

**ASSESSMENT OF THE PROGESTIN POLYMERIC VAGINAL FILMS BY AN
OPTIMIZED SOLVENT-CAST PLATFORM**

by

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Background: Although many birth control options are provided for women nowadays, the rate of unintended pregnancies remains high internationally and nationally. Novel contraceptive methods, such as contraceptive vaginal films, are needed to meet the users' biological, social and economic needs. An established solvent-cast platform can potentially be an option for vaginal administration of progestins for contraception (1). The goal of this work is to the feasibility and versatility of film platform for incorporating progestins. **Methods:** A panel of progestins, desogestrel (DES), etonogestrel (ENG), dienogest (DNG) and ulipristal acetate (UPA) were chosen based on their different physiochemical properties and availability. The progestins were incorporated into a fast-dissolved film formulation and the films were evaluated for appearance; weight; thickness; puncture strength; water content; disintegration time and drug content. Stability for each of these parameters was monitored for 3 months under two storage conditions (25°C/65%RH; 40°C/75%RH). Additionally, *in vitro* dissolution was investigated using a USP 1 method. **Results:** All four progestins could be successfully incorporated into the film formulation, despite their different lipophilicities. When comparing across progestin films for two dosing levels, differences were observed with respect to the physical characteristics of the progestin film. A decrease in drug content was observed for desogestrel (DES) films over 3 months. However, other progestin films remained stable throughout 3 months. Film physical properties were consistent throughout this time frame. The *in vitro* dissolution study showed that all four progestins released 80% of the drug within 15 minutes. **Conclusion:** Results demonstrated that the progestins could be formulated as

polymeric vaginal films, despite their different physiochemical properties. For ENG, DNG and UPA films, the physical characterizations of the progestin films remained stable for at least 3 months. However, the amount of progestin located into the film impacted the puncture strength and water content of the films. This project demonstrated the capacity of the film to be applied for vaginal delivery of progestins.

Keywords: progestin; polymeric vaginal films; formulation modification; physical characterizations

TABLE OF CONTENTS

PREFACE.....	XI
1.0 INTRODUCTION.....	1
1.1 CONTRACEPTON	1
1.1.1 The importance of birth control	1
1.1.2 Mechanisms of contraceptive agents: menstrual cycles, ovulation and hormones	2
1.1.3 Estrogen and Progestin: Benefits and Risks.....	4
1.2 DOSAGE FORMS FOR HORMONAL CONTRCEPTION	7
1.2.1 Transdermal patch (Ortho Evra® and Xulane®)	7
1.2.2 Birth control implant (Nexplanon®/ Norplant)	9
1.2.3 Vaginal Ring (NuvaRing®)	11
1.2.4 Intrauterine device (IUD).....	12
1.3 POLYMERIC VAGINAL FILMS	16
1.3.1 N-9 vaginal films (Non-hormone contraception films)	16
1.3.2 The polymeric vaginal film: advantages and disadvantages	17
1.3.3 The progestin vaginal films	18
2.0 MATERIALS AND METHODS	21
2.1 MATERIALS	21
2.2 METHODS.....	21
2.2.1 Film formulation	21
2.2.2 Film characterizations	23

2.2.2.1	Weight and thickness.....	23
2.2.2.2	Water content.....	23
2.2.2.3	Mechanical properties	23
2.2.2.4	Drug content.....	24
2.2.2.5	Target product specifications	24
2.2.3	Short-term stability study	25
2.2.4	Dissolution study	25
2.2.5	Statistical analysis	26
3.0	RESULTS	27
3.1	THE FILM PLATFORM CAN PRODUCE CLINICAL DOSE PROGESTIN FILMS	27
3.2	THE FILM PLATFORM CAN PRODUCE EQUAL DOSE PROGESTIN FILMS	29
3.3	PROGESTIN FILMS OF TWO DOSING LEVELS HAVE DIFFERENT PHYSICAL CHARACTERIZATIONS	31
3.4	THE CLINICAL DOSE PROGESTIN FILMS REMAIN STABLE FOR AT LEAST THREE MONTHS	35
3.5	PROGESTINS DO NOT AFFECT THE RELEASE PROFILE OF THE POLYMERIC VAGINAL FILMS.....	42
4.0	DISCUSSION	44
5.0	LIMITATION	51
6.0	NOVELTY AND SIGNIFICANCE.....	52
7.0	FUTURE PLANS	53

APPENDIX A	56
BIBLIOGRAPHY	57

LIST OF TABLES

Table 1.1.3.1. Classification of Progestins.....	5
Table 1.2.4.1 Summarized table of all hormonal IUDs on the U.S. market.	14
Table 1.3.3.1. Summarized table of four progestins used in this work.	19
Table 2.2.1.1. Formulation for Progestin Films.	22
Table 2.2.2.5.1 Summarized table of specifications for vaginal films.	25
Table 2.2.4.1. Summary of the Dissolution Study for Contraceptive Films.	26
Table 3.1.1. Summary of progestin used in clinical dose polymeric films	28
Table 3.1.2. Summary of the clinical dose progestin films' physical characterizations.	28
Table 3.2.1. Summary of the progestins used in equal dose polymeric films.	29
Table 3.2.2. Summary of the equal dose progestin films` physical characterizations.	30

LIST OF FIGURES

Figure 3.3.1 Physical characterizations of DNG films at two dosing levels.....	32
Figure 3.3.2 Physical characterizations of ENG films at two dosing levels.	33
Figure 3.3.3 Physical characterizations of UPA films at two dosing levels.	34
Figure 3.4.1 Weights (mg) of four progestin films stored under two conditions in 3 months.	36
Figure 3.4.2 Thickness (mm) of four progestin films stored under two conditions in 3 months.	37
Figure 3.4.3 Puncture strength (kg/mm) of four progestin films stored under two conditions in 3 months.....	38
Figure 3.4.4 Water Content (%) of four progestin films stored under two conditions in 3 months.....	39
Figure 3.4.5 Disintegration time (sec) of four progestin films stored under two conditions in 3 months.....	40
Figure 3.4.6 Drug content (w/w) of four progestin films stored under two conditions in 3 months.....	41
Figure 3.5.1 Dissolution curves of four progestin films in 6 hours.....	42

PREFACE

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1.0 INTRODUCTION

1.1 CONTRACEPTION

1.1.1 The importance of birth control

Reducing unintended pregnancy is one of the most important reproductive health goals identified by the United States Departments of Health and Human Services (2). Women who have an unintended pregnancy are at high risk of having adverse social, economic, and health outcomes for both the mother and the child (3, 4). The most common behaviors associated with unintended pregnancy include delayed or no prenatal care, smoking during pregnancy, and no breastfeeding. These behaviors subsequently cause low birth weight, preterm birth, miscarriage, infant death, or illness during childhood (5).

Besides the adverse outcomes for the mother and the child, unintended pregnancy also consumes a significant amount of federal dollars. One study (6) estimated the annual cost to taxpayers of unintended pregnancy and birth in the United States. This study indicated that for every dollar spent on voluntary family planning services to prevent unintended pregnancy, about \$4 are saved in short-term costs to the government for the medical care for the pregnancy and for

1 year of infant care after birth. Another report (7) indicated that every public dollar invested to avoid unintended pregnancies saves \$5.68 in Medicaid expenditures that otherwise would have gone to pregnancy-related care; in 2010, that amounted to a net government savings of \$10.5 billion. Overall, birth control can not only improve the health outcomes of the newborns but also decrease its financial burden to society.

However, family planning has remained as one of the most critical public health topics over the last century. According to a recent paper (2), which analyzed the incidence of unintended pregnancy through 2006, nearly half (49%) of the pregnancies in 2006 were unintended, slightly up from 48% in 2001. Another study (5) analyzed the unintended births in the U.S. from 1982 to 2010. The authors reported that about 37% of births (only accounted for the number of live births) were unintended at the time of conception. The overall proportion of unintended births has not declined significantly since 1982. Moreover, the number is likely underestimated (8). Since 2001, the U.S. has not made progress on the issue of controlling unintended pregnancies, with the overall rate remaining high. Thus, it is essential to find optimal contraceptive options for women to minimize the unintended pregnancy rate.

1.1.2 Mechanisms of contraceptive agents: menstrual cycles, ovulation and hormones

Among all the FDA-approved, reversible birth control options, all methods with low failure rate (<10 pregnancies expected per 100 women) contain hormones. Necessary background information on hormones will be provided in this section.

The menstrual cycle has four distinct phases: menstruation, the follicular phase, ovulation, and the luteal phase. Even though menstruation is generally considered as the first phase of the cycle, the follicular phase is the beginning of egg formation for fertilization. During this phase, the

pituitary gland releases follicle-stimulating hormone (FSH), causing 10 to 20 follicles to develop in the ovary. Usually, only one dominant follicle will develop into a mature egg when an appropriately high FSH level threshold is reached, while others die of atresia. The mature egg moves to the surface of the ovary, while other follicles will break down and be reabsorbed by the ovary. During this phase, the ovary produces oestrogen (estrogen), which causes the endometrium to thicken. Because of the increase in estrogen, the hypothalamus releases gonadotropin-release hormone (GnRH). The high-pulse release of GnRH, in turn, causes the pituitary gland to produce increased levels of luteinizing hormone (LH). This abrupt increase of LH triggers ovulation, which marks the end of the follicular phase. During ovulation, the mature egg enters the fallopian tube and moves towards the uterus. The lifespan of a mature egg is between 6 to 24 hours. If the egg is not fertilized, it will break down within 24 hours.

During the luteal phase, the remnant of the follicle which released the egg will release large amounts of progesterone and some oestrogen. These hormones further cause thickening of the uterus. If fertilization does not occur, progesterone level decreases and the corpus luteum breaks down. The shredded uterine lining flushes out of the vagina as a mixture of blood, endometrial cells, cervical mucus, and vaginal secretions. This phase is called menstruation.

Fertilization requires mature eggs and sperms. Even though the mature egg disintegrates shortly after ovulation, the sperm can remain active for a much longer time. Sperm can remain motile in cervical mucus for seven or more days after insemination (9, 10). Further, sperm retains the ability to fertilize human ova *in vitro* after five days at room temperature (11). Thus, a critical step for contraception is to control ovulation.

The rapid drop in estrogen-to-progesterone ratio suggests the luteinization of the ovarian follicle. Moreover, the LH peak correlates with the day of ovulation (12). This evidence

demonstrates how hormones control the menstrual cycle, including ovulation. On one hand, the increased amounts of estrogen will inhibit pituitary production and secretion of FSH and LH, particularly during the mid-cycle surge of those hormones. The decreased levels of FSH and LH further lead to the inhibition of the follicular development, ovulation, and corpus luteum formation (13). On the other hand, increased level of progesterone results in inhibition of ovulation, and changes in cervical mucus and the endometrium (14). Finally, increased amounts of estrogen and progesterone can inhibit the fertilization. These biological effects demonstrate the contraceptive uses of estrogen and progesterone.

1.1.3 Estrogen and Progestin: Benefits and Risks

Estrogens impact various reproductive tissues, including the uterus and breast. For example, they cause vaginal cornification and uterine growth. They are also highly involved in hormone-responsive cancers, resulting in cell replication in breast or endometrial tumors (15). The initial action of estrogen is considered to be the same in each targeted tissue. Estrogen first binds to the nuclear estrogen receptor (ER). Following which an estrogen ligand causes conformational changes, resulting in interaction with either DNA sequence directly, or the promoter region of estrogen-responsive genes. Although there are two ERs, ER α and ER β . They both belong to the steroid/thyroid hormone superfamily of receptors and share a common structural architecture. Therefore, the majority of estrogens bind to ER α and ER β with similar affinities or potencies.

Estrogens are proposed to be classified into two categories based on the structure of their responsive receptor complexes: the diethylstilbestrol type (Class 1) and the triphenylethylene type (Class 2) (15). The Class 1 estrogens are planar. They bind to an activated activating function 2 (AF2) and produce an ER complex to initiate estrogen action. The Class 2 estrogens are angular.

These estrogens bind to an activated AF2b site. However, despite the structural differences, they act as contraceptive agents through the same endogenous effect, which is the inhibition of ovulation by preventing the production of FSH and LH (14).

Progesterone is a natural hormone found in the human body, but not commonly used for therapy. The most commonly used synthetic hormones, which have properties similar to the progesterone, are progestins. The reason for their use is that compared to progesterone, the synthetic progestins have a more extended half-life and stable plasma levels for long-term use (16). One widely used purpose of progestin in the clinic is contraception. Similar to progesterone, progestin can inhibit the ovulation. Moreover, increased progestin levels can affect the cervix by decreasing the quantity and changing the quality of cervical mucus leading to interference with the sperm (17). Besides progesterone, there are different classes of progestins, such as retroprogesterone, progesterone derivatives, 17 α -hydroxyprogesterone derivatives, 19-norprogesterone derivatives, 19-nortestosterone derivatives, spiro lactone derivatives. A review of these classes is shown in Table 1.1.3.1. Examples were obtained from the literature (18).

Table 1.1.3.1. Classification of Progestins.
Examples are taken from reference (18).

Progestin Class	Example
Progesterone	Natural progesterone
Retroprogesterone	Dydrogesterone
Progesterone derivative	Medrogestone
17 α -Hydroxyprogesterone derivatives (pregnanes)	Medroxyprogesterone acetate, megestrol acetate
17 α -Hydroxyprogesterone derivatives (norpregnanes)	Gestonorone caproate, norgestrel acetate
19-Norprogesterone derivatives (norpregnanes)	Demegestone, promegestone, nesterone
19-Nortestosterone derivatives (estranges)	Norethisterone, norethisterone acetate
19-Nortestosterone derivatives (gonanes)	Norgestrel, levonorgestrel, desogestrel, etonogestrel, dienogest
Spirolactone derivative	Drospirenone

The pharmacodynamics properties of progestins depend on not only their chemical structures but also the route of administration. For example, several studies (19, 20) demonstrated that after oral administration, synthetic progestins are rapidly absorbed, and maximum serum concentration is reached within 2-5 hours, which is faster than that observed with parenteral, vaginal, intramuscular, or transdermal administration. Many the synthetic progestins are metabolized in the liver and excreted via the urine. As for the biological activities of progestins, they are dependent on the binding affinities and binding efficiencies to the progesterone-receptors. There are different forms of progesterone receptors (PR), PR-A and PR-B. The two receptors are encoded by a single gene in human (18). Because of the control of distinct promoters, the difference in the two receptor types lies in the amino-acid sequence differences between the A-form and the B-form, with molecular masses of approximately 81kDa and 115kDa respectively (21). PR-B can mediate the intermediate agonistic effects in several organs, while PR-A can act as a dominant repressor of PR-B with extensive responses to diminish other hormone (22). The progestin-receptor (P-PR) effects are tissue specific. PR presence has been demonstrated in multiple areas of the central nervous system as well as the gastrointestinal system (23, 24). Literature also indicates that progestins have the neuroprotective role in models of traumatic brain injury (25, 26).

Either estrogen and progestin alone can prevent ovulation. The combined use of both estrogen and progestin is commonly implemented in a number of birth control products given that this combination can promote antigonadotropic and ovulation-inhibitory effects (14). For example, combined oral contraceptives can suppress the secretion of FSH and LH, lowering the systemically circulating concentrations of both FSH and LH levels within the first day of administration (27). However, continuous use of the combined oral contraceptives is required for inhibition of

ovulation. More importantly, in some cases, where a portion of women who cannot use estrogen, the single use of progestin is recommended. The majority women requiring progestin-only contraception have the potentials to develop deep vein thrombosis or estrogen-related cancers. Another reason may be breastfeeding since estrogen-containing products should not be used during this time. As a result, the progestin-only products have also been developed.

The progestins, as the major active pharmaceutical ingredients (API) for most contraceptive methods, have been formulated in various drug delivery systems (DDS). According to a report by the United Nations (UN), *Trends in Contraceptive Use Worldwide 2015*, female fertilization (19%) and intra-uterus devices (IUD) (14%) are two common methods used by married or in-union women worldwide. Long-acting or permanent contraceptive methods are especially popular in Asia and North America, including sterilization, IUD, and implants. No single birth control option can meet the need for everyone, therefore short-term contraceptive methods have also been developed and are acceptable to some women. Reversible contraceptive methods, including transdermal patches and vaginal rings, are also worth mentioning, as they produce another acceptable product option to women.

1.2 DOSAGE FORMS FOR HORMONAL CONTRACEPTION

1.2.1 Transdermal patch (Ortho Evra® and Xulane®)

The transdermal contraceptive patch is a thin plastic film that is adhered to the skin of a user. Just like oral contraceptive pills, the patch contains hormones, usually a combination of estrogen and progestin. Therefore, the contraceptive effects come from both estrogen and progestin. The

contraceptive patch needs to be changed weekly. After use for three (3) weeks continuously, the woman should leave the patch off for one week before starting a new dose. The failure rate of patches is 9% for typical uses and 0.3% for perfect use (28). The birth control patch is not only effective and convenient for contraception, but it also has other health benefits, such as relieving the menstrual cramps and alleviating bleeding.

Transdermal drug delivery systems can avoid the difficulties in absorption and first pass metabolism in the gastrointestinal tract (29). The patch also has been shown to result in good patient compliance. In two clinical trials involving healthy adult women of child-bearing potential in the United States and Canada (30, 31), compliance was higher with the patch than with oral contraceptives. However, additional studies are needed to determine whether the patch can offer significant efficacy or safety advantages over other contraceptive methods. On the other hand, the patch is not as effective in overweight women (weighing more than 90 kg) as in women with lower body weight (32). The patch also has some common adverse reactions caused by hormones, such as breast symptoms, headache, application-site reactions, and dysmenorrhea (33). But it should be noted that these side effects would accompany any estrogen containing product.

Ortho Evra® is the first transdermal patch approved for prevention of pregnancy(33) with a contact surface area of 20 cm². It contains 6mg of norelgestromin (NGMN) and 0.6 mg of ethinyl estradiol (EE) and releases 150 µg/d and 20 µg/d, respectively (29). Ortho Evra® is no longer available in the U.S. but still available in Europe and Canada. Currently, the only available contraceptive patch in the U.S. is Xulane®, a generic Ortho Evra® Patch marked by Mylan Pharmaceuticals. Xulane® is a 14 cm² patch containing the same hormones as Ortho Evra® with different dosage (4.86 mg NGMN and 0.53 mg EE). It will release approximately 150 mcg/d NGMN and 35 mcg/d EE. Pooled data from the clinical studies demonstrated that the steady state

of serum drug concentration is reached within two weeks of application. Serum concentrations of both APIs dropped slightly after patch replacement but recovered within 12 hours. Following removal of the patch, the contraceptive effects can be discontinued quickly (half-life for NGMN and EE are 28 hours and 17 hours, respectively). Since the patch will be attached to the skin, specific environmental changes may impact drug release. For example, increased EE exposures were observed associated with higher body temperature. Moreover, due to the estrogen component, Xulane®'s effects include increased cardiovascular risks or blood clotting among certain users.

There are other combined estrogen and progestins patches on the market, but not for contraception. CombiPatch® is a prescription medicine used to treat severe hot flashes associated with menopause containing estradiol and norethindrone acetate. Another similar hormone patch is Climara Pro®, which contains estradiol and levonorgestrel. It is also used to treat menopause symptoms like hot flashes and to prevent osteoporosis (bone loss).

1.2.2 Birth control implant (Nexplanon®/ Norplant)

Birth-control implants are long-acting reversible contraceptive (LARC) forms with normally four years of contraceptive effect. The implants are usually small rods (for example, Nexplanon®: 4 cm in length with a diameter of 2 mm) which are inserted under the skin of a women's upper arm. The implant is unnoticeable, providing women a discreet contraceptive option. At the product's expiration date, the old implant must be removed, and a new one is re-inserted if the woman wishes to continue contraception. Women are no longer under the protection of implants as soon as they are removed, and pregnancy may occur shortly after removal if no other form of contraceptive protection is used.

The mechanisms of contraceptive implants are similar because they all contain progestin solely as the active pharmaceutical ingredient (API). Ovulation suppression is the primary contraceptive mechanism. This can be full or partial suppression because of different progestins used. The evidence of ovulation is found in about 10% of cycles of a woman using implants in the first year (14). Another contraceptive mechanism of progestin is thickening of the cervical mucus. This inhibits sperm penetration, leading to prevention of fertilization (34).

Nexplanon® is the only implant that is available in the U.S. It can prevent pregnancy for three years with the efficacy higher than 99%. It contains 68 mg of the synthetic progestin etonogestrel (ENG). Once inserted, it will release ENG from 60-70 mcg/day (in week 5-6) to 25-30 mcg/day (at the end of the third year). Norplant was the first contraceptive insert approved by FDA, but is not currently available in the U.S. Norplant contains levonorgestrel (LNG) with the similar progestin level to that of the progestin-only oral contraceptives (75 mg of LNG). The contraceptive effects of Norplant are maintained for five years. In the first year, ovulation happens about 10% of cycles of a woman using Norplant. The LNG blood levels decline, and the ovulation occurs in 30% to 75% of cycles by the fifth year (35). Implants are the most effective forms of contraception with a one-year failure rate of around 0.05% (36). The reason is that, in the late stage, the implant remains effective through the progestin effects other than ovulation inhibition, probably through the inadequate development of a secretory endometrium (37). The major downside of the implant is that both insertion and removal need to be performed by professional healthcare providers.

1.2.3 Vaginal Ring (NuvaRing®)

Intravaginal rings (IVR) are small flexible polymeric ring-shaped platforms which provide controlled release of drugs. IVRs provide protective therapeutic effects for one month or longer. There are several IVRs currently marketed, including: Estring® (containing estradiol), Femring® (containing estradiol-acetate), Progering® (containing progesterone) and NuvaRing® (containing progestin and estradiol). However, Estring® and Femring® are mainly used for the treatment of vaginal atrophy. Progering® is only available in Chile and Peru. That makes NuvaRing® is the only contraceptive intravaginal ring available in the U.S.

NuvaRing® is a non-biodegradable, flexible, transparent vaginal ring with an outer diameter of 54 mm and a cross-sectional diameter of 4 mm. It contains 11.7 mg etonogestrel (ENG) and 2.7 mg ethinyl estradiol (EE). After insertion, the ring will release 0.120 mg/d of ENG and 0.015 mg/d of EE on average. Like other combined hormonal contraceptive methods, NuvaRing® stops ovulation, as well as thickens the cervical mucus.

For regular use, the ring stays inside vagina continuously for three weeks. After a three-week period of use, it is removed for a 1-week break. During the break, the user will experience a menstrual period. The user should insert and remove the ring on the same day of the week and the same time. The failure of removing the ring for more than four weeks may result in no protection from pregnancy.

Obviously, controlled release of hormones is one of the ring's strengths. It provides a steady serum concentration of both estrogen and progestin. Also, the ring can be left in the vagina during intercourse. Most couples report no interference or discomfort, with neither partner feeling the presence of the ring in many cases. The ring can be removed before/ during intercourse if that is preferred. However, the discontinued time should be within 3 hours to maintain the efficacy of

birth control. The handling of NuvaRing® is its downside. It requires proper disposal. Even though the exact location within the vagina is not critical for clinical efficacy, the presence of the ring should be maintained for contraception. Thus, the user should check ring placement before and after intercourse. To maximize the ring's contraceptive effects, on-time insertion, and removal are both crucial. Because of the 3-week-on, 1-week-off pattern, users might need reminders to stay on schedule, which is not suitable for those who have a hectic schedule and cannot stay on top of using NuvaRing® correctly.

Similar to other combined hormone contraceptive options, NuvaRing® has some mild to serious side effects. Specific groups (women who smoke cigarettes and are over 35 years old) should consider the potential cardiovascular side effects due to estrogen in the ring. Other common adverse side effects include irregular bleeding, nausea or headaches, which usually subside after 2 or 3 months.

1.2.4 Intrauterine device (IUD)

An intrauterine device (IUD) is a small T-shape plastic/ metal device inserted into the uterus to provide birth control effects. As one of the long-acting reversible contraceptive (LARC) methods, IUDs result in great satisfaction among users (38). There are two types of IUDs available on the market: Non-hormonal copper IUDs; and IUDs with progestins.

ParaGard® is the only non-hormonal copper IUD available in the U.S. With correct use. It can protect users from pregnancy for up to 12 years with a one-year failure rate around 0.7% (39). The failure rates will increase over 12 years because of the decrease in copper, but the cumulative 12-year failure rate is still low (2.2%) (40). It can also be used for emergency contraception up to 5 days post sexual intercourse with the failure rate of 0.09% (41). Copper IUDs work primarily by

disrupting the mobility and morphology of sperms. Increasing the level of copper ions within the uterus acts as a spermicide (42). Several studies(43, 44) showed that the sterile foreign body reaction in the uterine cavity causes biochemistry and cellular changes which can influence the number of sperm reaching the uterine cavity and fallopian tubes. The changes can also damage the sperm itself, causing head-tail separation, which makes the sperm unable to fertilize (14).

There are four brands of hormonal IUDs: Mirena®, Liletta®, Kyleena®, and Skyla®. The dosage and approval length for each hormonal IUD are summarized in Table 1.2.4.1. As shown in the table, all four IUDs contain the same progestin, levonorgestrel, but may differ in progestin loading level and release profile.

Mirena® is a levonorgestrel (LNG)-releasing IUD with a failure rate less than 1%. It can prevent pregnancy for up to 5 years or even longer (45). The IUD requires insertion by a healthcare provider. After five years, Mirena® must be removed and replaced with a new IUD if continued contraceptive protection is desired. The most common adverse reactions are alterations of menstrual bleeding patterns, abdominal/ pelvic pain, amenorrhea, and headache/ migraine. The most important safety concern is that it may cause pelvic inflammatory disease (PID). Even though only 1% of the users get this serious pelvic infection, women should not use Mirena® if they have a history of pelvic infection or are at increased risk of infection. Since PID is often associated with a sexually transmitted infection (STI), which IUDs cannot prevent from, full health background screening should be performed before IUD insertion. The health care providers should also rule out other risk factors, like leukemia or acquired immune deficiency syndrome (AIDS).

Liletta® is a pharmacologic equivalent to Mirena® with the same total amount of hormone. It releases slightly less hormone per day and has other differences, so it is not a generic to Mirena®. Liletta® serves as a cheaper substitute compared to Mirena®. Kyleena® is a newly approved IUD

manufactured by Bayer®. It is approved for the same length as Mirena® but releases fewer hormones. This device is a better option for women who would want to keep regular menstrual periods. Skyla® also releases fewer hormones than Mirena®, meaning more women will keep getting their periods, which is the same purpose as Kyleena®. Skyla® is smaller than other IUDs, making it more suitable for women who have not had their cervix dilated. However, Skyla® is only approved for three years, which is the shortest therapeutic period among all the IUDs.

Table 1.2.4.1 Summarized table of all hormonal IUDs on the U.S. market.

* Additional studies show that the devices are effective even longer than originally approved for: 7 years for Mirena®(45), and five years for Liletta®(46).

Name	Hormone	Manufacturer	Dose	Approved Length
Mirena®	Levonorgestrel	Bayer®	20 mcg/d (52 mg total)	5 years (7 years*)
Liletta®	Levonorgestrel	Allergan® and Medicines360®	18.6 mcg/d (52 mg total)	3 years (5 years*)
Kyleena®	Levonorgestrel	Bayer®	17.5 mcg/d (19.5 mg total)	5 years
Skyla®	Levonorgestrel	Bayer®	14 mcg/d (13.5 mg total)	3 years

For all IUDs, both insertion and removal require professional health care provider participation. Women may experience cramping or pain during the IUD insertion/ removal process and immediately after (47). Other common side effects include irregular bleeding and spotting and increased chance of infection. All IUDs have risks of expulsion (the IUD is expelled from the uterus) and perforation (the IUD moves through the wall of the uterus). Copper IUD (ParaGard®) use may result in increased blood flow during menstrual periods and worse menstrual cramps. If women are opposed to the heavy menstrual bleeding, they can switch to a hormonal IUD. In fact, Mirena® is also FDA-approved for the treatment of heavy menstrual bleeding or menorrhagia.

There used to be controversial theories on whether IUDs are abortifacient or not. Some experts in human reproduction believed that IUDs with copper could disrupt implantation (48). There has been no definitive evidence showing that the IUD users have higher embryonic loss rates (42). Nowadays, the majority of people believe that the IUD works on the early stage of human reproduction as noted by the WHO scientific group, “They (IUDs)...interfere with steps in the reproductive process that take place before the ova reaches the uterine cavity”(43). The non-abortifacient mechanism was also confirmed by American College of Obstetricians and Gynecologists (ACOG) in 1987 (14). The conclusion is useful for the users of specific religions as it may eliminate the fear of using IUDs.

Since the development of combined estrogen and progestin oral contraceptive over 50 years ago, almost all innovations of contraceptive methods have revolved around progestins. Novel progestins have been designed to decrease side effects, increase potency and half-life, and to manipulate agonist/ antagonist profiles to achieve optimal contraceptive effects (17). Also, modern delivery systems have achieved significant advances, specifically for progestins. Non-oral delivery systems (patch, ring, implant and IUD) will continue to be developed for progestins. These delivery systems can provide steady, continuous, safe administration and achieve higher user compliance. Polymeric vaginal films, a novel topical drug delivery system, may provide as a new birth control option for women.

1.3 POLYMERIC VAGINAL FILMS

1.3.1 N-9 vaginal films (Non-hormone contraception films)

The use of the polymeric film as a drug delivery system has attracted considerable attention in recent years. Many orally-dissolving products have been developed, such as Listerine PocketPaks®, TheraFlu® ThinStrips®, and Sudafed PE Quick Dissolve Strip®. Other than oral delivery, vaginal delivery routes have also been studied for polymeric films. The Vaginal Contraceptive Film® (VCF®) is the only FDA-approved vaginal film used for contraception. VCF® is a two-inch square soft soluble film that is manually inserted into the vagina at least 15 minutes before intercourse. VCF® contains Nonoxynol-9 (N-9), an FDA-approved organic compound that can be used as a spermicide. N-9 can immobilize sperm and is widely used in three vaginal contraceptive forms: suppositories, gels, and films.

VCF® is small and can be discreetly used. It is quickly dissolved by body fluids in the vagina, so it will not be noticed by most women and their partners during sex. VCF® washes away with the natural body fluids, so the users do not need to worry about removal or disposal. Use of this product does not require a prescription, and it is even available online. The easy-to-get, use-on-site, and unnoticeable nature of VCF® makes it acceptable among some women. However, as a spermicide, VCF® has a relatively higher failure rate, compared to other birth control methods. A clinical study indicated that five vaginal contraceptives containing N-9 as spermicide have typical-use failure rates ranging from 10% to 20% (49). The N-9 VCF® has also been associated with increased risk of STIs. One WHO report indicated that N-9 does not prevent HIV infection and may even favor infection if used frequently. The sole use of VCF® will not prevent pregnancy efficiently. Therefore, other birth control options are strongly recommended.

1.3.2 The polymeric vaginal film: advantages and disadvantages

The polymeric film delivery system is a thin and flexible strip of polymer which incorporate the API within. The API may be either dissolved or suspended in the polymeric film. It is a solid pharmaceutical product, and therefore APIs which are susceptible to hydrolysis can be stabilized using this dosage form. Low water content provides films with no need to incorporate preservatives for longer shelf-life. Additionally, polymeric films can be administrated with precise dose, easily transported and conveniently stored (5). As mentioned above, the use of oral-releasing polymeric films has been well-established. As a drug delivery site, the vagina presents several advantages, such as large surface area, abundant blood supply, no first-pass metabolism, and relatively high permeability to several drugs (50). This biological advantage allows some APIs to be quickly absorbed through the vagina into the blood. The APIs are then distributed systemically. Thus, the polymeric vaginal film is an ideal dosage form for both topical and systemic drug delivery. Moreover, compared to other vaginal DDSs, like vaginal gels and foams, the polymeric vaginal film causes less messiness and leakage (51, 52). Unlike vaginal gels or vaginal creams, vaginal films do not require applicators for product insertion. The films can be individually wrapped in flat, sealed packages. The flexibility makes films more resistible to physical forces, compared to tablets. Their portability provides advantage over gels, creams, and foams. Additionally, several published studies and products prove that the polymeric vaginal film has good acceptability (5). On the other hand, culture and religion differences, personal hygiene issues, and insertion difficulties should all be considered when using the polymeric vaginal film (53).

1.3.3 The progestin vaginal films

The polymeric vaginal film, as a drug delivery system, has many advantages as mentioned above. It has potential for local and systemic drug delivery through the vagina. The literature reported that polymeric films could successfully incorporate a range of compounds, including peptides and virus-like particles (VLP) for prevention of HIV (5, 52, 54-56). In the hope of achieving dual prevention products, our laboratory previously combined levonorgestrel (LNG) and dapivirine (DPV) into one film (57). The combined effects of contraception and HIV-prevention can be further developed as multi-purpose prevention therapy (MPT). To better understand the feasibility and versatility of this film platform, progestins other than LNG need to be tested. Since there are various classes of progestins on the market, the choice of progestins used for this work is critical. Lipophilicity was the main physicochemical characteristic considered. Given the solvent-casting manufacturing method used for films typically utilizes an aqueous-based polymer solution, the lipophilicity of progestins may affect the dispersion. A panel of progestins with different lipophilicities was selected in this work. (Table 1.3.3.1) As mentioned above, ENG is used in Nexplanon®, a long-acting contraceptive implant. Nexplanon® contains 68 mg of the progestin and releases ENG 60-70 mcg/day. DES, sold under the brand names Cerazette® and Micertte®, is used in birth control pills for women. The daily-used tablets contain 0.15 mg of DES. DNG is also used in contraceptive pills and is sold under the brand name Natazia® and Qlaira®. The medication is available both alone and in combinations with estrogens. The progestin-only tablets contain 2 mg of DNG. UPA is a medication used for emergency contraception. It is sold under the brand name Ella®. As emergency birth control product, it should be used within 120 hours of sex. A vaginal ring, releasing 2.5 mg/d UPA, is currently under a Phase II clinical trial. The four progestins, chosen in this project, also span different progestin generations. (Table 1.3.3.1)

Table 1.3.3.1. Summarized table of four progestins used in this work.

* Clinical daily doses of each progestin were calculated based on other contraceptive products.

Agent	Generation	LogP	Target Clinical Dose* (mg/1”X2” film)
Etonogestrel (ENG)	3 (LNG derivative)	4.2	0.06
Desogestrel (DES)	3 (LNG derivative)	6.6	0.15
Dienogest (DNG)	4 (estrane)	1.9	2
Ulipristal Acetate (UPA)	N/A (Selective PR modulators)	4.5	3

Other than APIs, film formulations usually include water-soluble polymers, plasticizers, fillers, colorants and flavoring agents (54). Water soluble polymers should be non-toxic; non-irritant; possess good wetting, spreadability, peelability; exhibit moderate mechanical properties; inexpensive to manufacture (58). Plasticizers are added to provide flexibility and pliability (54). Other excipients can be included for better patient’s compliance. Among all the excipients, the type of polymer and its molecular weight dramatically influences the properties of the film (50). Polyacrylates, polyethylene glycol, polyvinyl alcohol (PVA), and cellulose derivatives are the polymers commonly used in vaginal films (59). Our lab has already developed a solvent-cast (SC) polymeric vaginal film platform (1). This PVA-based formulation was originally used for vaginal delivery of dapivirine (DPV), a potent anti-retroviral molecule. This quick dissolve film provides rapid drug release, lacks toxicity to the innate microflora, maintains stability and retains bioactivity of APIs (52). Therefore, this SC platform was chosen as a platform incorporation of the progestins. Hence, the goal of this project was to investigate the feasibility of application of the SC platform for progestin drugs.

Technological characterization of developed films on various esthetic, chemical, physical, mechanical, and performance parameters was tested (1, 60). One paper (50) summarized all the

characterizations for vaginal films in one table. Appearance, color, and transparency are the main characteristics to be evaluated for films. Thickness and surface morphology are physical parameters that characterize vaginal films. Controlling of the water content of films is essential, as water content impacts mechanical properties of the film. In addition, high water content might contribute to microbial growth over time, low water content might affect disintegration (1, 61). Drug content, content uniformity, *in vitro* drug release and film pharmacokinetics are highly dependent on film chemical and physical properties (61). One behavioral clinical study (62) asked participants to compare a variety of vaginal films using a set of *in mano* perceptibility survey items. Women most frequently preferred vaginal films to be thin and smooth and translucent. The size of films mattered to some women, but the preferred size differed individually. In conclusion, physical properties, including thickness, weight, puncture strength, water content, disintegration time and drug content should be tested for controlling the product quality. These attributes can be defined prior to product development as Target Product Specifications.

Our goal is to demonstrate the capacity of this film platform for application to vaginal delivery of progestins. To achieve this goal, we propose that the platform can incorporate a range of progestins and generate polymeric vaginal films with desired physical properties.

2.0 MATERIALS AND METHODS

2.1 MATERIALS

Desogestrel (DES) was obtained from Tokyo Chemical Industry company (Japan). Etonogestrel (ENG), dienogest (DNG) and ulipristal acetate (UPA) were obtained from LGM Pharma (Erlanger, KY, US). Ethanol (HPLC grade), acetonitrile (ACN, HPLC grade), trifluoroacetic acid (TFA) were obtained from Fischer Scientific (Pittsburgh, PA, US). Polyvinyl alcohol (PVA 40-88), propylene glycol and glycerin were purchased from Spectrum (Gardena, CA, US). Creamophor (Kolliphor® RH 40) was purchased from Sigma-Aldrich (St. Louis, MO, US). Polyethylene glycol (PEG 8000) and hydroxypropyl methylcellulose (HPMC E5) were obtained from Dow Chemical Company (Midland, MI, US). All other chemicals were analytical grade. The filters (Millex®-LG low protein binding hydrophilic LCR(PTFE) membrane) were obtained from Sigma-Aldrich. Ultrapure water was obtained from an in-house Milli-Q® water purification system (Millipore Sigma Advantage A10).

2.2 METHODS

2.2.1 Film formulation

Formulation was adapted based on an established platform(54). Polyvinyl Alcohol (PVA) and Hydroxypropyl Methylcellulose (HPMC E5) were selected as film-forming polymers. Ethanol was

added as solubilizing/dispersing aid. Other excipients, polyethylene glycol (PEG) 8000, propylene glycol, and glycerin were also composed in the formulation as described in Table 2.2.1.1.

Table 2.2.1.1. Formulation for Progestin Films.

Ingredient	Role
Polyvinyl Alcohol (PVA 40-88)	Film Forming
Hydroxypropyl Methylcellulose (Methocel E5)	Film Forming
Polyethylene Glycol (PEG) 8000	Disintegration Agent
Propylene Glycol/ Glycerin	Plasticizer/Dispersing Aid
Drug	Effective Agent
Ethanol	Solubilizing/Dispersing Aid

The progestin film was prepared by the solvent-cast (SC) method. Briefly, PVA was dissolved in Milli-Q water, and to expedite dissolution, the mixture was placed in a hot water bath (90°C) until the PVA was completely hydrated. HPMC E5 was then slowly added into the PVA solution with moderate stirring using an overhead mixer. After the polymer solution became uniform and homogenous, the plasticizer was added. The mixture was allowed to stir overnight in order to remove any entrapped air bubbles. In the end, the progestin dissolved in ethanol was added. The mixture was stirred for no less than three hours to obtain a uniform polymer solution. The solution was then cast onto a polyester substrate attached to the hot surface of an automatic film applicator (Elcometer 4340 Automatic Film Applicator) using a 4-inch or 8-inch doctor blade. The solvent evaporated after 15 minutes, leaving a thin layer of film. The film sheet was removed from the polyester substrate and cut into 1”X2” rectangle pieces using a die press. The films were separately packed into aluminum pouches.

Two dosing levels for each progestin were manufactured, clinical dose films and equal dose films. Clinical daily doses were meant to reflect the doses which are available clinically. The clinical doses were calculated based on products which are on the market. Progestin films incorporated with clinical doses were named as clinical dose films; To better investigate whether the progestin impacts the film, a same dose was chosen for all progestins. The polymeric films incorporated with the equal dose were named as equal dose films.

2.2.2 Film characterizations

2.2.2.1 Weight and thickness

The film weight was measured by scientific balance (Mettler Toledo, XS205); The film thickness was measured at three different locations (two corners on one diagonal line and center) by thickness gauge (Mitutoyo, Absolute).

2.2.2.2 Water content

The water content was measured by Karl-Fischer Titer apparatus (Metrohm, 890 Titrando) in accordance with the titration method specified by the manufacturer.

2.2.2.3 Mechanical properties

Puncture strength and disintegration time of the film were tested using a Texture Analyzer (Texture Technologies, TA. XT Plus). Briefly, to test the puncture strength, the film was fixed on a film extensibility rig with only a flat round surface of the film was exposed. A probe was used at a perpendicular angle with increasing force. The analyzer recorded the force as puncture force when the film was penetrated. Puncture strength (kg/mm) is puncture force (kg) divided by the film

thickness (mm); For determination of the disintegration time, the film was fixed on the rig in the same way as mentioned above. 15µl Milli-Q water was first applied on the film, and then a probe was used at a perpendicular angle with a constant force. The apparatus measured the time it took for the probe penetrated the film, named as the disintegration time.

2.2.2.4 Drug content

The drug content was analyzed by High Performance Liquid Chromatography (HPLC) system. The film was fully dissolved in 4 ml of 50% ACN solution (in MilliQ water) via a vortex mixer. After sitting still for 4 hours or more, the upper clear solution was extracted. The solution was filtered and aliquoted for HPLC analysis. The HPLC system (Thermo Scientific, Dionex UltiMate 3000) equipped an auto injector, a quaternary pump, and a diode array detector was used. Chromeleon 7 Software was used for controlling the HPLC system, as well as calculating the drug content based on the total peak area. A C18 reverse phase column (Waters, xBridge C18, 5µm, 2.1X50mm) was used for separating the compound of interest at room temperature. The mobile phase consisted of (A) 0.1% TFA in MilliQ water and (B) 0.05% TFA in ACN using a gradient elution at a flow rate of 1.0 ml/min. The DES was determined by Ultra-Violet (UV) detection at 210 nm, the ENG at 242 nm, the DNG at 275 nm, the UPA at 275 nm.

2.2.2.5 Target product specifications

Physical properties, including thickness, weight, puncture strength, water content, disintegration time and drug content were selected as Target Product Specifications (Table 2.2.2.5.1). The specifications were used for stability study of the progestin film.

Table 2.2.2.5.1 Summarized table of specifications for vaginal films.

Test	Specification
Weight	80 -120% of the value at Time 0
Thickness	80 -120% of the value at Times 0
Puncture Strength	80 -120% of the value at Times 0
Water Content	Water Content < 10%
Disintegration	No more than (NMT) 250 seconds
Drug Content	80 -120% of the value at Times 0

2.2.3 Short-term stability study

After film manufacture, each film was separately packaged in an aluminum foil pouch. For each progestin group, films were randomly selected and stored under two conditions ([25°C/65% RH]; [40°C/75% RH]). The stability of the film was monitored for three months (Times 0; 1 week; 2 weeks; 1 month; 2 months; 3 months). At each time point, the physical characterizations and drug content were determined for all groups.

2.2.4 Dissolution study

To determine the kinetics of drug release, an *in vitro* release study was conducted using a USP 1 apparatus (Distek dissolution system 2100C). In this method, the film was placed in a 40-mesh standard basket, which was fully emerged in a certain amount of 1% Cremophor® solution (1g Cremophor® in 100 mL Milli-Q Water). The baskets were set to rotate at 100 rpm for 6 hours. 0.5 mL of the media was sampled at each time point (15 minutes; 30 minutes; 1 hour; 2 hours; 3 hours;

4 hours; 5 hours; and 6 hours), and 0.5 mL of the fresh 1% Cremophor® solution was replaced at each time point. The study design is shown in Table 2.2.4.1.

The drug concentration at each time point was tested using HPLC. The cumulative released drug amount was calculated. The theoretical drug content was estimated by the film weight and the average ratio of drug content/ film weight (w/w, %). The percentage of released drug was calculated by the accumulated released drug amount/ the theoretical drug content.

Table 2.2.4.1. Summary of the Dissolution Study for Contraceptive Films.

*Sink condition media volume is at least 3 times greater than the volume present in the saturated solution.

Dissolution Study for Contraceptive Films				
API Name	Log P	Solubility (ug/mL)	Clinical Dose (mg/Film)	Sink Condition Media Volume* (mL)
DNG	1.9	49.84	2	150
ENG	4.2	93.51	0.06	5
UPA	4.5	86.88	3	150
DES	6.6	716.75	0.15	5

2.2.5 Statistical analysis

For comparison of the progestin film at two dose levels, statistical analysis was performed using a two-tailed Student's t-test. A P-value < 0.05 was considered statistically significant.

3.0 RESULTS

3.1 THE FILM PLATFORM CAN PRODUCE CLINICAL DOSE PROGESTIN FILMS

These progestins have already been used in products which are on the market. To demonstrate that the film platform can incorporate the progestins, we would like to use the doses which are used clinically. These clinical doses were calculated based on the approved length of the product and the amount of progestin incorporated in products respectively, as shown in Table 3.1.1. The physical characterizations were tested for all four films. For each physical characterization, three films were randomly selected. All the physical characterizations and drug contents are shown in Table 3.1.2. The target dose (w/w) in Table 3.1.2 was the clinical dose (mg/film) divided by the average film weight (mg/film).

Despite of the different doses, all four progestin films can be manufactured with targeted dosing levels.

Table 3.1.1. Summary of progestin used in clinical dose polymeric films

Progestin	Log P	Clinical Dose (mg/film)
Etonorgestrol (ENG)	4.2	0.06
Desogestrel (DES)	6.6	0.15
Dienogest (DNG)	1.9	2
Ulipristal Acetate (UPA)	4.5	3

Table 3.1.2. Summary of the clinical dose progestin films' physical characterizations.

Data was presented as Mean \pm SD. N=3 for each group.

	DNG (XT-1-137)	ENG (XT-1-159)	UPA (XT-1-161)	DES (XT-1-139)
Log P	1.9	4.2	4.5	6.6
Weight (mg)	178.14 \pm 17.22	182.51 \pm 16.00	139.54 \pm 17.19	131.34 \pm 21.34
Thickness (mm)	0.13 \pm 0.01	0.13 \pm 0.02	0.11 \pm 0.01	0.11 \pm 0.02
Puncture Strength (Kg/mm)	14.25 \pm 2.06	15.47 \pm 1.39	13.62 \pm 0.47	14.62 \pm 1.40
Water Content (%)	4.46 \pm 0.32	2.19 \pm 0.13	1.55 \pm 0.06	4.01 \pm 0.12
Disintegration (sec)	151.76 \pm 21.16	244.70 \pm 28.33	123.96 \pm 5.36	80.88 \pm 43.18
Target Dose (w/w; μ g/mg Film)	12.30	0.40	18.40	0.90
Assayed Dose (w/w; μ g/mg Film)	14.00 \pm 0.30	0.50 \pm 0.00	23.80 \pm 1.50	1.10 \pm 0.00

3.2 THE FILM PLATFORM CAN PRODUCE EQUAL DOSE PROGESTIN FILMS

In order to further investigate the progestin's effects on physical properties of the film, this panel of progestins were incorporated in films at the same dose. These progestin films with the same dosing level were named as the equal dose progestin films. This dose was chosen based on the mid-range clinical dose, which is the clinical dose of DES. Table 3.2.1 shows the lipophilicity and dose of progestins. All the physical characterizations and drug contents are shown in Table 3.2.2. The target dose (w/w) in Table 3.2.2 was the equal dose (mg/film) divided by the average film weight (mg/film). For each physical characterization, three films were randomly selected.

Table 3.2.1. Summary of the progestins used in equal dose polymeric films.

Progestin	Log P	Equal Dose (mg/film)
Etonorgestrol (ENG)	4.2	0.15
Desogestrel (DES)	6.6	0.15
Dienogest (DNG)	1.9	0.15
Ulipristal Acetate (UPA)	4.5	0.15

Although the progestins have various lipophilicities, the equal dose progestin films can be produced. All four progestin films are at the same drug loading level. In addition, the equal dose films have similar physical characterizations—weights, thickness, water content, puncture strength, and disintegration time. The data demonstrate that the progestin film platform can produce equal dose progestin films with similar physical properties.

Table 3.2.2. Summary of the equal dose progestin films` physical characterizations.

Data was presented as Mean \pm SD. N=3 for each group.

	DNG (XT-1-67-2)	ENG (XT-1-83-2)	UPA (XT-1-82-2)	DES (XT-1-103)
Log P	1.9	4.2	4.5	6.6
Weight (mg)	164.34 \pm 12.76	152.67 \pm 10.72	162.47 \pm 14.70	174.14 \pm 12.96
Thickness (mm)	0.11 \pm 0.01	0.10 \pm 0.01	0.11 \pm 0.01	0.13 \pm 0.01
Puncture Strength (Kg/mm)	25.23 \pm 0.97	21.20 \pm 1.44	16.88 \pm 1.90	15.21 \pm 0.11
Water Content (%)	2.09 \pm 0.04	1.56 \pm 0.20	1.88 \pm 0.04	2.06 \pm 0.12
Disintegration (sec)	142.33 \pm 31.74	125.25 \pm 15.38	127.54 \pm 27.34	165.84 \pm 18.61
Target Dose (w/w; μ g/mg Film)	0.90	0.90	0.90	0.90
Assayed Dose (w/w; μ g/mg Film)	0.70 \pm 0.10	0.70 \pm 0.00	0.80 \pm 0.09	0.80 \pm 0.00

3.3 PROGESTIN FILMS OF TWO DOSING LEVELS HAVE DIFFERENT PHYSICAL CHARACTERIZATIONS

For DNG, ENG, and UPA, polymeric films were manufactured at two dosing levels. To study whether the amount of progestin impacts the physical characterizations of the films, we compared DNG films, ENG films, and UPA films across two dosing levels respectively. Physical characterizations of DNG films are shown in Figure 3.3.1. Physical characterizations of ENG films are shown in Figure 3.3.2. Physical characterizations of UPA films are shown in Figure 3.3.3.

Compared across the two dosing levels, significant differences were observed on puncture strength and water content for DNG, ENG and UPA films ($p < 0.05$). All three clinical dose films had lower puncture strength. For DNG and ENG films, clinical dose films had higher water content. The ENG clinical dose films had significantly longer disintegration time ($p < 0.05$), compared to the ENG equal dose films. Other physical characterizations, such as weight and thickness, remained the same for progestin films of two dosing levels.

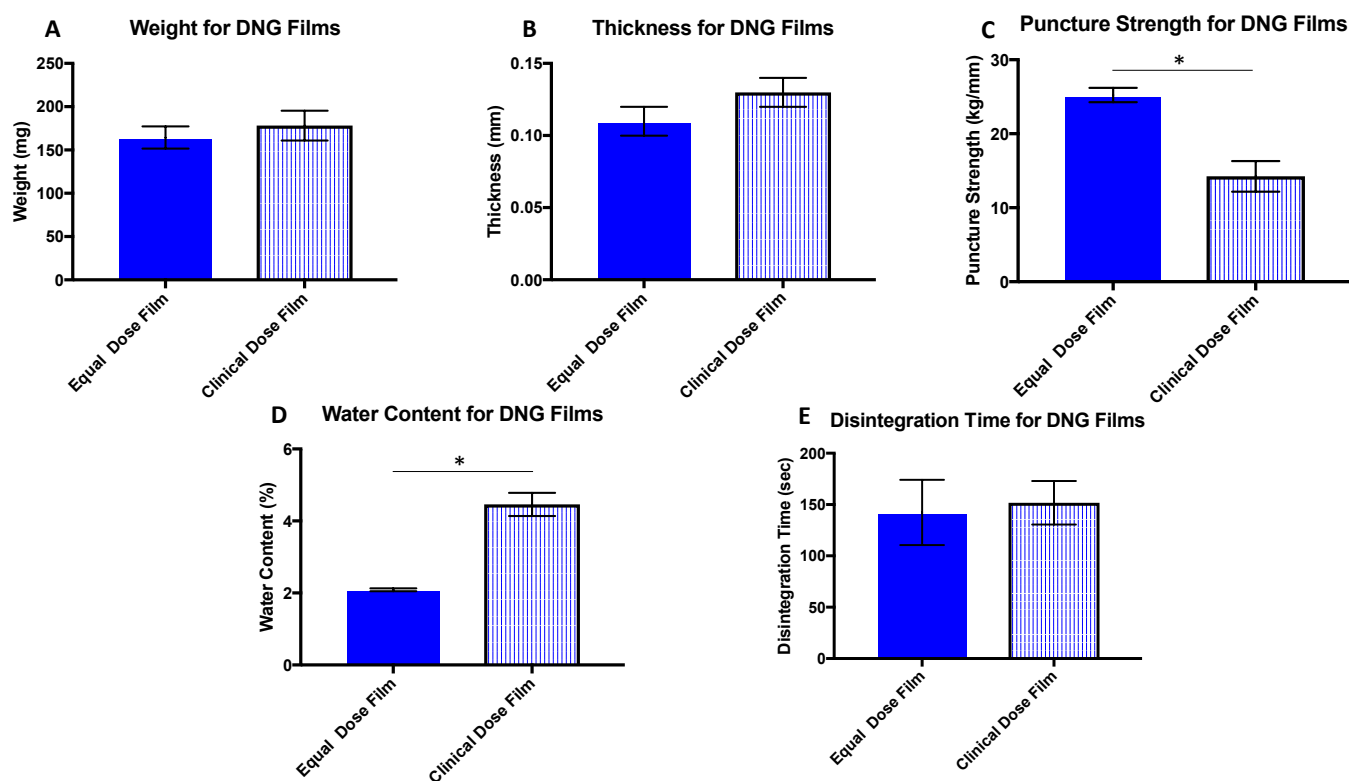


Figure 3.3.1 Physical characterizations of DNG films at two dosing levels.

A. Weight (mg) was measured by scientific balance; B. Thickness (mm) was measured by thickness gauge; C. Puncture strength (kg/mm) was analyzed by Texture Analyzer at each time point. The puncture strength (kg/mm) was the puncture force (kg) divided by the film thickness (mm); D. Water content (%) was measured by Karl-Fisher Titer apparatus; E. Disintegration time (sec) was analyzed by Texture Analyzer. Significant differences were observed for puncture strength and water content. Data was presented as Mean \pm SD. N=3 for each group. * P -value < 0.05 .

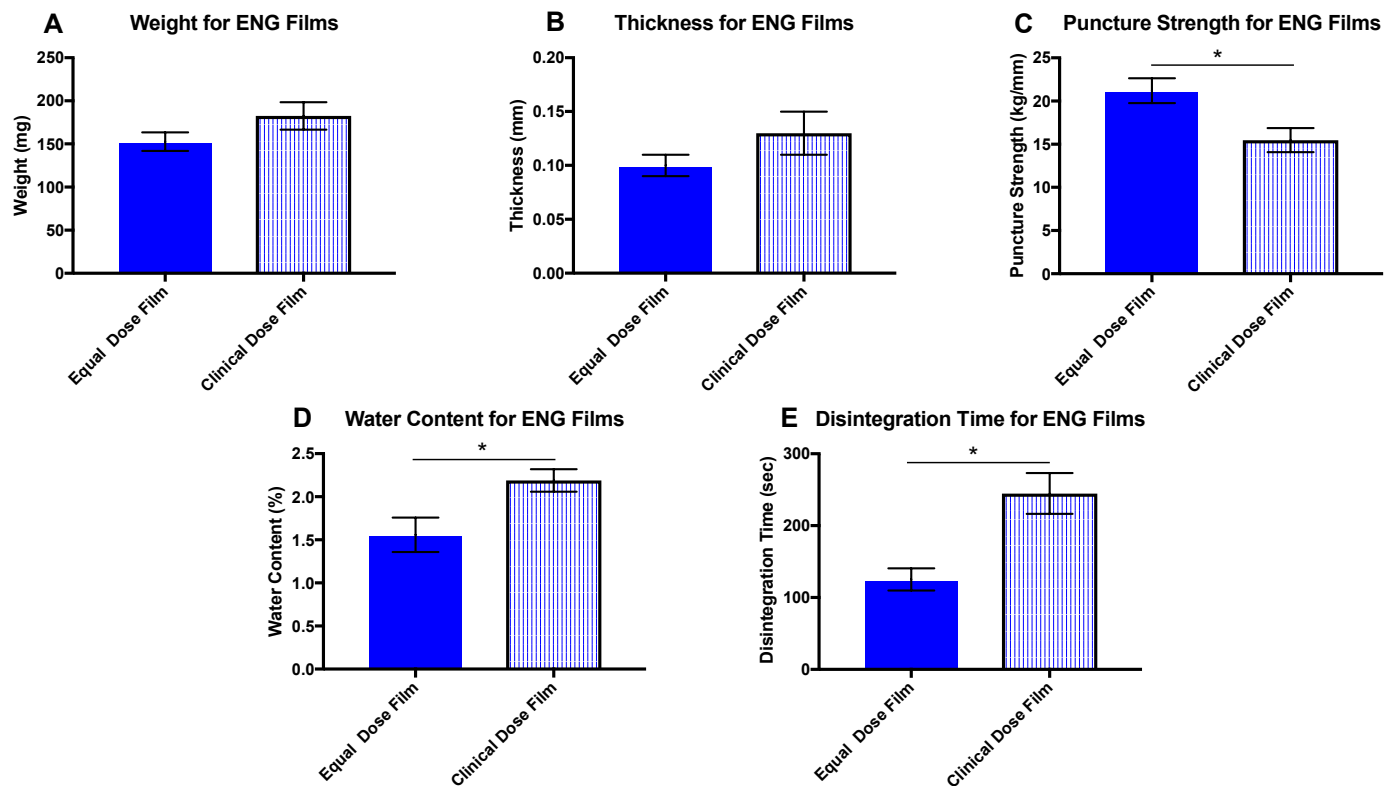


Figure 3.3.2 Physical characterizations of ENG films at two dosing levels.

A. Weight (mg) was measured by scientific balance; B. Thickness (mm) was measured by thickness gauge; C. Puncture strength (kg/mm) was analyzed by Texture Analyzer at each time point. The puncture strength (kg/mm) was the puncture force (kg) divided by the film thickness (mm); D. Water content (%) was measured by Karl-Fisher Titer apparatus; E. Disintegration time (sec) was analyzed by Texture Analyzer. Significant differences were observed for puncture strength, water content and disintegration time. Data was presented as Mean \pm SD. N=3 for each group. * P -value < 0.05 .

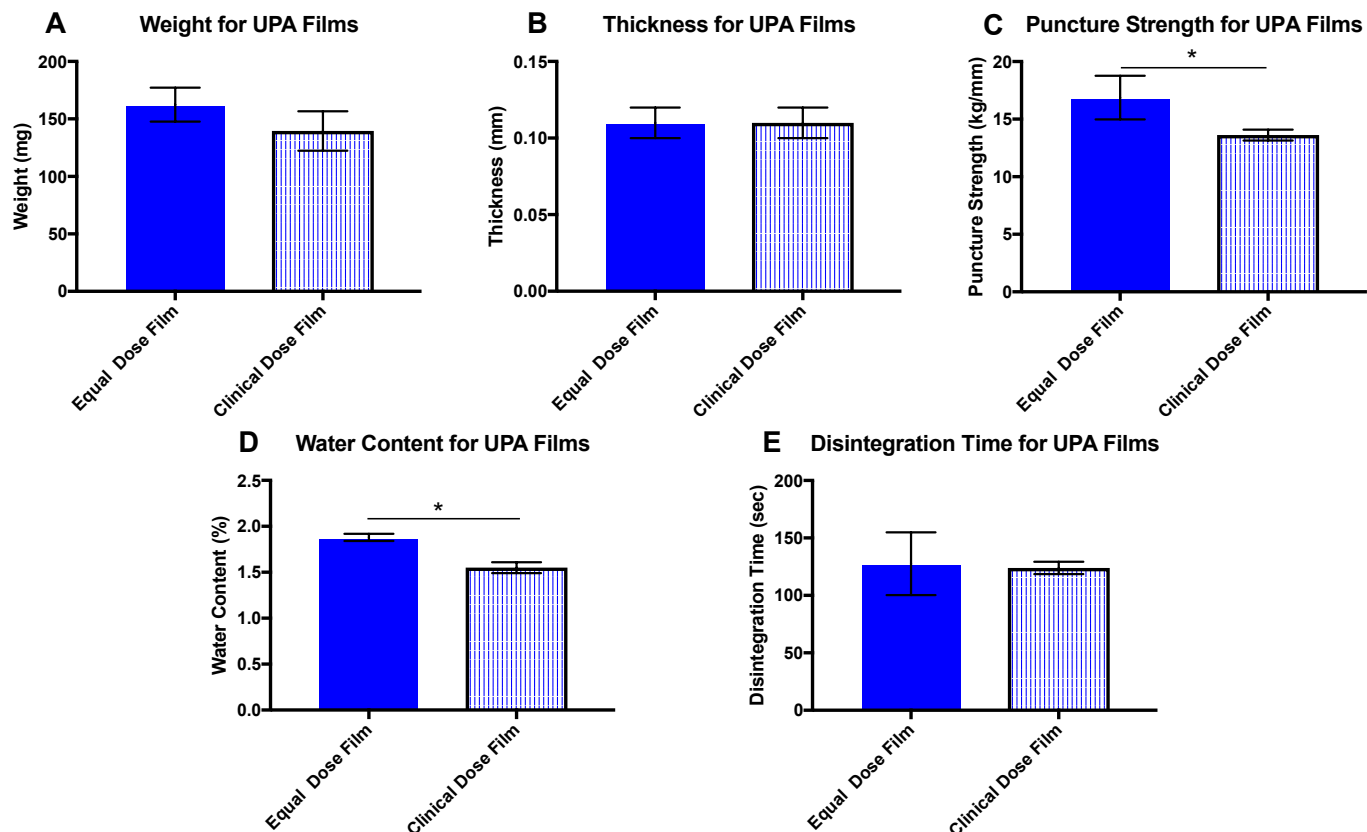


Figure 3.3.3 Physical characterizations of UPA films at two dosing levels.

A. Weight (mg) was measured by scientific balance; B. Thickness (mm) was measured by thickness gauge; C. Puncture strength (kg/mm) was analyzed by Texture Analyzer at each time point. The puncture strength (kg/mm) was the puncture force (kg) divided by the film thickness (mm); D. Water content (%) was measured by Karl-Fisher Titer apparatus; E. Disintegration time (sec) was analyzed by Texture Analyzer. Significant differences were observed for puncture strength and water content. Data was presented as Mean \pm SD. N=3 for each group. * P -value < 0.05 .

3.4 THE CLINICAL DOSE PROGESTIN FILMS REMAIN STABLE FOR AT LEAST THREE MONTHS

The clinical dose progestin films were randomly divided into two groups, stored under two conditions ([25°C/65% RH]; [40°C/75% RH]) as per ICH guidelines. The films were monitored for short-time stability (3 months). At each time point (Time 0; 1 week; 2 weeks; 1 month; 2 months; 3 months), films were analyzed for physical characterizations. The data of films' weight is presented in Figure 3.4.1; Thickness in Figure 3.4.2; Puncture strength in Figure 3.4.3; Water content in Figure 3.4.4; Disintegration time in Figure 3.4.5; Drug content in Figure 3.4.6.

For all four progestin films, the film weights, thickness and puncture strength remained constant through three months. The water content remained below 10% at all time. The disintegration time remained less than 250 seconds. Based on the target product specifications (Table 2.2.2.5.1), these physical properties were all stable through three months. For DNG, ENG and UPA films, the drug content remained between 80% - 120% for three months. For DES films, drug content remained constant over a 1-month period but was significantly decreased ($p < 0.05$) at three months. In addition, the films were stored under two different conditions, 25°C/65%RH and 40°C/75%RH. No difference on physical characterizations was observed between films stored under these two conditions. Similarly, all the drug contents of the progestin films were not affected by the temperature and humidity.

The developed film platform can incorporate all four progestins with consistent physical characterizations through 3 months, regardless of the various physicochemical properties. The data illustrate that the SC film platform can produce stable progestin films.

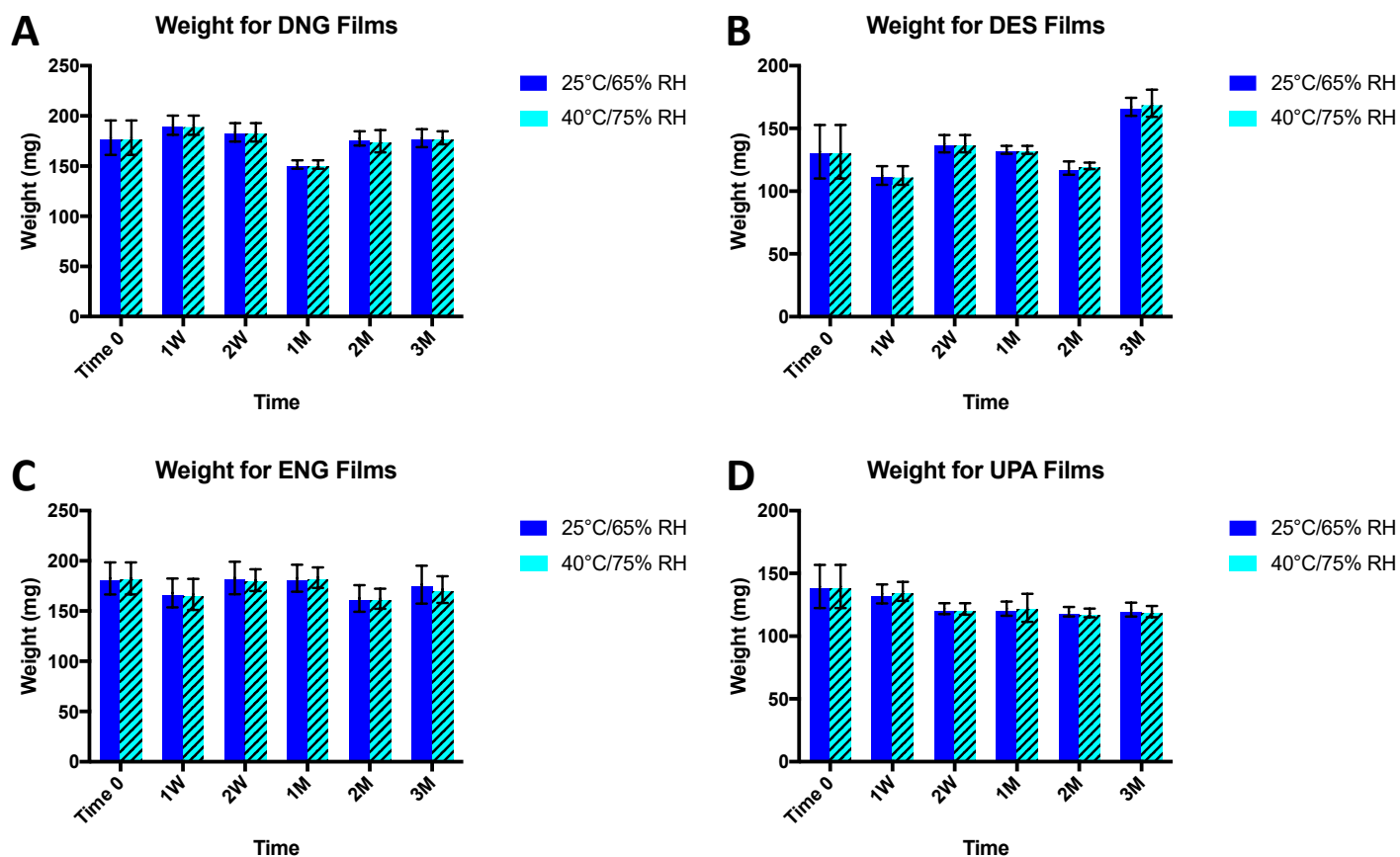


Figure 3.4.1 Weights (mg) of four progestin films stored under two conditions in 3 months. A. DNG films; B. DES films; C. ENG films; D. UPA films. The weight (mg) was measured by an analytical balance at each time point. Data was presented as Mean \pm SD. N=3 for each group.

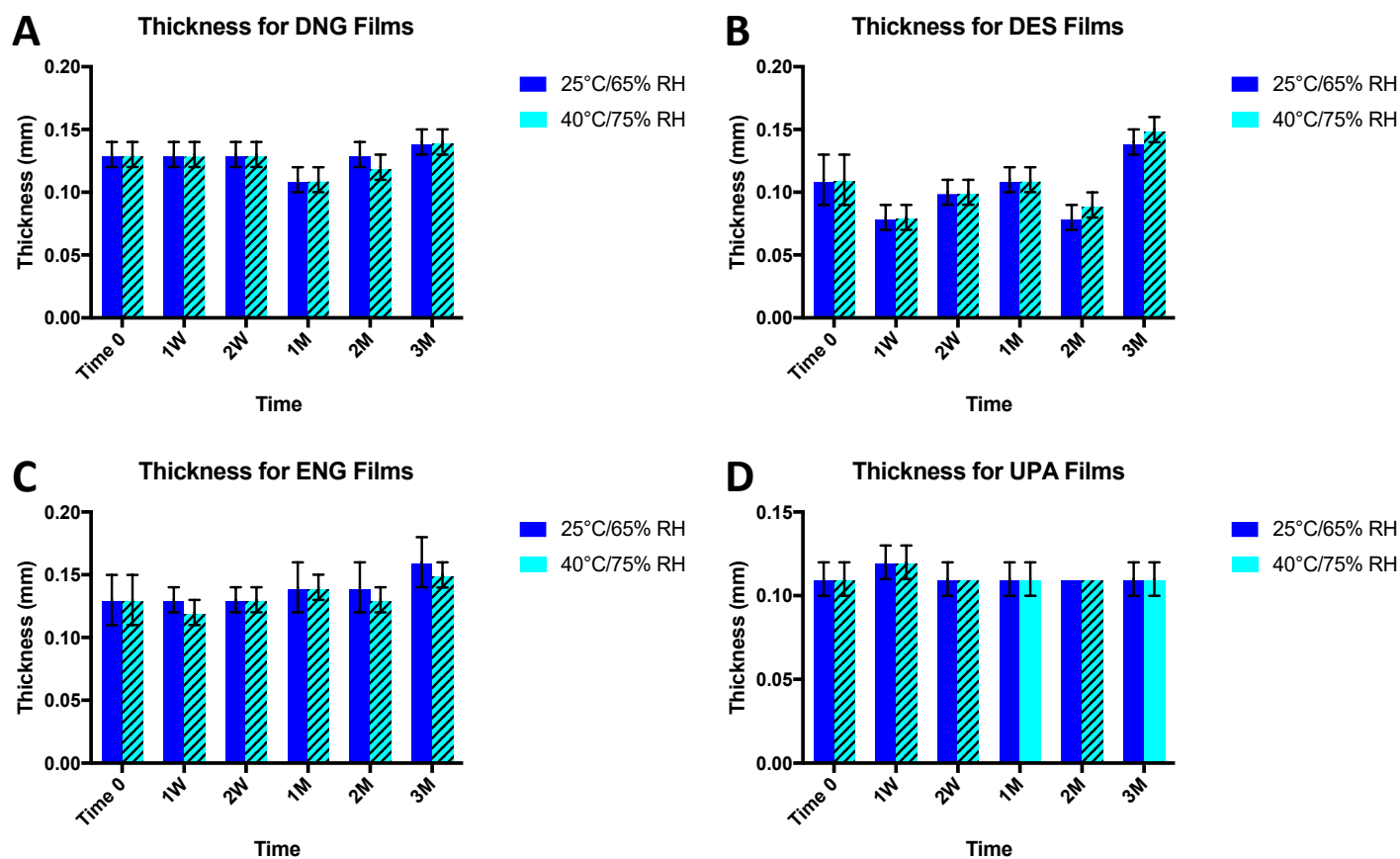


Figure 3.4.2 Thickness (mm) of four progestin films stored under two conditions in 3 months. A. DNG films; B. DES films; C. ENG films; D. UPA films. The thickness (mm) was analyzed by thickness gauge at each time point. Data was presented as Mean \pm SD. N=3 for each group.

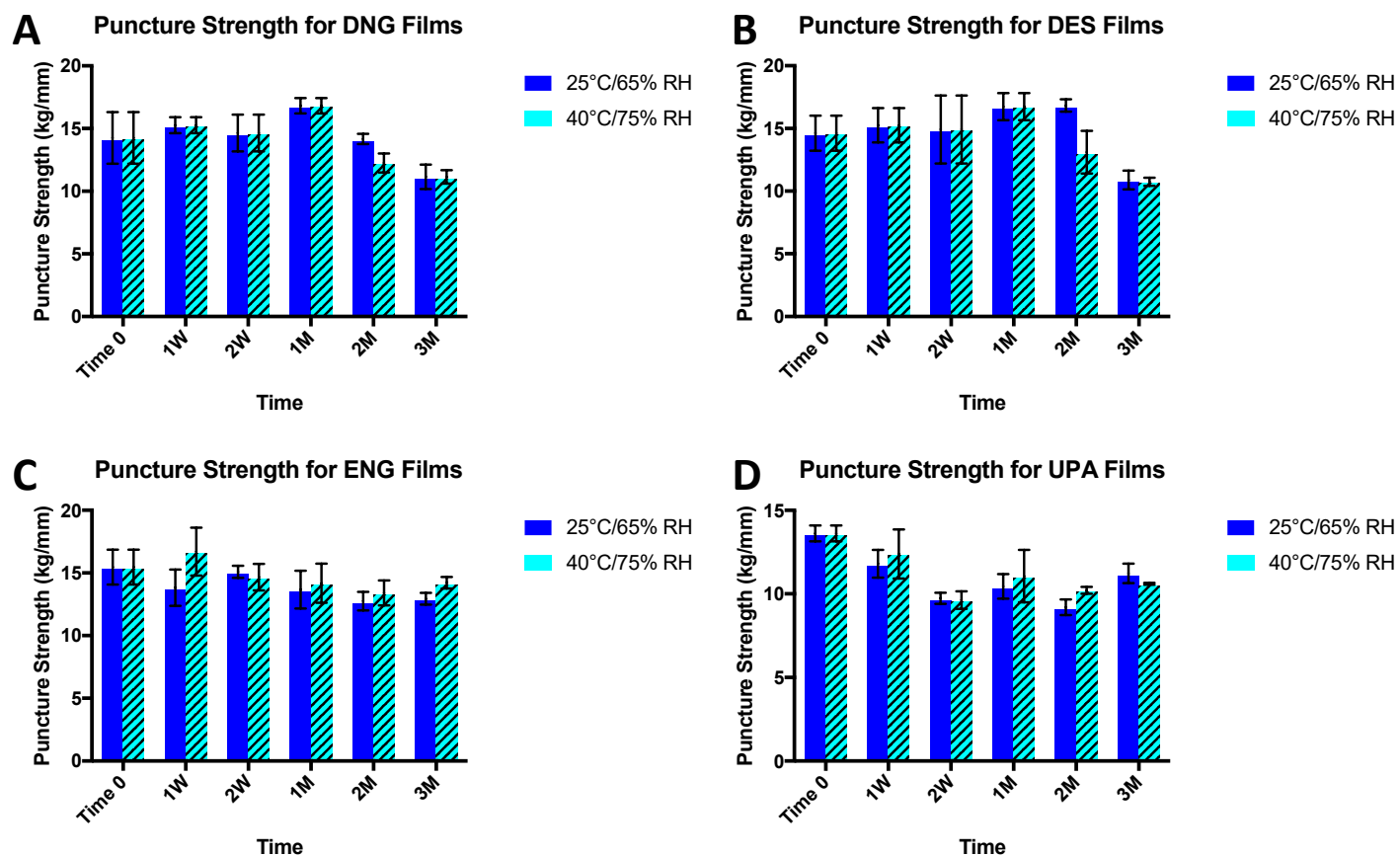


Figure 3.4.3 Puncture strength (kg/mm) of four progestin films stored under two conditions in 3 months. A. DNG films; B. DES films; C. ENG films; D. UPA films. The puncture force (kg) was analyzed by Texture Analyzer at each time point. The puncture strength (kg/mm) was the puncture force (kg) divided by the film thickness (mm). Data was presented as Mean \pm SD. N=3 for each group.

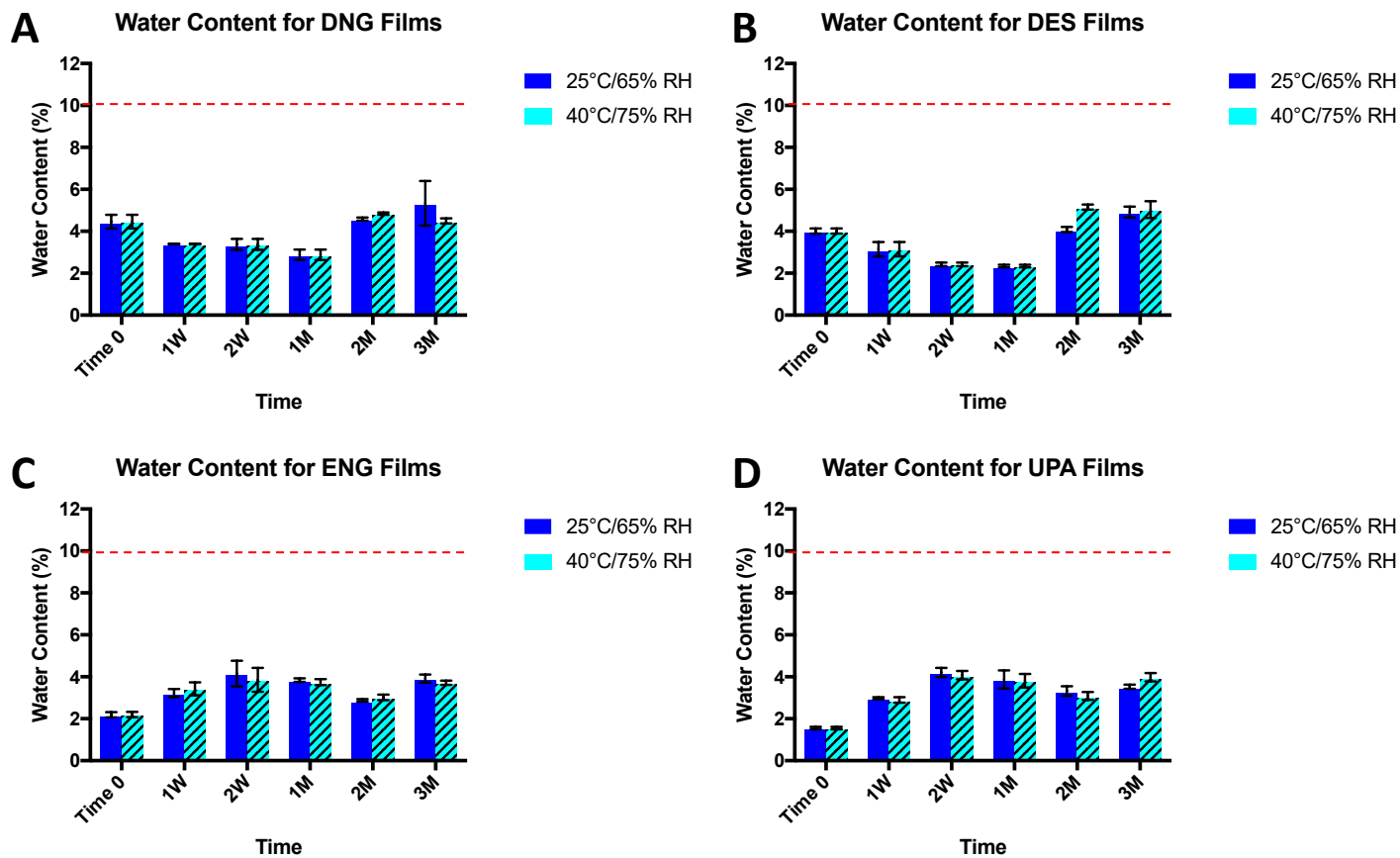


Figure 3.4.4 Water Content (%) of four progestin films stored under two conditions in 3 months. A. DNG films; B. DES films; C. ENG films; D. UPA films. The water content (%) was analyzed by Karl-Fisher Titer apparatus at each time point. The red dashed lines indicate the Target Product Specification. All water content was < 10%. Data was presented as Mean \pm SD. N=3 for each group.

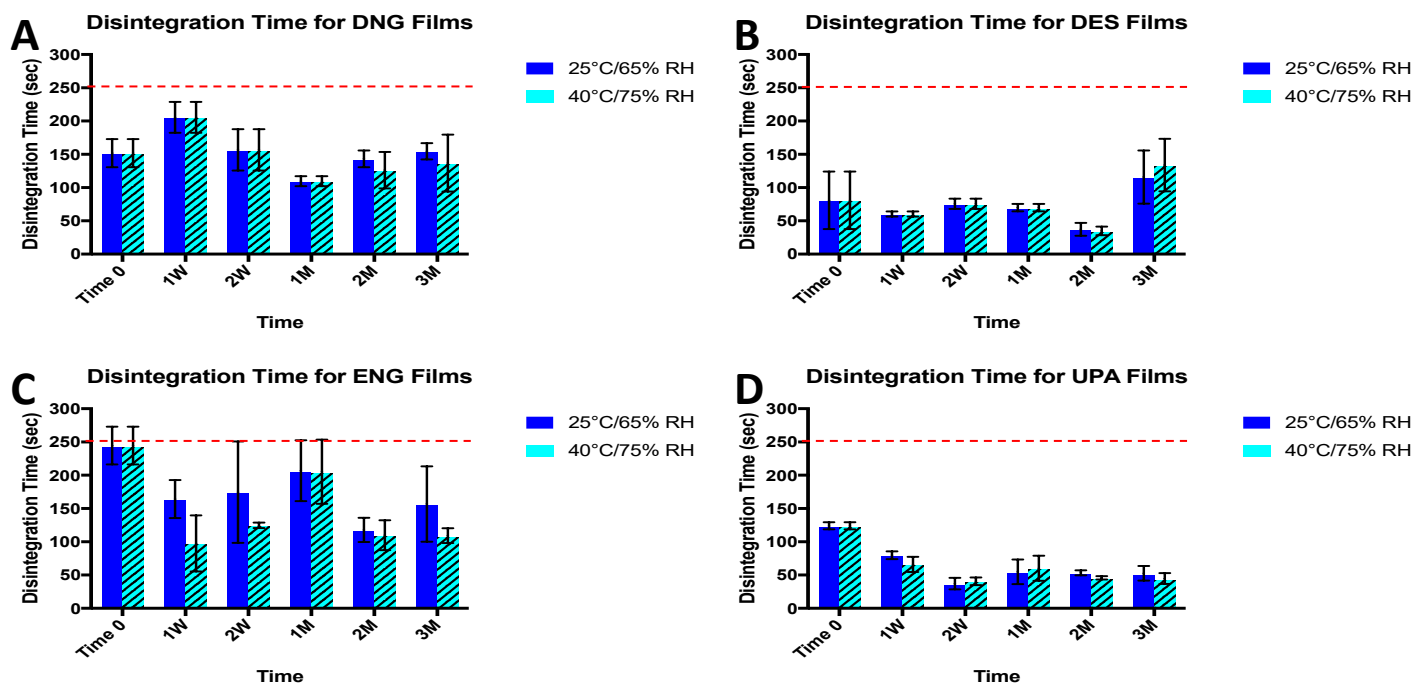


Figure 3.4.5 Disintegration time (sec) of four progestin films stored under two conditions in 3 months. A. DNG films; B. DES films; C. ENG films; D. UPA films. The disintegration time (sec) was analyzed by Texture Analyzer at each time point. The red dashed lines indicate the Target Product Specification. All disintegration time was NMT 250 seconds. Data was presented as Mean \pm SD. N=3 for each group.

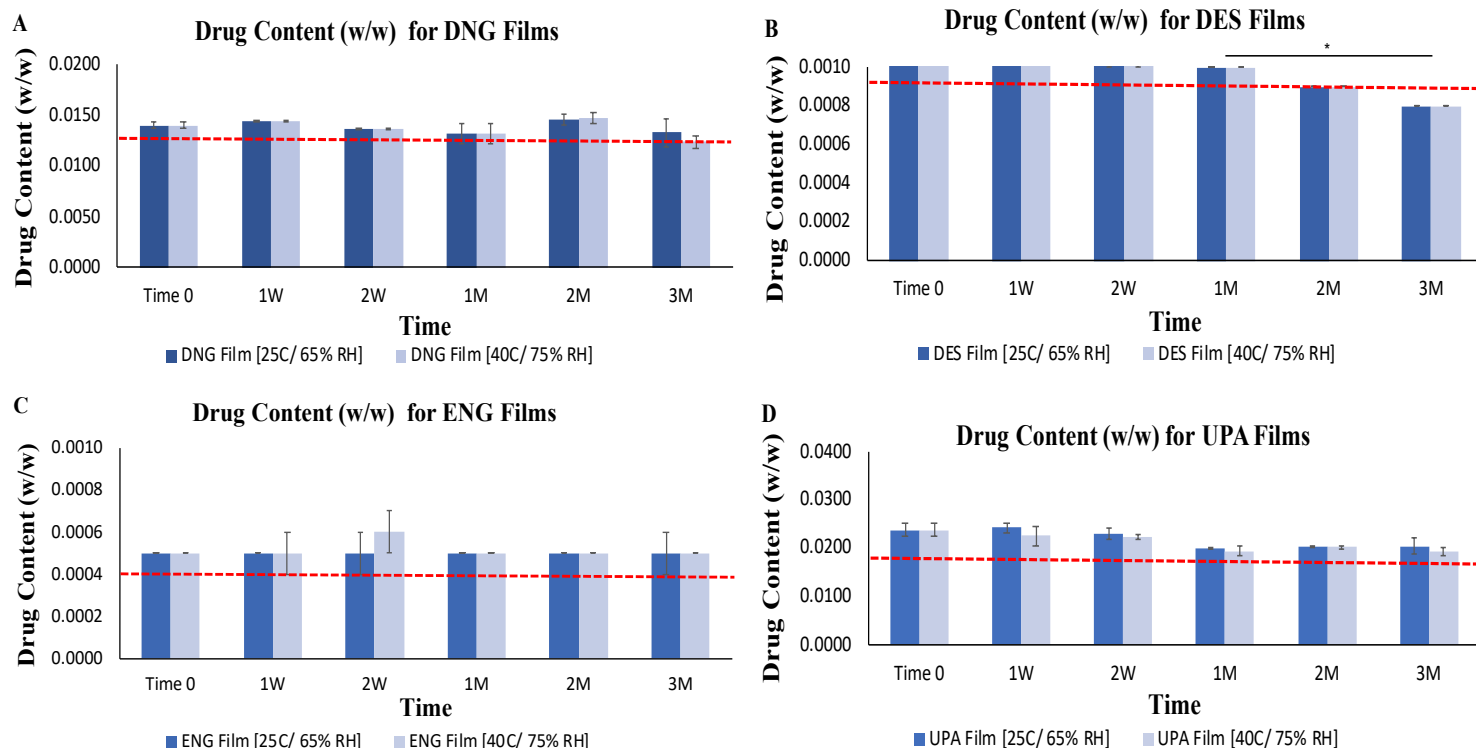


Figure 3.4.6 Drug content (w/w) of four progestin films stored under two conditions in 3 months. A. DNG films; B. DES films; C. ENG films; D. UPA films. The drug amount was analyzed by HPLC at each time point. Drug content (w/w) was the drug amount (mg) divided by the film weight (mg). The red dashed lines indicate the targeted drug content (w/w) respectively (A: 0.0123; B: 0.0009; C: 0.0004; D: 0.0184). Data was presented as Mean \pm SD. N=3 for each group. For DNG, ENG and UPA films, drug content remained between 80% -120% through 3 months. For DES films, the drug content at 3 months was significantly different compared to that at 1 month. * P -value < 0.05.

3.5 PROGESTINS DO NOT AFFECT THE RELEASE PROFILE OF THE POLYMERIC VAGINAL FILMS

The *in vitro* drug release profile is an important specification for this film platform. Because intended formulation was fast dissolve films (1), incorporation of hydrophobic drugs should not alter the release profile. To determine the release profile for the four progestin films, an *in vitro* dissolution study was performed using a USP 1 basket method. The clinical dose films were chosen for the study. For each group, three films were randomly selected. The drug content of the samples was tested by HPLC. The dissolution results are shown in Figure 3.5.1.

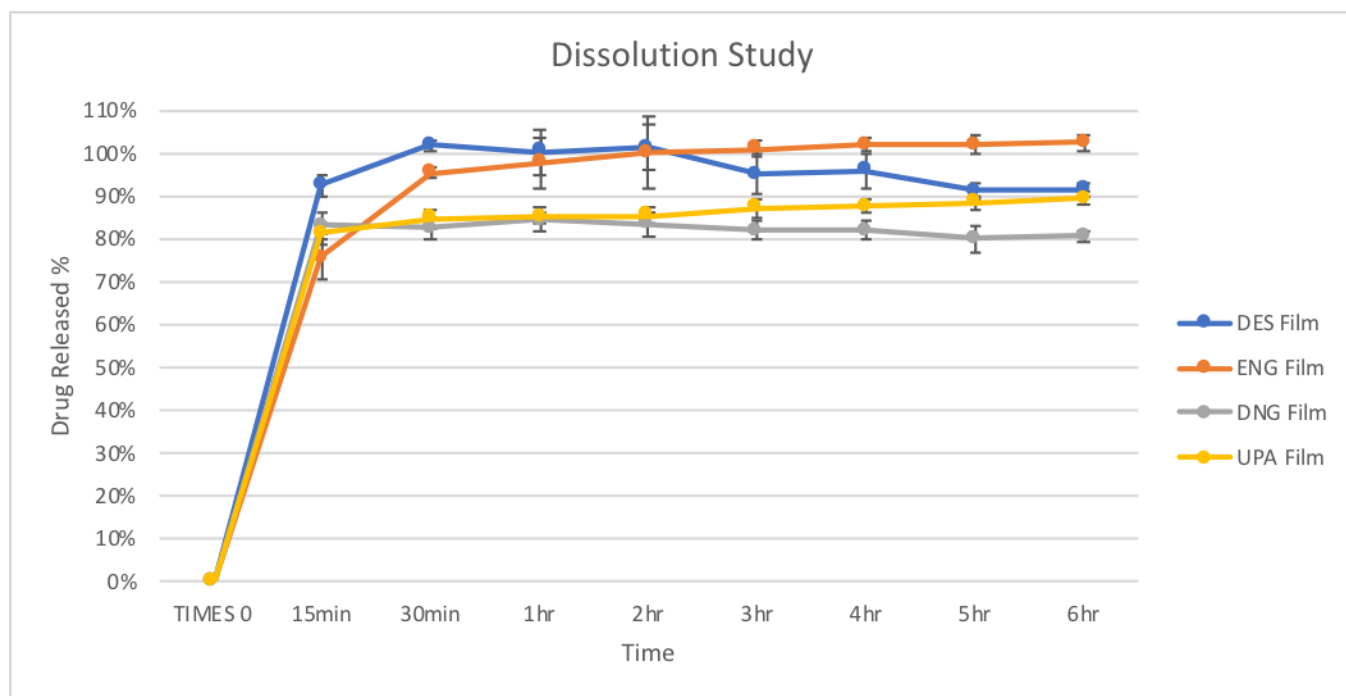


Figure 3.5.1 Dissolution curves of four progestin films in 6 hours.

In vitro dissolution study was conducted using a USP 1 apparatus (Distek dissolution system). Sink conditions were maintained for all films. 1% (w/v) Cremophor solution was used as dissolution media. The baskets were set to rotate at 100 rpm for 6 hours. 0.5 mL of the media was sampled at each time point (15 minutes; 30 minutes; 1 hour; 2 hours; 3 hours; 4 hours; 5 hours; and 6 hours), and 0.5 mL of the fresh 1% Cremophor solution was added. The accumulated drug amount was calculated at each time point.

Within 15 minutes, at least 70% of the drug was released from the film. After 30 minutes, the released drug amounts of ENG, DNG and UPA films plateaued. For DES and ENG films, the drug amounts both reached 100% within 1 hour. However, the cumulated drug amount of DES decreased slightly (around 10%) after 2 hours. For UPA films, the released drug amounts accumulated above 90% in 6 hours. For DNG films, the accumulated drug amount was 84% and remained around 80%. These dissolution profiles followed the same fast release trend, which was expected as the films used in this study were designed to be fast-dissolved films. The various physiochemical properties of these progestins did not affect the drug being quickly released from the film.

4.0 DISCUSSION

Reducing unintended pregnancy has remained to be one of the most important reproductive health goals identified by the United States Departments of Health and Human Services since last century (2). Even with various contraceptive options existing globally, a considerable portion of pregnancy is unintended (unwanted or mistimed). To this end, novel drug delivery systems, such as polymeric vaginal films, need to be developed for incorporation of contraceptive agents. Beyond that, polymeric vaginal films can provide a platform for design of combination products of contraceptive agents and anti-retroviral agents to achieve preventions of both unintended pregnancy and HIV infection. The first step of this goal is to manufacture stable polymeric films containing hormones, specifically in this project, progestins.

One behavioral clinical study (62) asked participants to compare a variety of vaginal films. Women mostly frequently preferred vaginal films to be thin, smooth, and translucent. The size of films did matter to some women, but the result was debatable. Some women preferred 2"X2" square films as they thought it might be easier to insert and cover more vaginal area so that it would work better. Others argued that smaller sizes were more discreet and would not be felt inside the vagina, as 2"X2" square films might be too obvious. In this project, we chose to manufacture smooth, translucent rectangular films of a 1"X2" size.

Film formulations usually comprise water-soluble polymers, plasticizers, fillers, colorants and flavoring agents (54). Water soluble polymers should be non-toxic; non-irritant; possess good wetting, spreadability, peelability; exhibit moderate mechanical properties; and be inexpensive to manufacture (58). Plasticizers are added to provide flexibility and pliability (54). Disintegrants can be used to provide rapid film disintegration once in touch with fluids. Other excipients may be

included to enhance patient compliance. The formulation used in these studies was adapted from the formulation previously developed for dapivirine (1). All excipients in this formulation have a history of use and were found to be safe. Polyvinyl alcohol (PVA) is a polymer base which is used in several currently marketed vaginal film products (1). This film combines PVA with hydroxypropyl methylcellulose (HPMC) as a copolymer. The ratio of polymers used was based on their ability to improve film properties, such as tensile strength, elongation, toughness, and elastic modulus (54). In addition, given the hydrophobic nature of progestin and hydrophilic nature of the polymer base, progestin exists as a dispersion in the film product. Therefore, the polymer concentration is essential to provide adequate viscosity to maintain the drug dispersion during the manufacturing process. However, the high viscosity of polymer solution can result in entrapped air during film manufacture, which will affect the physical properties of the film. To this end, sedimentation studies are required to ensure adequate dispersion of progestin in the polymeric solution through the film product manufacturing process.

Propylene glycol and glycerin were used as plasticizers in the formulation. The plasticizers enhance film flexibility, mechanical properties and softness. Moreover, propylene glycol was used in the formulation to facilitate the dispersion of the hydrophobic drug (1). Since dapivirine has similar lipophilicity ($\log P=5.3$) as the most hydrophobic progestins used in this project ($\log P$ ranging from 1.9 to 6.6), it was initially attempted to utilize propylene glycol as a dispersant for the progestin prior to addition into the polymer solution. Unfortunately, this process resulted in drug content of less than 74%. The limited drug incorporation illustrated that propylene glycol was not an adequate dispersant for progestin. Ethanol, a commonly-used solvent for hydrophobic drugs (63), was then evaluated as a dispersing/dissolving aid for progestins. Incorporation of ethanol increased drug content to greater than 80%. Based on this finding, ethanol was incorporated to

avoid drug precipitation. The good miscibility of ethanol with water also facilitated formation of a homogenous solution, resulting in better uniformity of drug disposition in films.

To study whether the type of progestin impacts the physical characterization of the film, films were manufactured at an equal dose level (Table 3.2.1). Despite their hydrophobic chemical nature, all progestins could be successfully manufactured into solvent-cast films as expected (Table 3.2.2). This demonstrated the wide versatility of this aqueous-based film platform. When the progestins were incorporated into the film at the same level (Table 3.2.2), no differences were observed in film weight, thickness or water content ($p>0.05$). Comparisons across this panel of progestin films at an equal dosing level demonstrated that progestin incorporation did not impact physical characterizations of the film. All film properties tested met the established primary target product specifications (Table 2.2.2.5.1).

To investigate the impact of progestin amount on physical characterizations of the film, we compared the films at two dosing levels (Figure 3.3.1, Figure 3.3.2 and Figure 3.3.3). The clinical dose films had lower puncture strength ($p<0.05$) as well as higher water content ($p<0.05$) for ENG and DNG films. It was reported that the type and amount of API in the film may impact physical properties, such as mechanical strength and swelling (64). We also considered that there may be a correlation between high water content and low puncture strength in these films. Water could act as a plasticizer (65). The water in the film may compete with API for hydrogen bonding with polymers (66). Given the hydrophilic nature of the polymer base (PVA) and the hydrophobic nature of progestins, polymers had a decreased ability to form hydrogen bonds when more progestin was included in the formulation. Thus, polymers have to form hydrogen bonds with water. The lack of hydrogen bonds will form a weak network structure (67). Therefore, an increased amount of progestin resulted in high water content and low puncture strength. In addition,

the clinical dose film was manufactured on a larger scale (five times of the scale of equal dose films). In a large-scale manufacturing process, more ethanol was used for dispersing the hydrophobic progestin. One study (68), analyzing hydrophilic zein (a class of protein found in maize) films made by either acetone or ethanol, indicated that ethanol affected mechanical properties of the film. Therefore, the different amounts of ethanol may also contribute to the differences in physical properties, such as puncture strength, water content and disintegration time.

The stability study demonstrated that the clinical films were stable for at least three months and were not impacted by temperature. Based on target product specifications (Table 2.2.2.5.1), physical characterizations of films, including weight (Figure 3.3.1), thickness (Figure 3.3.2), water content (Figure 3.3.4) and disintegration time (Figure 3.3.5), remained stable. For ENG, DNG and UPA films, drug content remained between 80% - 120% of Time 0. However, a decrease in drug content (Figure 3.3.6 B) was observed for DES films ($p < 0.05$). DES is the pro-drug of ENG. It can be rapidly transformed into one of its metabolites, ENG (etonogestrel/ 3-keto-desogestrel), by CYP3A4 (69). In addition, DES is susceptible to oxidation and it is reported that DES is incompatible with strong oxidizing agents (70). Compared to other hormones used in that study (EE and LNG), DES was easily degraded under 1% hydrogen peroxide, while others remained stable (71). Therefore, this decrease in drug content could be due to the oxidation of DES. To stop the degradation of DES, anti-oxidants can be added in the formulation (72). In contrast, ENG, UPA, and DNG all have a keto group on C3 position which is a more stable functional group (Appendix A). Ketone is a well-known, stable form. Particularly in these three progestins, the keto form can share the π bond with the nearby alkene group (carbon-carbon double bond). Also, as a hydrogen bond acceptor, the 3-keto group might increase the stability of ENG, UPA, and DNG. Therefore, no decrease was observed in drug content for these three progestin films. Moreover, no

differences in drug content were observed between progestin films stored when stored at either of the two test conditions.

To appropriately determine if developed vaginal products will effectively distribute drug to target tissues and the systemic compartment, two critical factors must be considered. The first being the efficiency on rate of drug release from the vaginal product and the second being the ability for drug to permeate the mucosal epithelium (73). The primary methods in a preclinical study to evaluate these critical components of delivery are dissolution and *ex vivo* permeability assessments. Dissolution testing was conducted for the progestin films developed. In this project, we first demonstrated that the polymeric films were fast-dissolving films, which was consistent with our hypothesis. According to set Target Product Specifications, the fast-dissolving films should release at least 80% of the drug within the first 30 minutes (74). *In vitro* dissolution study showed that all the four progestin films released over 80% of the drug within the first 15 minutes (Figure 3.4.1), indicating that progestin could be quickly released from the film. However, differences can be observed among these release profiles. The cumulated drug amount of DES decreased after 2 hours. This phenomenon may be due to the instability of DES in the medium (w/v 1% Cremophor solution). Cremophor is polyethoxylated castor oil, commonly used for poorly-water soluble drugs (75). Because of the hydrophobic nature of some progestins, it was necessary to use Cremophor in the dissolution medium to maintain sink conditions throughout the study. However, Cremophor would not be a biologically relevant condition. One study (76) found that it has systemic toxicity, as Cremophor caused oxidative damage to red blood cells. Another study (65) also demonstrated the oxidative nature of Cremophor. As mentioned above, DES is susceptible to oxidation. The Cremophor in the dissolution media may cause oxidation of DES, leading to the decrease of cumulative drug amount.

Although progestins are Biopharmaceutical Class System (BCS) II drugs, which have good permeability, the stratified multilayer vaginal epithelium may represent a barrier for progestin permeation. For this reason, in addition to the dissolution study, the permeability of progestin in both the formulated and unformulated state should be evaluated using an excised cervical tissue to establish the permeability profile for progestins.

Physiological factors will also affect the absorption of progestin, including the thickness of epithelium layer, the volume of vaginal fluid, and alteration of vaginal pH. These factors vary individually, resulting in different contraceptive effects on different patients. In order to minimize the individual differences and achieve more consistent contraceptive effects, the progestin dose in the film is essential. The doses that we determined in this project as clinical doses were calculated based on products on the market. Besides UPA, which clinical dose was calculated based on a vaginal ring, other clinical doses were calculated based on non-intravaginal drug delivery systems. For example, ENG dose was based on an implant; DNG and DES doses were based on pills. Indeed, different administration routes will result in pharmacokinetics differences. Compared to oral drug delivery system, such as pills, vaginal delivery offers certain advantages, such as avoidance of gut and hepatic first-pass metabolism (73). One recent study indicated that the mucoadhesive tablets had higher mean residence time (MRT) in the blood and higher bioavailability with oral progesterone administration (77). After implant insertion, ENG is rapidly absorbed into the circulation and becomes almost 100% bioavailable (78). Compared to this subcutaneous drug delivery system, vaginal delivery shows a potential limitation in absorption and distribution, because progesterone can accumulate in the uterine tissue after vaginal application (79). Thus, it is difficult to quantitatively estimate the distribution of a drug after an intravaginal administration based on available data from non-vaginal drug delivery systems for contraception. Therefore, in

future studies, the effective clinical doses will need to be established through established *in vivo* animal studies.

5.0 LIMITATION

In order to incorporate all the progestins in this SC platform, ethanol was used as dispersing/dissolving agent. Ethanol belongs to Class 3 solvents (solvents with low toxic potential). For a Class 3 solvent, the allowable limit of residual amount in the final product is 0.5%. Therefore, a methodology of testing residual ethanol in the film must be developed. In addition, progestins, such as DES, had limited solubility in ethanol. Other solvents, such as ethyl ether and acetone, should be considered and analyzed for their compatibility with progestins (70). Further, undissolved progestins may exist in the film in either the crystalline or amorphous state (80). It was reported that crystal form may impact the drug solubility (81). Polymorphs can also impact the bioavailability and stability of poorly soluble drugs (82). The crystal structure of progestins in the film should be analyzed by X-ray crystallography or differential scanning calorimetry.

Given the hydrophilic nature of the polymer base (PVA) and hydrophobic nature of some progestin (such as DES), sedimentation studies should be carried out to ensure adequate dispersion of progestins in the polymeric solution throughout the film product manufacturing process. In addition, DES is susceptible to oxidation. Antioxidants, such as Vitamin E, should be considered in the formulation for stabilizing the DES. Moreover, Cremophor may oxidize DES leading to a decrease in released drug amount. Other surfactants without any potential to impact the API should be used in dissolution testing of products containing DES.

6.0 NOVELTY AND SIGNIFICANCE

This study investigated the SC platform for incorporating progestins into polymeric films. The polymeric films we generated had similar and stable physical characteristics. The successful incorporation of a panel of progestins with various physiochemical properties demonstrated the versatility of this platform. Moreover, these progestins are currently used as contraceptive agents. On one hand, the clinical progestin films can serve as a practical solution for the urgent need of more novel birth control options. On the other hand, the data with progestin films developed in this project illustrated that this SC platform is capable of application of dual prevention (contraception and HIV prevention) therapies.

7.0 FUTURE PLANS

The vagina can be used to achieve both local and systemic drug exposure. The use of vaginal administration for systemic drug delivery has been well established. The vagina also contains a vast network of blood vessels making systemic delivery of drugs possible (83). This route offers certain advantages, such as avoidance of gut and hepatic first-pass metabolism (73). A study (84) of the pharmacokinetics of vaginal maraviroc gels in rhesus macaques indicated that the peak plasma concentration was achieved 2 hours after gel application. Another study (85) investigated the pharmacokinetics of quick-dissolving vaginal films incorporating a poorly soluble antiretroviral drug, IQP-0528. In this study, drug concentration monitored in the vaginal fluid illustrated that the films could deliver similar levels of drug as gels, for up to 24 hours after application. These studies demonstrate feasibility for delivery of progestins within the vagina and rapid uptake into the systemic circulation.

The primary mechanism of contraception for progestin is to inhibit ovulation, which depends on the negative feedback of high levels of progestin on hypothalamus and pituitary. Therefore, to achieve contraceptive effects, it is desirable that progestin obtain stable high serum levels continuously. The fast-release progestin films we manufactured in this project deliver a daily dose of progestins. For daily-use dosage forms, such as pills and vaginal films in this project, timing and duration of administration are critical (17). For example, the biological half-life of ENG is about 25 hours. The steady-state levels of ENG are achieved after about 8 to 10 days of daily administration (86). In order to achieve optimal contraceptive effects, these progestin films should be used daily at the same time of the day.

A study (38) showed that long-acting reversible contraception (LARC) lead to better patient compliance than daily used contraceptive methods. To this end, the progestin polymeric vaginal films can be further developed as extended-release (ER) progestin films for contraception. Ideally, an extended-release (ER) vaginal film can stay in the vagina to achieve controlled progestin release. Similar to contraceptive implants, IUDs, an ER vaginal film would not require frequent administration, resulting in better patient's compliance (38).

The data generated in this project can also be applied for multi-purpose prevention therapy (MPT). The goal of MPT is to achieve multiple prevention effects at the same time. By combining APIs with different pharmacological effects, one dosage form can achieve MPT. The solvent-cast film platform used in this project was initially evaluated in the clinic for vaginal delivery of dapivirine (DPV), a non-nucleoside reverse transcriptase inhibitor (1) for HIV prevention. This film platform has already been studied in *in vitro* evaluations for its capacity to deliver multiple APIs in one film (51). Over sixty-two percent of women of reproductive age currently use contraception, 10.6 million women of which are using hormonal contraceptive methods. Therefore, combining a hormonal contraceptive film with an anti-retroviral agent could be developed to prevent both unintended pregnancy and HIV infection.

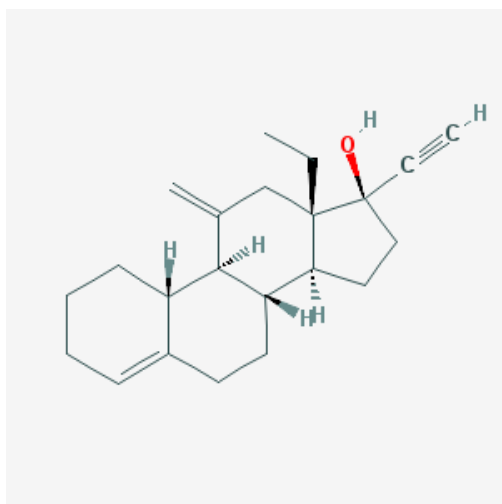
In addition, there are certain considerations should be addressed in future film development. The normal vaginal pH level is between 3.8 and 4.5. The slightly acidic environment is critical to avoid infections and diseases (87). Thus, vaginal films should maintain an optimal pH for vaginal health. To further ensure the safety of vaginal films, standard microbicide safety tests should be performed to assess *Lactobacillus* compatibility (1); *in vitro* and *in vivo* toxicity studies should also be considered as criteria in the evaluation of the vaginal film.

Moreover, early work by Johnson and Masters (88) showed that the drug distribution and coverage of vagina tissue vary considerably with the nature of the delivery system. Therefore, in addition to those common criteria mentioned above, other critical film properties are required to achieve intended effects. For example, the drug should penetrate the epithelium in order to achieve systemic effects. Therefore, permeability profiles should be tested; the drug should distribute uniformly throughout the vaginal cavity if local effect is intended. Thus, study of the products ability to uniformity distribute drug throughout the vagina is critical for films with intended topical drug delivery. For controlled/extended films, bio-adhesion is a preferable film characterization. However, at the same time, it may serve as a permeability barrier for drugs (73). Therefore, film swelling (swelling index), bio-adhesion (bio-adhesive strength), and retention (drug amount retained on vaginal mucosa) studies should be performed as critical film characterizations (89).

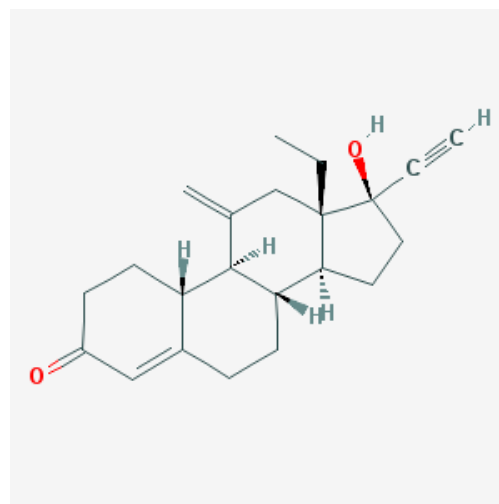
APPENDIX A

2D CHEMICAL STRUCTURE OF PROGESTINS(70)

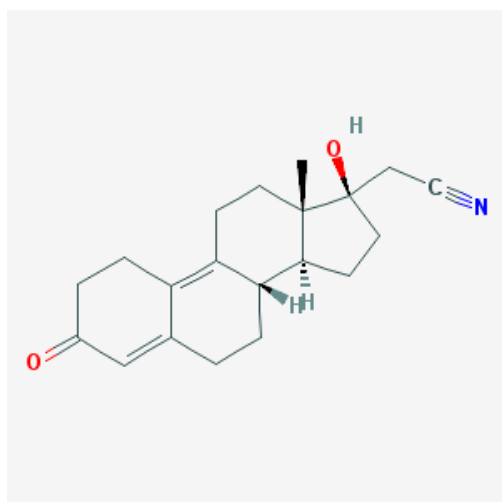
Desogestrel (DES)



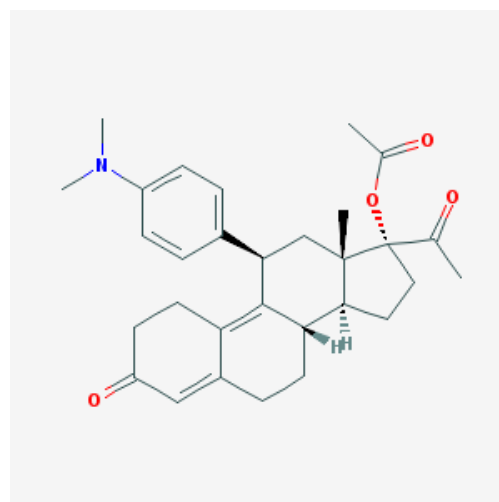
Etonogestrel (ENG)



Dienogest (DNG)



Ulipristal Acetate (UPA)



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