also (Table 23) but it was not significant. Recall from previous chapters that increased water content can act as a plasticizer in films which can impact mechanical properties of films like puncture strength. HPMC K4M was second in rank order of significance for dissolution rate constant. This high molecular weight polymer significantly decreased release rate constant, consistent with theories that molecular weight is inversely

correlated with release rate. These three parameters, viscosity, disintegration and dissolution, were prioritized for the development of a lead film in these studies, but data generated from all other tests can be used for future formulation development as each of these parameters can be tailored to fit the needs for the dosage form.

In addition to all response parameters measured as part of the DOE, imaging studies were conducted to see if polymer ratios impacted solid film surface morphology. Before these studies, it was unknown if polymer network formation results in surface pores, so scanning electron microscopy was conducted. Based on time and resources, three formulations were selected for SEM imaging. These formulations differed based on polymer levels and are indicated in Table 21. Interestingly, it was found that there was no pore formation at the surface of any of these films (Fig. 42), but that there are differences in the surface topography based on formulation. SEM was unable to quantify these differences, so atomic force microscopy was used to quantify the differences in surface topography of the selected formulations. Using the Gwyddion 2.47 software, AFM images were quantified for average roughness and root mean square roughness (Fig. 43), the two most commonly used parameters to measure roughness [214]. Changes in surface roughness, as measured by average surface roughness and root mean square roughness, were observed based on formulation inputs and AFM was sensitive enough to capture these changes.

The lead formulation candidate selected based of all data and models generated had high levels of all three input polymers. High polymer content in solid dosage forms has been shown to decrease erosion and dissolution over time, which was reflected in disintegration time and dissolution rate. Drug loading in all DOE films was kept consistent, but a 24 mg loading level was targeted for a 2" x 2" film size. Actual drug loading in the film was greater than this. A

higher drug loading level was also targeted and the film was able to accommodate approximately 100 mg of MK-2048 in a 2" x 2" film. This shows that the film dosage form was capable of loading very high levels of a hydrophobic agent even when using a solvent cast method of manufacture. This high level of MK-2048 was not compatible with all strains of *Lactobacillus*, so the lower loading dose was chosen for further study. The high drug loading in the film also had a higher IC₅₀ when compared to the lower drug loading level, which was more similar to the IC₅₀ of pure drug substance. This shift in IC₅₀ from 3.58 nM to 11.13 nM may not constitute a major shift and could be a result of deviations between replicates in the experiments as seen by the deviations in Fig. 46.

In vivo film retention and drug release studies revealed surprising findings when the proposed weeklong film was actually retained for 24 days (n=5) and up to 30 days (n= 2) in some animals (Fig. 47a). Not only was the physical film product present, but drug levels of MK-2048 in vaginal fluid remained above the IC₅₀ for this compound over this duration as well (Fig. 47b), showing that the film can provide extended release of the API. Levels of MK-2048 in the vaginal fluid dropped rapidly after the film was no longer detected in the vagina after day 24 (Fig. 47). Levels in the CVF on days 27-36 show a rapid decline in concentration which coincides with the disappearance of the film product. 1 macaque, R06303, had noticeably shorter duration of film retention with lower drug levels in CVF compared to other animals (Fig. 47). Upon further inspection, it was found that this animal had learned to physically remove the film from the vagina. Results from this animal were not analyzed in MK-2048 CVF concentration because of this. Compared to the films generated in the Chapter 3, the lead MK-2048 film was retained for nearly 20 days longer in the NHP. One of the concerns raised with the films generated in the thick film platform was the acceptability of films that were 2.5-5 times thicker

than what was developed for coitally dependent usage. The films developed in this chapter have similar thicknesses to the coitally dependent films while still offering the increased retention time, thus overcoming the potential issue raised with thick film platform.

The DOE provided invaluable information for formulation development of a novel polymeric film platform using polymers with different molecular weights, hydrophilicities and solubilities. These studies, taken together, show that small formulation changes impact film parameters and that these parameters can be adjusted or tailored based on the goal or need of the film. A DOE uses select experiments to create a larger data space and is an efficient tool in formulation development. The lead film selected from these experiments could deliver MK-2048 vaginally to a NHP for up to a month at values about the IC_{50} . This study confirms the utility of DOE in formulation development for vaginal drug delivery. These studies also show that the lead formulation platform was able to accommodate ranging levels of MK-2048, a hydrophobic agent. While the highest level loaded into the film, 100mg/ 2" x 2" inch film, was not compatible with all strains of *Lactobacillus*, the manufacturing method and platform were still able to be used for this loading level.

There were limitations in the studies conducted here. Only one grade of each polymer was used instead of various grades and percentages. Using multiple grades of each polymer would cause the matrix of the DOE to expand exponentially and the scope of work would be nearly impossible to undertake. Eudragit RS 30D is a 30% dispersion and this 30% was not accounted for when using percentages in the DOE. If conducting these experiments again, this dispersion percentage should be accounted for. There were also limitations to modeling of dissolution. With any model, there are shortcomings and limitations to the model selected. The Weibull and Higuchi models were attempted for the analysis of the dissolution data but using

these models, no significant comparisons between release profiles could be made which was the goal of modeling the data. First order release is usually used with water soluble compounds in porous matrices [186] which was not the case for the MK-2048 films in these studies. There is however merit in using this model because there is a concentration driven gradient between the dosage form and biological environment and as the dosage form swells and the polymer chains relax, pore and channel formation could facilitate the release of API from the dosage form. Another limitation is in the animal studies. Animal studies were only conducted in 3 animals per group, and there was an issue with film removal with one animal. This study could be repeated to see if results can be replicated, and the animal that removed the film should not be included in future studies.

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6.0 SUMMARY OF MAJOR FINDINGS, CONTRIBUTIONS AND FUTURE DIRECTIONS

6.1 INTRODUCTION

Numerous strategies in HIV prevention are being pursued to curb infection rates so that this devastating virus will one day no longer threaten millions of lives around the world. Vaginal delivery of antiretroviral (ARV) compounds is a focus of HIV prevention research since “unprotected heterosexual intercourse is the leading cause of HIV acquisition in women” [98]. Various dosage forms are under investigation for the vaginal delivery of these ARVs including coitally dependent and independent products. Gels were the first delivery platform investigated as coitally dependent vaginal microbicide products. Then tenofovir gel was found to be safe and effective in clinical trials when the dosing regimen was followed, with greater efficacy seen with higher adherence rates [26, 30]. Overcoming some of the hurdles to usage seen with gels, films are another product being developed for the vaginal delivery of ARVs. Films alleviate issues with leakiness or messiness reported with gel use since they are a small volume, solid dosage form as opposed to a semisolid gel product [28]. Films do not require an applicator, which is needed for gel application, they are low cost to manufacture, discrete to use, easily portable and have been shown to be preferred over other vaginal dosage forms [28, 79, 96, 97]. In a clinical study which compared a film and a gel product containing DPV, both showed efficacy against

HIV in *ex vivo* testing, but the film had a smaller impact on innate anti-HIV activity in cervicovaginal fluid (CVF) [89]. Another clinical study compared a vaginal gel and a vaginal film product which both contained TFV. This study assessed the pharmacokinetics and pharmacodynamics after single administration of either the TFV vaginal gel or film and it was found that after film administration TFV levels in the cervicovaginal fluid and cervical tissue were higher than after gel dosing [215]. Both the TFV and DPV films were designed to be used in a coitally dependent fashion.

Dosing frequency has been shown to impact adherence with intermittent dosing leading to higher levels of adherence compared to daily dosing schedules [119]. This led the field to switch towards the development of longer acting products which did not require as frequent dosing. Capable of delivering APIs for periods of a month or longer, intravaginal rings (IVR) are a coitally-independent dosage form whose long duration of action makes them attractive alternatives to aid in overcoming adherence issues with daily and on-demand dosing [31]. Two studies, ASPIRE (MTN-020) and The Ring Study (IPM 027) showed an ARV containing ring could reduce HIV incidence 37% and 31% for these Phase 3 studies, respectively [31, 32]. Adherence to product use was again closely tied with efficacy, and in the ASPIRE (MTN-020) study, the efficacy of HIV protection was 56% with an adherence rate of greater than 80%, and the efficacy of HIV protection of -27% in those with an adherence rate of less than 80% [31]. Rings are still an attractive long-acting strategy for HIV prevention.

A one size fits all model will not work for every woman, due to many social and cultural differences. For this reason it is important that different product types be developed. It is especially important to use what we have learned from clinical trials and acceptability data to inform product design. Combining as many positive attributes into a single dosage form is a

strong strategy to increase user compliance and therefore efficacy of a microbicide product. This dissertation focuses on the modification of a well-accepted dosage form platform, the vaginal film, to transform it from a quick dissolve, fast release coitally-dependent product into a product capable of extended release.

To confirm production of an extended release vaginal film, we aimed to develop novel methods to evaluate film performance both *in vitro* and *in vivo*. An *in vitro*, quantitative disintegration method was developed using a quality by design (QbD) approach. Previous disintegration methods were non-specific to films and/or introduced user bias, so a need existed to develop a more robust and reproducible method. Further, a gap existed for an *in vivo* evaluation of film retention and drug release. The NHP model provided a useful surrogate for humans to study *in vivo* product functionality. The disintegration and visualization methods were used in the design and evaluation of extended release films in this dissertation.

The development of extended release films was divided into two main aims; (I) Establish the use of the vaginal polymeric film as an extended release dosage form for delivering potent ARVs through geometric manipulation, (II) Develop an extended release film through polymeric modifications to slow release rate from films. Aim I manipulated the manufacturing process to produce thicker films which contained a single ARV or a combination of ARVs, to determine if this resulted in slower erosion and increased film retention. Through a carefully constructed design of experiments, aim II developed an extended release film platform. Three polymers were selected for inclusion in the design of experiments (DOE) based on different strategies to extended film retention and drug release. Using high molecular weight polymers and polymers which contain functional groups that lead to mucoadhesive interactions, were two strategies employed to slow erosion of the dosage form and to increase mucoadhesive interactions for film

retention. Another polymer with limited solubility was used to further slow film erosion and drug release. Formulations generated from the DOE were evaluated for a range of physiochemical outputs which determined a lead formulation platform which was further evaluated in a NHP model for safety and pharmacokinetics. Taken together, these studies achieved the goal of demonstrating the feasibility of designing an extended release vaginal film product for prevention in women.

6.2 SUMMARY OF FINDINGS

In the pursuit of developing novel extended release vaginal films, methods had to be developed and validated to evaluate potential products. Polymeric thin films have predominantly been designed as quick dissolve delivery systems, and drug release is highly dependent on dosage form disintegration. However, methods to evaluate this disintegration in a quantitative, reproducible manner were not available. A novel disintegration testing method was developed using a Texture Analyzer instrument to fill this gap. This method used a force and amount of fluid which were both biologically relevant to the vagina, but which can be tailored for other films depending on indication or route of delivery. The disintegration method was applied to evaluate the two most clinically advanced antiretroviral containing films, the TFV and DPV films. This method was further able to distinguish changes in disintegration time when these films were altered based on formulation or excipient. Another method which was applied in this dissertation work was a novel visualization technique with drug quantification to study the retention and drug release of films in the NHP. This method used colposcopy to determine film disintegration, in

conjunction with drug content analysis of vaginal fluids to create a complete understanding of film product functionality *in vivo*. After methods had been developed to evaluate crucial film parameters, films could be developed. At first, current film manufacturing parameters were manipulated to create thicker films with increased polymer concentrations to slow film disintegration and therefore drug release. Films were cast at the maximum thickness for each formulation using solvent casting. Hydrophobic DPV and hydrophilic TFV model compounds were incorporated into previously established formulations, and evaluated using the developed disintegration and visualization techniques. These extended release TFV and DPV films had increased disintegration times and were retained in the NHP vagina for longer periods when compared to the original, coitally dependent thin films. Additionally, a combination product containing MK-2048 and DPV, compounds which target HIV at two different points of the lifecycle, was also developed for extended release. This film also had a longer disintegration time than previously developed thin films. These studies demonstrate feasibility that extended release can be achieved by increasing the thickness of the film and therefore overall polymer content.

An alternative approach to varying thickness, was to modify film polymeric constituents to decrease dosage form disintegration and slow drug release rate. The properties which govern drug release from solid, swellable, polymeric dosage forms are well understood and can therefore be manipulated to slow this rate. Using high molecular weight polymers with functional groups that favor mucoadhesive interactions, using polymers with hydrophobic functional groups and using insoluble polymers were three strategies employed in a single formulation to decrease dosage form erosion and drug release rate. The completed DOE produced a remarkable amount of data showing that small changes in formulation components

and ratios can impact final film attributes such as mass, thickness, viscosity, puncture strength, disintegration, water content, mucoadhesion, first order release rate, and surface topography. Data generated from the DOE provided further support that excipient type and ratio can be tailored to achieve a specific performance/functionality parameter (thickness, viscosity, disintegration, etc.).

As the performance goal for this project was to extend drug release, a lead film was selected based on disintegration and dissolution outputs. This lead candidate was tested in a NHP and found to be safe. The most impressive finding was the *in vivo* retention and drug release of the lead film formulation selected. *In vivo* data showed that films were retained in the vagina and drug levels of MK-2048 remained above its IC₅₀ in vaginal fluid for up to one month. These films, unlike films in aim I, preserved their thin film qualities; they were only approximately 100 µM, soft, and flexible. Combined, these data show that the polymeric, vaginal, thin film can be used to extend the delivery of a potent ARV for HIV prevention.

6.3 LIMITATIONS

While promising data were generated throughout this dissertation research, there were limitations to the performed work that should be recognized.

The NHP studies with the increased thickness films showed the feasibility of increasing film retention through the alternation of a physical film parameter. Drug release from films and drug presence in vaginal fluid was confirmed through the API quantification from swabs taken from the vagina of these animals. However, it should be noted that initial studies did not control for the weight of the secretions on the vaginal swabs so the concentration of the drug is in

weight/swab. If swab weight is controlled for it can provide a more accurate representation of drug release. This was an initial oversight in study design but can be controlled for in future studies. Further, due to the high cost and limited availability of macaques, a small sample size was used for all NHP studies. This can result in greater variability in findings and small changes may not be as evident as they would be in larger studies.

In aim II, formulation strategies which varied film content to include polymers employed to prolong film retention in the vagina and increase mucoadhesive interactions between the film and vaginal mucosa. The lead film generated from the DOE was found to be retained in the NHP for 24-30 days. In this DOE, in terms of inputs, formulations could have been made with the incorporation of various other polymers in aim II. Different grades of HPMC could have been used (even higher molecular weights), although the viscosity of the polymer melt may have increased to a point that casting a film may not have been feasible. Other film forming polymers such as PVA or mucoadhesive polymers such as sodium carboxymethylcellulose [216] could also have been included in a DOE. In the scope of this work, too many input polymers would have caused the DOE to grow exponentially and would not have been feasible to complete, but including only three input polymers was a shortcoming of the project. Other extended release polymers could have been used to provide an even more extended release profile.

Finally, the lead candidate film produced by the DOE when evaluated in the NHP, MK-2048 levels were measured in vaginal fluid, not in cervical or vaginal tissue. MK-2048, as an integrase inhibitor, has an intracellular site of action, so tissue sampling may have provided more supporting data for enhanced delivery of this compound. Everyday tissue sampling for each NHP was not feasible over the duration of this study due to the limited number of animals and the limited number of biopsies which can be collected from each animal. However, analysis from the

vaginally applied TFV gel showed that levels in the cervicovaginal fluid can be 1-3 logs higher than what is observed in vaginal tissues [217], thus showing that concentrations of MK-2048 at its site of action could be lower than anticipated.

6.4 SIGNIFICANCE TO THE FIELD

This dissertation work produced methods and significant data which will help the microbicide and film formulation fields continue to grow and advance. Two novel methods were developed as part of this work. The *in vitro* disintegration method developed can be used not only for vaginal films but for films intended for other routes of delivery such as oral or topical. The parameters of the disintegration method can be tailored based on route of administration and product indication. This method introduces less user bias and is specific to the film dosage form, as opposed to using methods designed for other dosage forms. The disintegration method allows for development of more specific and measurable product target specifications. The *in vivo* visualization method using the NHP is specific to vaginally delivered films, and is valuable in evaluating film functionality and performance. Further, this work demonstrated that traditionally applied *in vitro* dissolution methods were not predictive of *in vivo* behavior of film products. This observation led to the development of a more bio-relevant *in vitro* dissolution method.

Overall, the main contribution to the field provided by this work is the feasibility of developing extended release film platforms either through increased thickness or through formulation excipient selection. It was demonstrated that the *in vivo* retention of a coitally dependent film could be increased by simply increasing film thickness. However through component formulation changes, even greater increase in film retention and longer drug release

profiles could be obtained. We have modified a highly advantageous dosage form, the vaginal film, to provide extended drug release. Feasibility studies in the NHP with an extended release MK-2048 film achieved drug retention and release for up to a month. This time frame is equivalent to most IVRs. The work of this film lead to the development of a prototype film which is positioned for advancement to the clinic.

6.5 FUTURE DIRECTIONS

Data presented in these studies are encouraging, but to assess if a true extended release platform has been developed there are future studies to be completed with the film produced from the DOE in aim II. To test the versatility of the platform, other compounds will need to be incorporated into the lead film formulation. Hydrophobic, hydrophilic, small and large agents should be incorporated into and tested for the compatibility of the formulation to accommodate these agents. If these agents can be successful incorporated into the platform and also show favorable release profiles, it can be concluded that the platform is an extended release platform regardless of API. Full validation with the low volume *in vitro* release using the Distek basket method should also be completed.

Further studies should also be completed in the NHP model. These studies should address the impact of coitus on film platform retention and MK-2048 pharmacokinetics. Given that this product is intended to be utilized in the context of sexual intercourse it is imperative to develop a better understanding of its functionality under this condition to ensure product efficacy. Additionally, in these studies it was found that the film remained in the vaginal vault for a period of 21-34 days. This time frame may not be appropriate for acceptable or desired

windows for product use. Therefore, studies should be done to optimize film retention time and MK-2048 drug release profile to meet the needs of women's preferred dosing regimens.

After demonstrating acceptable safety and retention profiles in the NHP model through the suggested additional studies, clinical studies should be conducted. Clinical acceptability of this extended release platform should be evaluated to ensure that this film, if development is continued, would be acceptable to target populations. Feasibility studies assessing the safety and functionality of the film product should be conducted. Currently, there are clinical trials planned for the placebo and drug loaded film (extended release platform) of the lead formulation platform. The first trial is to be conducted in HIV-uninfected women aged 18 – 45 years old at Magee- Womens Hospital (Pittsburgh, PA USA) using the placebo film and will assess safety, vaginal microbiome, acceptability and film persistence. Barring any major safety or acceptability concerns, future studies are planned using a drug loaded MK-2048 extended release film.

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