DOCTOR OF PHILOSOPHY

A modular device for vaginal administration of multiple therapeutic agents

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A modular device for vaginal administration of multiple therapeutic agents

by

Vicky-Leigh Young
BSc. MSc.

A thesis submitted for the award of the degree of
Doctor of Philosophy
at
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Abstract

In this project, the aim was to develop a new multipurpose prevention technology (MPT) platform to help address the global health burdens associated with sexually transmitted infections (e.g. HIV-1, HSV-2, HPV) and unmet need for contraception, which disproportionately affect young women in low and middle-income countries. The new MPT product concept is based around an innovative vaginal ring in which drug- containing cores are physically inserted into and retained within a ring-shaped frame using 'interference fits'. Compared to more conventional vaginal ring designs, this vaginal ring technology platform offers ease of fabrication of complex devices containing multiple drugs and multiple materials and can offer different kinetics and mechanisms of drug release within the same device. Various model drugs have been used to demonstrate the concept: MIV-150 is an experimental antiretroviral drug owned by Population Council, New York; Nestorone (NES) is a contraceptive progestin; metronidazole (MET) is an antibiotic commonly used to treat bacterial vaginosis; and lactic acid (LA) and its cyclic dimer form lactide (LT) can maintain protective vaginal pH and provide an anti-inflammatory effect.

Firstly, UPLC methods for all model drugs were developed and validated to ensure all data collected was precise and accurate. Secondly, various preformulation studies were conducted including assessing solubility of the model drugs in different polymers, an important factor in considering polymers for both the core insert and ring body and determining a suitable medium to use throughout this project for in vitro release. Other studies included using a model prototype skeleton body to access the reduction of drug release when a drug loaded core in placed inside a ‘ring body’ and using drug loaded reservoir cores to evaluate drug release in relation to surface area.

Once a mold was designed and manufactured so that the prototype vaginal ring body could be injection molded, mechanical testing was conducted on ring bodies manufactured from various thermoplastic polymers. Comparative tests such as a 5 mm compression and clinically relevant tests (e.g., a 28-day static

compression) were used to compare various polymers mechanical strength and recovery. From these studies three polymers (Vistamaxx™ (VX), Hydtrel® (HY) and Desmopan® (DS)) were chosen to bring forward into *in vitro* release studies.

Three sets of *in vitro* release studies were conducted. The first set of studies involved drug loaded (2.5% w/w) silicone cores (1 x ½ or 2 x ¼ cores) of MET, MIV-150 and NES separately into a ring body manufactured from either VX, HY or DS. *In vitro* release was conducted for a period of 10 days, after which the ring body and cores were evaluated for residual drug. From this study it was found that the polymer HY provided adequate flexibility while having reduced drug uptake.

The second *in vitro* release study focused four formulations allowing co-release of MET, NES and MIV-150 from drug loaded cores placed into a HY ring body. Core lengths and loadings were modelled from data obtained in the previous in vitro release study. This approach allowed co-release and independent control of each model drug while evaluating release rates for 29 days. The fourth formulation (containing all model drugs) was evaluated for stability after 1 month at 45 ºC/ 75% RH. This stability study also explored if there was a different if the cores where stored placed in the ring body or separately. With the last *in vitro* release study in Chapter 9, involving a modified formulation to maintain and control drug release of all three model drugs over a period of 78 days.

By applying a modular construction approach based around a common architecture, this device may allow healthcare professionals to select the appropriate combination of therapies to suits women's circumstances and family planning aspirations.

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Introduction

1. 1 Human immunodeficiency virus and acquired immunodeficiency syndrome

The human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), one of the most devastating worldwide public health pandemics in recent history. The virus attacks certain cells of the immune system reducing its ability to fight infections. Over time, the continued damage to the immune system leads to increased risk of developing common infections (such as tuberculosis), other opportunistic infections and tumours that rarely affect people who have fully functional immune systems. The development of late-stage symptoms of infection defines the clinical onset of AIDS.

In 2019, approximately 37.9 million people worldwide were living with HIV with 1.7 million new infections that year [1]. New infections were reduced 40% compared with peak incidence in 1997, mostly on account of increased access to antiretroviral therapy. Just over half of the people living with HIV are women and 2.1 million are children. 32 million people have died from AIDS related deaths since the start of the epidemic.

Eastern and southern African regions account for 43% of all new global HIV infections, and more than half of those cases are attributed to women and girls [2]. Women are significantly more susceptible to sexual acquisition of HIV, attributed to a range of biological factors that are still not fully understood. For example, it has been reported that women are twice as likely to become infected with HIV than men after a single sexual encounter [3]. In recent years, inflammation of the female genital tract caused by microbial imbalance (dysbiosis) in the vagina and sexually transmitted infections (STIs) have been identified as a major risk factors for increased incidence of HIV acquisition [4].

While biological factors clearly contribute to increased prevalence of HIV in young women, social factors are also important. Discrimination against women and girls in access to healthcare and education is common in low- and middle-income countries. Often females receive no inheritance compared to their male siblings or relatives [5]. As a result of this discrimination, young women often form relationships for money

and social security [6]. Relationships like these, usually with older partners, have been associated with an increased risk of acquiring HIV. Young girls who acquire HIV usually drop out of school, leading to an increased education deficit [7,8]. Domestic abuse victims have been shown to be another high-risk group for acquiring HIV. Fear and violence mean they have little or no choice when a sexual act is asked of them. This also discourages them from disclosing their current HIV status [9]. Due to the economic climate in sub-Saharan Africa, some women are forced to earn a living as commercial sex workers [10], significantly increasing their risk of contracting HIV, STIs or having an unintended pregnancy.

Global organisations have vowed to invest greater resources in advancing HIV treatment and preventive measures. A target set globally for 2020 was the 90-90-90-treatment goals: 90% of people living with HIV will know their status, 90% of HIV positive people will have the ability to access the necessary treatment, and 90% of these people will have suppressed viral loads [11]. Achieving these goals seems possible; new HIV infections have declined by 29% in the last six years and AIDS related deaths have fallen by 48% since the peak in 2005, suggesting that some measure of control is being brought to the epidemic.

1.2 Sexually transmitted infections

Sexually transmitted infections (STI) are a major global health problem. The consequences of contracting a STI can range from a treatable infection to an acute illness. If left untreated, some STIs can result in infertility and death [12]. STIs that cause inflammation of the genitals increase the risk of the infected individual infecting others and acquiring other infections themselves [12–15]. In particular, there is a growing body of evidence supporting a causal link between genital inflammation and increased risk of HIV acquisition [17–19].

Bacterial vaginosis is a common vaginal infection caused by a change to the normal microbial balance in the vagina. This imbalance is caused by the displacement of normal lactic acid-producing vaginal lactobacilli with anaerobic bacteria [20,21]. Although not usually classed as an STI, bacterial vaginosis has been found to cause significant genital tract inflammation [17, 19,21].

Herpes simplex virus type 2 (HSV-2) is an incurable infection that, although often asymptomatic, can cause painful blisters or ulcers in the genital area. In sub-Saharan Africa, it is estimated that up to 80% of sexually active women and up to 50% of sexually active men are infected with HSV-2. HSV-2 has been linked with 3.4 fold increase of HIV acquisition [22].

Human papillomavirus (HPV) is a group of viruses capable of infection and replicating in the genital epithelium. A review of the association between HPV and HIV found that the risk of HIV doubled with HPV infection. Also, certain high-risk HPV types have been shown to cause cervical cancer [17–19]. In the UK, it is standard practice for young girls aged 11–16 years to receive the HPV vaccine, which if extended worldwide could significantly reduce incidence of HPV-mediated HIV acquisition.

Women in low and middle-income countries experience high rates of unintended pregnancies, STIs and HIV infection. The Guttmacher Institute reported that 41% of 208 million pregnancies that occurred during 2008 were unintended, and 20% of these unintended pregnancies were terminated [26]. Pregnancy termination practices are often unsafe and can lead to serious medical complications, future infertility or maternal deaths [21, 22]. Although a myriad of contraceptive methods and products is available globally – including oral pills, long-acting injections, barrier methods, and vaginal rings – women in many countries have very limited choice. For example, in many sub-Saharan African countries, long acting injectables are the most common form of contraception; oral pills and other forms of contraception are rarely available or offered. The levels of protection afforded by contraceptive products also vary considerably by type and use. Even in high income countries like the USA where many different forms are available, it is estimated that half of pregnancies are unintended at the time on conception [29]. Despite the effectiveness of barrier methods such as male and female condoms in preventing pregnancy and reducing the risk of HIV infection, there are still very considerable barriers and resistance to their widespread use. Other forms of contraception generally do not offer any degree of protection against HIV infection, and modern hormonal forms of oral contraception have inevitably led to a significant reduction in condom use globally [30].

1.3 Pre-exposure prophylaxis

Pre-exposure prophylaxis is a type of treatment plan aimed at those who are at high risk of HIV-1 acquisition. It aims to prevent HIV-1 infection, whether through sexual transmission or engagement in injectable drug use [31]. Over the past twenty years, considerable efforts have been made to reduce sexual transmission of HIV, including promotion of behavioural change (encouraging sexual abstinence, promoting fidelity in relationship and reducing number of sexual partners, and advocating condom use) and development and testing of a wide range of biomedical strategies (vaccines, vaginal microbicides, oral pre-exposure prophylaxis, treatment-as-prevention etc.) [11,32–35].

Behavioural changes

Since the start of the HIV epidemic, behavioural changes have been widely advocated to reduce the spread of the disease. One of the most popular public health campaigns was 'ABCCC' which promoted Abstinence, Be Faithful, Condomise, Counselling and Circumcision. However, due to gender power imbalances, women often do not have a voice in many of these issues; for example, they may not be able to negotiate condom use or guarantee mutual monogamy [29,36].

Treatment-as-prevention

Treatment-as-prevention (TasP) refers to HIV prevention methods and programmes that use antiretroviral medicines to reduce the risk of HIV transmission. Evidence obtained with serodiscordant couples has shown that HIV-positive individuals on effective antiretroviral treatment with an undetectable viral load showed reduced transmission of HIV to their partner by up to 96% and significantly reduced AIDS-related deaths [37]. Currently, 57% of all HIV-positive individuals worldwide benefit from antiretroviral therapy with commitments to further increase its use [2].

Oral pre-exposure prophylaxis (Oral PrEP)

Numerous clinical trials have been completed to assess HIV prevention options for women. Many have focused on administering antiretroviral drugs either orally (so-called oral PrEP) or vaginally to allow women to directly control their own risk of

acquiring HIV. The Partners PrEP and Botswana TDF2 trials demonstrated oral PrEP as an effective prevention treatment in women [38,39]. However, other trials, including the VOICE trial, found that oral PrEP showed no protective effect over placebo [40]. This disappointing result was attributed to low levels of adherence and suggested that oral PrEP, despite its effectiveness, might not be suitable for all women. Despite the positive results from use of Truvada® in clinical trials, only the Food and Drug Administration (FDA) has approved this oral PrEP, leaving a gap in the preventive treatment of HIV-1 in other countries [41].

Preventative vaccines

Historically, vaccines are the most effective, safe and wide-reaching way of preventing infectious diseases. Ideally, a HIV vaccine would control the pandemic by evoking an immune response capable of fighting off the infection in those most at risk of acquisition. The vaccine would also be capable to suppressing viral loads in those already infected, slowing down disease progression [32,42]. A total of 256 trials involving 44,000 healthy volunteers have been conducted since 2014, with six of these trials progressing to Phase IIb and III. Of these six trials, one showed partial efficacy (31.2%) and the rest showing no efficacy or increased risk of HIV infection [42].

1.4 Vaginal microbicides

Microbicides are chemical substances administered to either the vagina or rectum to prevent the sexual acquisition of HIV. As with commercial contraceptive products which offer a very broad method mix in terms of choice of product, vaginal microbicides are being developed using a very wide range of formulation options, including gels, tablets, films, long-acting injectables, and rings [33,43–45]. Most vaginal microbicide strategies are focused on the same types of antiretroviral drugs that are commonly used in HIV treatment.

For a microbicide to be effective at preventing HIV infection, it needs to act at one or more stages of the HIV life cycle. The first stage of the cycle involves viral binding, also referred to as ‘attachment’. Here, HIV attaches itself to the CD4 receptor on the surface of certain types of immune cells (such as T helper cells, monocytes,

macrophages, and dendritic cells). Next, the CD4 cell membrane fuses with the HIV envelope allowing HIV to enter the cell (stage 2, fusion). Once inside the CD4 cell, the virus uses enzymes (reverse transcriptase; stage 3, reverse transcription) to convert its own RNA genetic material into DNA. HIV DNA then enters the cell nucleus and integrates (via the integrase enzyme) with the cell's genetic material (stage 4, integration). Once DNA has been inserted, the cell replicates thereby producing the HIV proteins required as building blocks necessary for production of more viral particles (stage 5, replication). These proteins are then transferred to the cell surface and are assembled using via protease enzymes into new viral particles (stage 6, assembly), which ultimately bud from the cell and continue the infectious cycle (stage 7, budding). Microbicide products, either singly or in combination, are intended to disrupt one or more of these different stages of the HIV lifecycle [44,46–49].

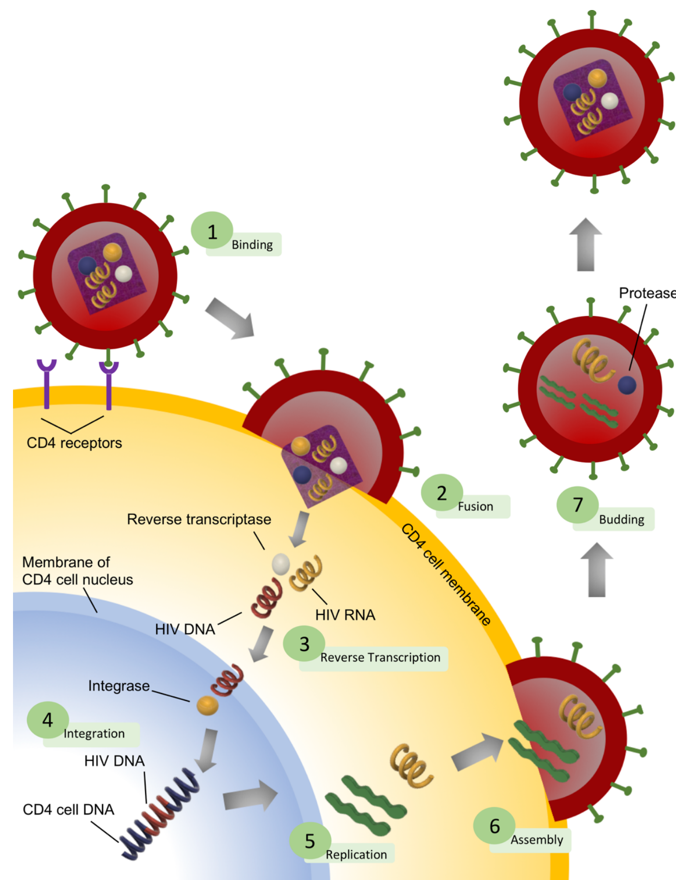


Figure 1. Depiction of HIV lifecycle. Stage 1- the virus cell attaches to CD4 receptors; Stage 2- CD4 membrane fuses with the HIV allowing the virus to enter the cell; Stage 3- the virus converts its own RNA into DNA; Stage 4- HIV DNA enters the cell nucleus and integrates with the cells genetic material; Stage 5- the cell replicates and produces more HIV proteins; Stage 6- Proteins are transferred to the cell surface and assembled into new viral particles; Stage 7- new virus particles bud from cell and continue the infectious cycle. Image reproduced from HIVinfo.NIH.gov [50].

Table 1. Summary of antiretroviral drugs that are being developed (or have previously been considered) as vaginal microbicides.

HIV life cycle stage	**Drug class**	**Mechanism of action**	**Examples**
binding	CCR5 antagonists (Entry inhibitor)	binds to CCR5 coreceptor to prevent HIV from gaining access to the cell	maraviroc [46,51,52] 5P12-RANTES [53,54] carrageenan [55–60] CMPD167 [61,62]
fusion	fusion inhibitors	peptides prevent conformational change and prevent desired structure for fusion	
reverse transcription	nucleoside reverse transcriptase inhibitors	inhibitor binds to DNA chain and terminates its production	tenofovir [48,49,63–66]
	non-nucleoside reverse transcriptase inhibitors	binds to the reverse transcriptase preventing the enzyme from catalysing DNA polymerisation	dapivirine [43, 46, 48,67,68] nevirapine [49] MIV-150 [58,59,69,70] MIV-160 [71]
integration	integrase inhibitors	inhibits integrase from having the ability to replicate the virus and infect new cells	MK-2048
replication	-		
assembly	-		
budding	protease inhibitors	binds to the active site of the protease enzyme preventing the maturation of the virus	saquinavir [49] darunavir [47]

Over the past twenty years, various microbicide candidates have been developed and tested in clinical trials. An aqueous vaginal gel containing nonoxynol-9 – a well-known spermicidal agent – was one of the first products to be tested [32–34]. However, repeated administration of the gel caused disruption of the vaginal mucosal epithelium and genital tract inflammation, ultimately leading to increased acquisition of HIV-1. Nonoxynol-9 and other surfactant-based microbicides were subsequently abandoned.

Carraguard®, a carrageenan-based vaginal microbicide, was also trialled as a HIV microbicide. The trial took place in five locations – three South African locations and two Indian locations – where women were at high risk of vaginal HIV transmission. The trial was prematurely halted due to concerns that the gel increased the risk of

transmission. It was later deduced that Carraguard® had no impact on rates of HIV acquisition. A smaller trial was also conducted testing Carraguard® for HPV-1 infection, demonstrating moderate efficacy [75].

The CAPRISA 004 trial was the first human clinical trial to demonstrate proof-of-concept of a vaginally administered microbicide in preventing HIV-1 infection. The antiretroviral agent tenofovir (TFV) had previously been shown to prevent vaginal transmission of simian immunodeficiency virus (SIV) in a macaque model when administered as a 1% w/w vaginal gel. In the subsequent phase I clinical study conducted in Africa, the same water-based gel was administered by women 12 hr before and after intercourse. Overall, the tenofovir gel reduced HIV acquisition by 39%. However, for those women who self-reported high levels of adherence, rates of protection were higher at 54% [66].

The 1% TFV vaginal gel was also assessed alongside two oral anti-retroviral drugs (TFV and Truvada®) in the VOICE trial (Vaginal and Oral Interventions to Control the Epidemic) [40]. However, unlike the CAPRISA 004 trial results, the VOICE trial showed no reduction in HIV incidence for any of the interventions compared to placebo, a result that was primarily attributed to poor user adherence [40]. For example, only 23% of women receiving the gel product had detectable levels of TFV in their blood at trial check points. The VOICE trial demonstrated unequivocally the correlation between adherence and product efficacy for HIV prevention products.

1.5 Intravaginal rings

Intravaginal rings (IVRs) are flexible torus-shaped devices – usually manufactured from either silicone elastomer or thermoplastic polymers – that are used to sustain release of active pharmaceutical ingredients (API) to either the vagina or the systemic compartment for a range of clinical indications. The first patent for an IVR was awarded to the UpJohn Company in 1970. The silicone elastomer device, comprising a stiff metal spring around which the silicone elastomer was molded, offered sustained release of the progestin medroxyprogesterone acetate over three months for hormonal contraception [76].

Currently, there are seven marketed (and one pending regulatory approval) IVR products incorporating a range of drugs in different polymers [32] (Table 2). One of the major advantages of vaginal ring compared to other products releasing similar drugs is that they are controlled by the woman. For example, for use as a contraceptive, IVR's are inserted and removed by the patient compared to having to attend a registered physician to have an IUD inserted. Another advantage is the local release of a small amount of specific drug compared to the systemic release of a large amount of drug e.g. the contraceptive pill.

Table 2. Marketed vaginal rings

Product	Active agent	Polymer	Ring type	Indication	Average release rate (µg/day)	Release time
Estring®	17ß-estradiol	silicone elastomer	reservoir	estrogen replacement therapy (local)	7.5	3 months
Nuvaring®	ethinylestradiol/etonogestrel	poly-ethylene-co-vinyl-acetate	reservoir	contraception	15/150	21 days
Ornibel®	ethinylestradiol/etonogestrel	polyurethane/poly-ethylene-co-vinyl-acetate	reservoir	contraception	15/150	21 days
Annovera®	ethinylestradiol/segesterone acetate	silicone elastomer	reservoir	contraception	13/150	21 days for up to 13 cycles
Femring®	17ß-estradiol-3-acetate	silicone elastomer	reservoir	estrogen replacement therapy (local and systemic)	50–100	3 months
Progering®	progesterone	silicone elastomer	matrix	contraception during lactation	10000	3 months
Fertiring®	progesterone	silicone elastomer	matrix	luteal phase supplementation in infertility treatment	10000	3 months
dapivirine ring	dapivirine	silicone elastomer	matrix	HIV-1 prevention	350–100	1 month

Polymers for the manufacture of intravaginal rings

To date, vaginal rings have primarily been manufactured from silicone elastomer materials and the thermoplastic polymer poly(ethylene-co-vinyl acetate) (EVA) [58, 61, 63, 68,69,77–79]. Both polymers are available as medical grade materials and are suitable for use in long-term implantable drug delivery systems. For example, Norplant® (no longer marketed) and Nexplanon® were/are subdermal implantable systems for contraception manufactured from silicone elastomer and EVA, respectively [80,81]. In recent years, thermoplastic polyurethanes (TPUs) have also been considered for fabrication of vaginal rings. For example, Ornibel® – a reservoir-type ring releasing etonogestrel and ethinyl estradiol – contains a hydrophobic TPU drug-loaded core. The polymers used for commercial vaginal ring products are summarised in Table 2.

Medical grade polymers for the manufacture of vaginal rings are required to meet the standards of United States Pharmacopeia (USP) Class VI. The different classes are based on end use and duration of expose to human tissues, with Class VI requiring the most stringent testing. Testing procedures include toxicity tests (to assess for toxic leachables), intracutaneous tests (to assess localised reaction of tissue to leachables), and implantation tests (to assess the reaction of the living tissue to the implant). Such stringent testing does not guarantee regulatory acceptance and extra testing should be carried out to meet ISO 10993 [82].

Thermosetting polymers

A thermosetting material is a polymer that when cured – by heat, UV light or chemical reaction – cannot return to its original non-cured state. This non-reversible reaction is caused due to the formation of strong chemical bonds – commonly referred to as crosslinks – between complementary reactive functional groups in the material. Thermoset materials are usually supplied in liquid or gel forms and can therefore be molded easily into the desired format.

Silicone elastomers are one of the most common biomedical thermoset polymers used in drug delivery and medical device applications. The relative lack of chemical functionality in the silicone elastomers polymer is responsible for their high

biocompatibility and bio-durability [83]. Five marketed vaginal ring products – Estring®, Femring®, Progering®, Fertiring®, and Annovera® – are currently manufactured using silicone elastomers, as is the experimental dapivirine ring currently undergoing regulatory review.

There are two types of silicone elastomers systems – addition-cure and condensation-cure systems. Addition-cure systems use a platinum-catalysed hydrosilylation reaction and do not produce any reaction by-product; these systems are particularly sensitive to catalytic poisoning by certain chemical functional groups. Condensation-cure systems are catalysed using a tin salt catalyst and produce an alcohol by-product. This alcohol by-product can be problematic, since drug(s) incorporated into the silicone are often highly soluble in the alcohol [77,84], leading to large burst effects.

Thermoplastic polymers

Thermoplastic polymers are materials that can be easily extruded or molded when melted/heated to a specific temperature. Upon cooling, the polymer then hardens again to take the shape of the injection mold or extrusion die [85]. Although a wide range of thermoplastic polymers are used for fabrication of medical drug delivery devices, only a very limited number are currently used in the manufacture of vaginal rings.

The most common thermoplastic polymers include polyethylene vinyl acetate (EVA), thermoplastic polyurethanes (TPU), nylons, polyethylene, polypropylene, polystyrene and polyvinyl chloride. In general, they consist of long molecular chains that are held together by weak intermolecular forces such as van der Waals forces, dipole bonds and hydrogen bonding. The weak nature of these intermolecular allows these polymers to melt and deform upon heating [86]. Depending on how their molecules are arranged, thermoplastics can be either crystalline or amorphous. A crystalline thermoplastic polymer will have a high mechanical resistance to stress and usually a high melting point due to a larger energy needed to break the ordered and compact arrangement of the polymer chains. Amorphous thermoplastic polymers are more flexible and elastic in nature due to the disordered arrangement of polymer chains.

Nuvaring® and Ornibel® – both containing etonogestrel and ethinylestradiol – are

manufactured using EVA and/or TPU polymers. Nuvaring® comprises a drug-loaded EVA core (containing 28% vinyl acetate (VA)) and a non-medicated EVA sheath (having 9% VA content). Ornibel® comprises of a TPU core and an EVA sheath containing 28% VA [87].

1. 6 Mechanisms for release of drug substances from IVRs

Molecular permeation – based on dissolving of drug in the polymer and subsequent passive diffusion – is the most common mechanism by which drugs are released from IVRs. Here, passive diffusion refers to the simple molecular diffusion of drug based on dissolution of drug and the establishment of a concentration drug concentration gradient between the ring device and the surrounding vaginal fluid/tissue (or *in vitro* release medium) [88].

Matrix-type rings are the simplest IVR design in which the drug(s) is evenly distributed throughout the entire ring volume. Two matrix rings have already reached market (Progering® and Fertiring®), and a dapivirine-releasing ring has recently been approved by regulatory authorities. Having the drug distributed throughout the ring body has implications for release, since, unlike reservoir rings, there is no distinct rate-controlling membrane. Typically, drug release rates matrix rings show a relatively high burst release followed by a steadily declining drug release rate (Figure 2) [46, 61, 63, 68,89,90]. The burst effect is due to the availability of drug in the surface layers of the ring immediately after manufacture. As drug is released from the surface layers over time via diffusion/permeation, a drug depletion zone forms through which the remaining drug molecules must first diffuse [90]. As the drug depletion zone increases over time, the drug release rate declines.

The drug substances contained in Progering®, Fertiring® and the dapivirine ring are deliberately incorporated into the rings at quantities above the solubility limit of the drug in the polymer, i.e. at least a fraction of the drug is present in the solid, and usually crystalline, state. Only the solubilised fraction of the drug in the ring is capable of diffusing and therefore releasing. As release continues, the solid drug particles in the polymer replenish the matrix with newly solubilised drug molecules and thus the

release is continued. In this manner, matrix type rings give rise to root time kinetics, also known as $t^{½}$ kinetics (Figure 2).

Progering® and Fertiring® contain relatively high initial loadings of progesterone (12–25% w/w), which translates into relatively high daily doses (~10 mg/day). Even at these high doses, progesterone is not excreted in the milk of breastfeeding mothers, making Progering® a useful form of contraception for breastfeeding mothers [91,92].

Reservoir-type IVRs consist of a drug-loaded polymer core which is encapsulated with a non-medicated, rate-controlling membrane (also known as a sheath). Only drug molecules that are molecular dispersed (i.e. dissolved) in the polymer can pass through the sheath; therefore, the rate of release is controlled by the thickness of the sheath and constant drug release will continue as long as there is drug present in the polymer core. Release rates are also dependent upon the partition coefficient of the drug across the device surface / release medium interface. Due to their design, reservoir-type IVRs offer much lower release rates compared with matrix-type rings which in some cases this may be desirable to achieve a therapeutic level. By inserting the drug into a reservoir core, the release rate can be controlled and slowed to achieve desired therapeutic limits.

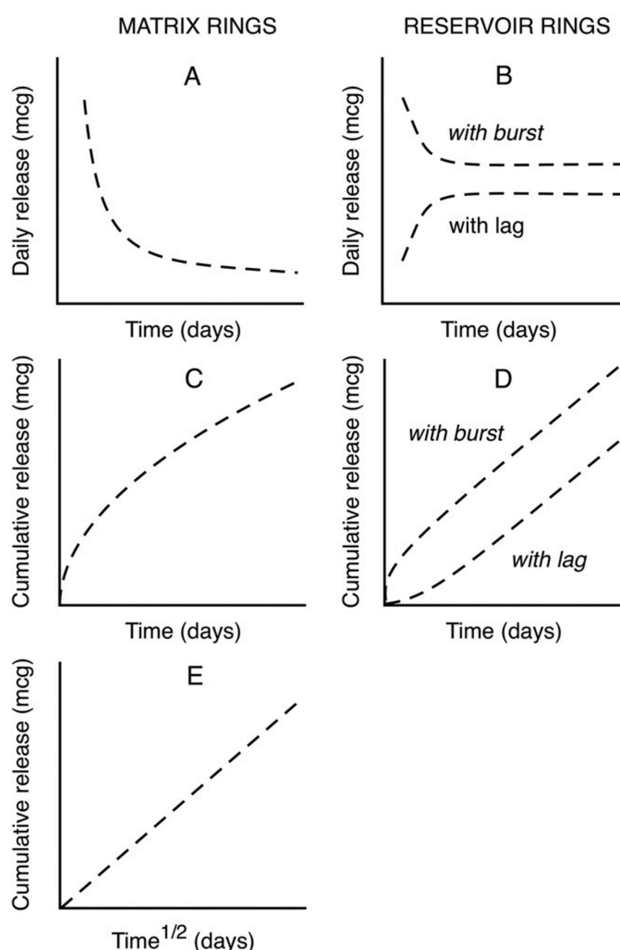


Figure 2. Representative daily and cumulative release vs. time profiles for non-swelling matrix and reservoir-type vaginal rings. Characteristic cumulative release vs root time kinetics is also shown. Image reproduced from Malcolm *et al.* [32].

Boyd *et al.* have reported the release of DPV and LNG from both matrix and reservoir-type rings. Day 1 DPV release from matrix rings was in the range 4132–6113 μg/day, compared with 61–131 μg/day for reservoir rings. These significant differences in drug release are characteristic of such ring designs, and usefully illustrate the release retarding effect offered by the non-medicated sheath [89]. The marketed vaginal rings Estring®, Nuvaring®, Ornibel®, Annovera® and Femring® are all reservoir-type devices; Progering®, Fertiring® and the 25 g DPV ring are all matrix-type devices.

Vaginal rings are also being developed containing drug-loaded pod inserts [49, 63,93–95]. The pods – comprising compacted drug powder and a semipermeable polymeric thin film with a hole drilled – are placed into a silicone elastomer ring body containing windows to expose the pods to the external fluid. Unlike conventional matrix and reservoir-type rings, the pod-insert rings are useful for sustained release of a drugs

having very different physicochemical properties, including hydrophilic and macromolecular drugs. By adjusting the size of the window in the ring, the surface area of the semipermeable membrane in contact with the media can be adjusted to modulate the drug release rate.

1.7 Manufacturing methods

Manufacture of thermosetting materials

Thermosetting silicone elastomers are the most common materials used in the manufacture of IVRs. Silicone elastomer are generally supplied as two-part kits, comprising Part A and Part B, which need to be mixed in a specific ratio. When using silicone elastomers for drug delivery applications, the drug material is added and mixed to both Parts A and B. Mixing can be achieved using manual mixing with a spatula or using a double-asymmetric centrifugal (DAC) Speedmixer® machine. When both parts A and B are mixed, the crosslinking reaction is initiated, and therefore the resulting active mix has a limited handling time. Typically, the active mix is then injected into a pre-heated custom mold assembly fitted to an injection molding machine to form the desired ring geometry. This injection molding process is outlined in Figure 3. For matrix rings, only a single injection step is required. For reservoir rings, the drug-loaded cores are manufactured first before being overmolded with non-medicated silicone in a further one or two-step process.

Drug-loaded silicone elastomer cores can also be manufactured using extrusion technologies. The active mix is placed in a temperature-controlled barrel and pushed down the barrel by an Archimedean screw. At the end of the barrel, the mix is pushed through a die of specific geometry and size to produce an extrudate having the desired cross-sectional profile. Often, the extrudate is then heat-treated in an oven in a final post-curing step. Although this method allows specific shapes and sizes to be produced for reservoir-type IVRs, it is significantly more labour intensive than the manufacture of matrix type IVRs.

The silicone elastomer NES/EE cores of the Annovera® IVR are manufactured via extrusion and the ring body via injection molding [96]. Estring® is also manufactured

using both extrusion and injection molding; the core is first extruded, cut to length and then overmolded with non-medicated silicone to produce the final ring.

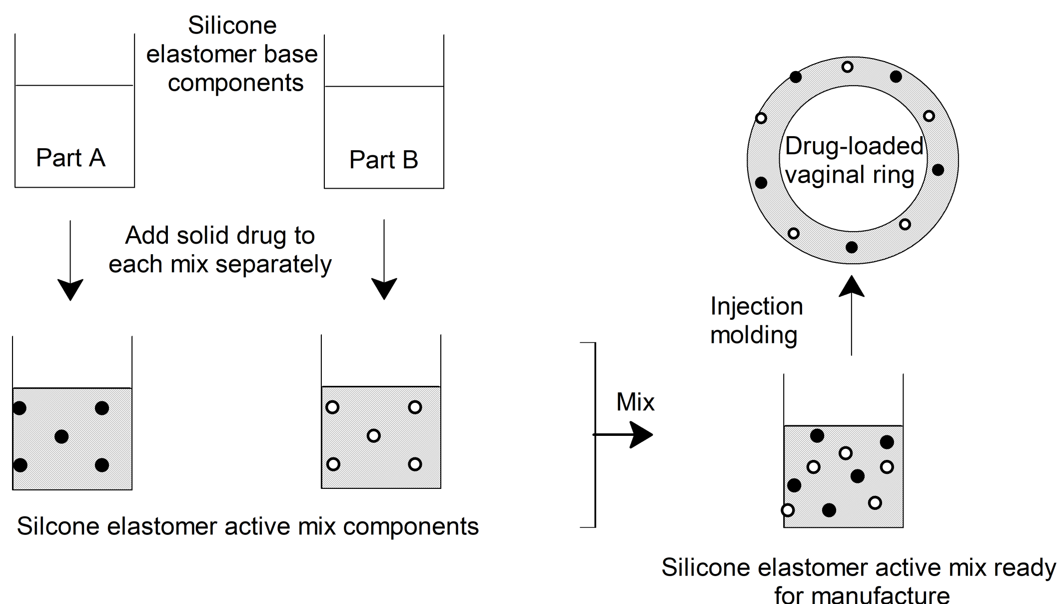


Figure 3. General method for the manufacture of silicone elastomer matrix rings. Additional, but similar, steps are required for the manufacture of reservoir rings.

Manufacture of thermoplastic materials

When manufacturing IVRs from thermoplastic materials, a first requirement is to incorporate the drug(s) into the thermoplastic polymer. Pellets of the selected thermoplastic polymer are mixed with the powdered drug and fed into a twin-screw extruder to form a compounded material in which the drug is dispersed throughout the polymer. This step is often repeated to assure uniformity of mixing. The compounded material is then extruded and the extrudate pelletised to produce drug-loaded thermoplastic pellets. These drug-loaded pellets can then be used directly for manufacture of vaginal rings using hot melt extrusion, injection molding or 3D printing techniques. The overall process is depicted in Figure 4.

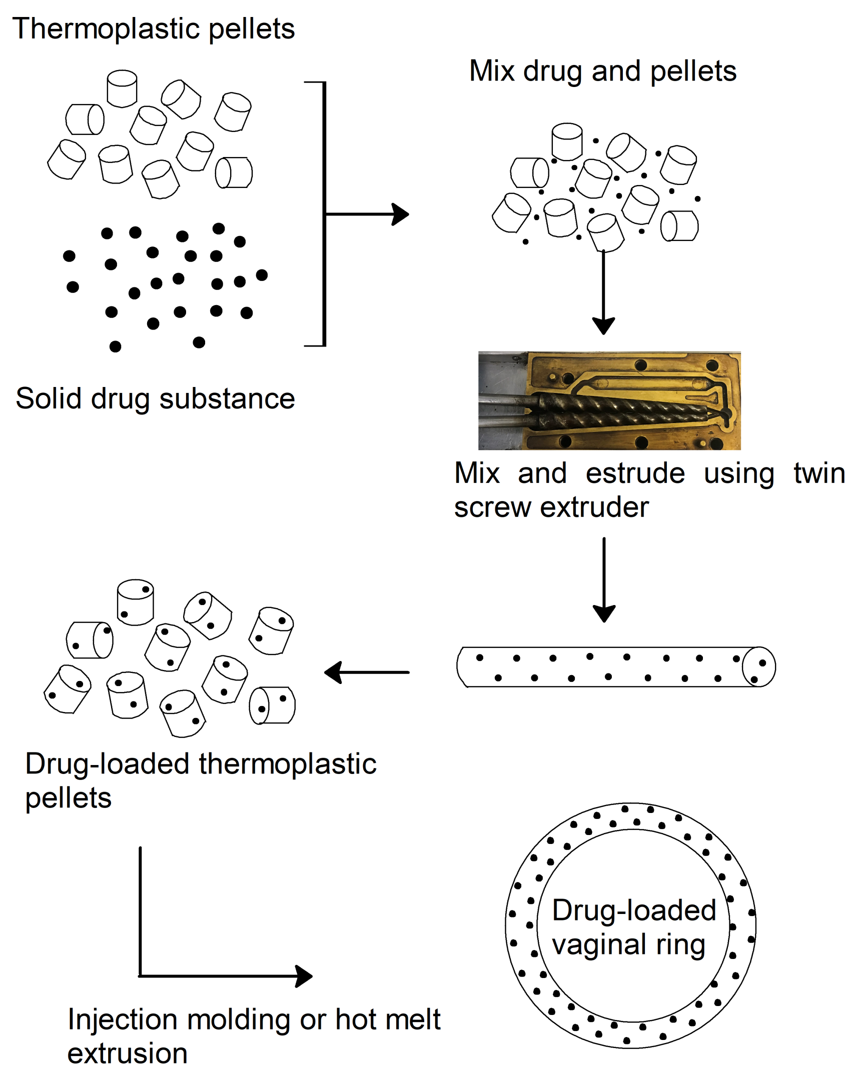


Figure 4. General method for the manufacture of thermoplastics matrix rings. There are additional steps for the manufacture of reservoir rings.

The marketed vaginal ring products Nuvaring® and Ornibel® are manufactured from thermoplastic polymers. Pelletised EVA and TPU (for Nuvaring® and Ornibel®, respectively) containing the actives are mixed using a twin-screw extruder and fed through a custom co-extrusion die. A second extruder then feeds non-medicated EVA to over mold the core providing the rate controlling layer [97,98].

1.8 Microbicide-releasing IVRs

Building on their success as drug delivery systems for contraception and estrogen replacement therapy, IVRs are being developed for sustained release of antiretrovirals as a microbicidal strategy for HIV prevention. A matrix-type silicone elastomer IVR

offering sustained release of nonoxynol-9 (N9) was first reported in 2003 [90]. Unusually, nonoxynol-9 is a liquid at room temperature, giving rise to unusual release characteristics when formulated in a ring. N9 was later discontinued as a microbicide candidate after it was shown to disrupt the vaginal mucosa and increase the risk of HIV acquisition [72,73].

Dapivirine (DPV), previously known as TMC120, is currently the lead HIV microbicide candidate and is being actively developed as a matrix-type silicone elastomer IVR formulation. The ring has been reported to provide long-term controlled release of the microbicide at 136 µg/day over 71 days. However, calculations showed that this release rate could be theoretically maintained for up to four years [68]. Baeten *et al.* and Nel *et al.* have reported the ring as releasing sufficient concentrations to prevent HIV acquisition [38,99].

MIV-150 is a NNRTI drug with similar properties to DPV. Singer *et al*. reported a daily release of 33–111 µg/day from a matrix-type EVA polymer containing 40% VA [69]. The study was able to demonstrate the protective potential of MIV-150 against vaginal immunodeficiency virus infection which reduced SHIV transmission in macaques by 58% compared to a placebo study.

Tenofovir (TFV), a nucleoside reverse transcriptase inhibitor, has been shown to be partially effective in preventing HIV acquisition in women when administered vaginally as a 1% TFV gel. However, a subsequent study found the gel to be no more effective than the placebo [66]. Johnson *et al.* have reported the release of TFV in the form of a reservoir ring [100]. The ring – comprising a water-swellable hydrophilic TPU and providing TFV release of ~10 mg/day over 90 days – has similar mechanical properties and dimensions as Nuvaring®. In a sheep pharmacokinetic study, TFV concentrations in the vaginal fluid were 1000-fold greater than those determined necessary to provide protection. More studies are planned to assess its candidacy as a microbial available for release from an IVR.

1.9 Multipurpose prevention technologies

Multipurpose prevention technologies (MPTs) are products designed to simultaneously provide protection against HIV infection, STIs and/or unintended pregnancy [101]. Although the MPT term is relatively new, this type of product has in fact been used for hundreds of years in the form of the male condom. There is ample evidence in the literature to support the effectiveness of male condoms for prevention of sexual transmission of HIV and other STIs. Despite their effectiveness, male condoms have not found widespread use. Men often object to using them and women often find it difficult to negotiate condom use with their male partners [30, 34–36,102,103]. Female condoms are also available, including new several new designs introduced in recent years [104–106]. However, they are not as common as their male counterpart, mostly due to higher costs and poor user acceptability. New MPT products, and particularly female-controlled products, are urgently needed, with a strong preference for those that offer long-acting protection and discrete use.

Contraceptive diaphragms are a barrier method of contraception. By covering the cervix during intercourse, they prevent sperm from passing through the cervix into the uterus and fertilising an egg. When used correctly (and often in combination with a spermicidal gel), diaphragms offer 92–96 % effectiveness at preventing pregnancy; however, under more typical use, effectiveness decreases to ~88% [107,108]. Diaphragms are usually inserted shortly before sex and removed within 6 hr after sex. A new diaphragm product, named CAYA® (previously SILCS), has recently been marketed. CAYA® comprises an anatomically contoured nylon-6,6 spring core that is overmolded with a silicone elastomer barrier sheath. The concept of reformulating the SILCS diaphragm to additionally provide controlled release of the antiretroviral microbicide dapivirine from the spring core has also previously been reported [109]. The adapted device contained 10 %w/w DPV incorporated into a polyoxymethylene (POM) spring encased with the original silicone sheath. *In vitro* release studies demonstrated continuous and controlled release of DPV over a 6-month period at rates close to 174 μg/day. Moreover, the modified device showed similar flexural and mechanical properties to the original SILCS diaphragm device. These results provide proof-of-concept for a non-hormonal, female-initiated device that can provide both protection from unwanted pregnancy and HIV-1 infection.

With the DPV-releasing vaginal ring having recently completed Phase III clinical testing, a next-generation MPT version of the ring is already in development offering simultaneous release of both DPV and the contraceptive progestin levonorgestrel (LNG) [43, 47, 67,68, 89, 99,109]. Levonorgestrel (LNG) has a long history of use in controlled release contraceptive products and is listed by the World Health Organisation as an essential medicine [110]. LNG is currently marketed in a range of products including the intrauterine device Mirena® (releasing 20 µg/day LNG) and the reservoir-type subdermal implant Jadelle® (releasing 35 µg/day LNG). Both products offer protection from pregnancy for up to 5 years [80,111,112]. A vaginal ring releasing 20 µg/day of LNG was previously developed and reported by the World Health Organisation, although it is not presently under development [113].

Simultaneous release of DPV and LNG from both matrix and reservoir-type silicone vaginal rings has also recently been reported [89]. Continuous release for 60 days was achieved with a silicone elastomer matrix-type vaginal ring, while a reservoir-type ring offered controlled release for up to 180 days with a mean daily release rate of 75–131 or 37–66 µg/day for DPV and 96–150 or 37–57 µg/day for LNG, depending on the configuration of the reservoir ring. The matrix ring, developed in partnership between Queen's University Belfast and the International Partnership for Microbicides, is currently progressing through early-stage clinical studies [114].

A segmented dual-reservoir MPT vaginal ring constructed from a thermoplastic polyurethane (TPU) has been reported for the co-administration of LNG and TFV [64]. A major obstacle in the development of this device was the significantly different hydrophilicities and potencies of the two drugs – LNG is highly hydrophobic and TFV is hydrophilic; and relatively large daily mg/day release rates are required for TFV, compared with µg/day quantities for LNG. A novel segmented ring design, containing discrete hydrophilic and a hydrophilic TPU components, was required to achieve release rates of 10 mg/day TFV and 20 µg/day LNG over ≥ 90 days in a rabbit model. The TFV segment comprised of a hollow core reservoir using a hydrophilic TPU. This TPU had the capability of providing a high drug loading capacity with sufficient release rate. The LNG segment is a solid core reservoir using a hydrophobic TPU with

design similar to NuvaRing. The preliminary data demonstrate the potential of a dual-segment ring for simultaneous release of different APIs. Phase 1 clinical trials are ongoing.

A novel silicone elastomer IVR containing multiple pod inserts containing TFV, nevirapine (NVP), saquinavir (SQV), estradiol (E2) and etonogestrel (ETG) has been reported [49,63]. The pods are made from compressing powder materials, including the drug substances, into a mold to form a pellet which is then coated with polylactic acid. The silicone IVR body was manufactured using a two-component silicone elastomer. In an ovine model, steady state drug delivery was maintained for 28 days, demonstrating the versatility of pod design rings to deliver antiretrovirals and a contraceptive.

Population Council has reported a novel multipurpose technology vaginal ring device aimed at preventing infection with HIV-1, HSV-2, HPV and reducing rates of unintended pregnancy [58]. The device incorporates four different active agents – MIV-150 (an experimental HIV microbicide), zinc acetate (ZA, to target HIV-1 and HSV-2), carrageenan (CG, targeting HPV and HSV-2) and LNG acting as a progestogenic contraceptive. The device comprises two components – a central core containing a compressed powder mixture of CG and ZA, and a surrounding EVA-28 matrix containing dispersed MIV-150 and LNG. Pores of various sizes are then drilled into the CG/ZA core to produce an access path from the surrounding liquid to the hydrophilic core. This ring design is particularly useful is permitting simultaneous release of both hydrophobic and hydrophilic active agents. *In vitro* release data using macaque prototype rings demonstrated steady state release of the hydrophilic APIs from the core. Levels of LNG achieved in the vaginal environment following ring administration in macaques were considered acceptable for contraception. Cell-based assays showed that all APIs retained their antiviral activity. The potential of this MPT ring is dependent on improving the ease of manufacture production and testing of a larger-sized ring in women.

1.10 Challenges facing MPT devices

With a number of new MPT devices in development, many obstacles need to be overcome in order to achieve success. Challenges include achieving therapeutic drug concentrations for all APIs while maintaining safe drug tissue levels, addressing adherence and acceptability of using the device, and careful consideration of issues around both viral and antibiotic resistance.

Achieving therapeutic drugs levels for different APIs

In general, therapeutic doses for vaginal administration of antiretroviral, antibacterial and contraceptive steroid drugs are very different. Steroids represent some of the most potent drugs currently used in therapeutics. For example, Nuvaring® provides daily administration over 21 days of 120 µg etonogestrel and 15 µg/day ethinyl estradiol; the Mirena® IUS offers uterine administration of LNG starting at 20 µg/day; and previous efforts to develop a LNG-releasing IVR targeted LNG release within the range 20–35 µg/day [112,115,116]. By comparison, many small-molecule reverse transcriptase inhibitors, such as DPV and TFV, require vaginal doses somewhere within the range 100–1000 µg/day. Further, many of the antibacterial agents commonly administered vaginally require doses in the range 1–1000 mg. Also, the intended duration of drug treatment is often different for the various clinical indications. Continuous, long-term administration of antiretrovirals and steroids is preferable, while antibacterials are usually only required over a shorter term for curative treatment. These issues highlight the very considerable challenges in the development of a practical and effective MPT formulation. Although drug release can be controlled in a myriad of ways, combining multiple strategies into a single product will prove very difficult.

Antimicrobial resistance to APIs used in MPT devices

Antimicrobial resistance occurs when microorganisms (such as bacteria or viruses) are genetically changed following the pressures of exposure to antimicrobial drugs (antivirals or antibiotics), resulting in the drugs no longer being effective for treatment [117]. This emergence of resistant strains of the microorganism makes it difficult to treat simple infectious diseases which leads to the spread of the disease, more

extensive treatment regimens and potentially more deaths. Although resistance occurs naturally over time as viruses and bacteria change genetically, the misuse of antibiotics and antivirals is accelerating the emergence of resistance. For this reason, antiretroviral and antibacterial APIs need to be carefully considered [118–122].

Adherence and acceptability

Adherence has been defined by the EMA as "the degree to which patient behaviours coincide with the healthcare providers and patients jointly agreed healthcare objectives and respective therapeutic regimen" [123]. EMA also defines acceptability as "the overall ability and willingness of the patient to use and its care giver to administer the medicine as intended." [124]. Adherence is a particular issue with self-administered products. The challenge of adherence and acceptability has been widely reported and is crucial in providing accurate data on efficacy and effectiveness of the product [32,33,73,79,118–123].

An important aspect in developing a new product is to assess its acceptability in the target population. For example, an acceptability study on the Annovera® vaginal ring across women in eight countries found that satisfaction rates were consistently high, ranging from 84–95% [128]. The study also reported that women were twice as likely to be adherent to the ring regime if they were satisfied with the product. For microbicide releasing vaginal rings, van Straten *et al*. has reported an adherence and acceptability study of a vaginal ring containing DPV and maraviroc. At the start of the phase I study in the US, the women expressed a range of minor concerns with the ring. However, during the study few concerns were actually experienced [52]. This issue has been widely reported in trial data however, once the trial starts, no problems arise [131–133].

During clinical trials, adherence is reported using either direct or indirect measures. Direct measures include biomarker and biometric measures of drug in bodily fluid, drug in hair levels or the body's response to the drug [134]. Indirect measures are sub divided into two categories. Self-reporting, which involves the participants completing questionnaires on how adherent they were to the product regime, and objective measures which require observations from clinicians [127]. Mensch *et al*. recently

reported on the topic of adherence during a DPV IVR phase 3 ASPIRE trial [135]. The trial used and compared direct and indirect measures of non-adherence. Direct measures involved reporting of residual DPV in the IVR and quarterly assessment of DPV plasma concentrations, while indirect reporting involved monthly self-report via questionnaire. Incidence of non-adherence was significantly higher for direct measures, suggesting that non-adherence was under-reported in the self-reporting measures. 11% of participants aged 18–21 and 7% of 22+ age range were considered non-adherent but rated themselves as adherent. So, although the ASPIRE study demonstrated ring safety and protection from HIV, it also highlighted the challenges of adherence and self-reporting [99]. Further studies, including the open-label HOPE trial for the DPV IVR, will make use of real-time drug monitoring and counselling to potentially overcome these problems.

Unsurprisingly, low adherence has been shown to correlate with reduced efficacy in HIV prevention studies. In a 1% tenofovir gel phase IIb clinical trial, CAPRISA 004, the gel prevented HIV acquisition by 39% overall [66]. However, when adherence categories were broken down into high (gel adherence > 80%), intermediate (gel adherence 50–80%) and low (gel adherence < 50%), a distinct correlation between adherence and effectiveness was revealed. 54% reduction in HIV incidence was reported for high adherers, compared to 38% and 28% reduction in acquisition for intermediate and low adherers, respectively. Although this study was the first to demonstrate that a topical antiretroviral gel could be used to reduce HIV acquisition, it also effectively highlighted the need for high adherence for precise clinical trial results.

Nel *et al.* reported a safety, acceptability and adherence study of DPV ring users in a clinical trial in multiple countries in Sub-Saharan Africa [136]. The trial assessed 280 HIV-negative woman aged 18–40 years over a 12-week period, half were enrolled with a 25 mg DPV ring and the other half, a placebo ring. Safety was assessed by direct methods while patient acceptability and adherence was assessed by indirect methods (self-report). After the 12-week period no safety concerns were found. Self-reports found the ring acceptable (97%) and adherence was very high. However, Baeten *et al*. reported on another DPV vaginal ring trial, the phase 3, randomised,

double-blind, placebo-controlled trial MTN-020-ASPIRE trial of the DPV ring. This trial recruited woman between the ages of 18 and 45 years in five African countries. Incidence of infection of HIV-1 in the DPV group was lowered by 37 % compared to the placebo group. However, woman over the age of 21 years showed a higher rate of protection than younger women (56 % compared to -27 %). This difference was correlated with reduced adherence among the younger woman. It was concluded that the DPV ring reduces the risk of HIV-1 infection demonstrating efficacy of the ring, but that efficacy was only evident in those groups having high adherence. Comparing these two trials of the same product show differences in adherence. Nel *et al.* reported adherence using indirect measures while Baeten *et al.* used direct measures. Self-report suggested 92% of participants fully adhered to the treatment for the entire trial irrespective of age, while Baeten's study indicated 70% adherence. These results align with data from other microbicide trials, demonstrating that self-report is not an accurate method for capturing information on user adherence. Combining direct and indirect measures may offer more accurate measures.

Potential strategies for more accurate measurement of adherence have been reported, including advanced vaginal gel applicators that contain dye or record time and date of application and temperature-logging vaginal rings [127,137]. For example, Boyd *et al.* have reported a temperature-monitoring vaginal ring, demonstrating that the ring was able to measure both accurately and continuously the environmental temperature and that the device responded quickly to temperature changes. *In vivo* studies in macaques also showed high sensitivity to daily changes in body temperature as well as ring removal. Given the previously mentioned problem of adherence, a temperature-recording vaginal ring could be used to accurately monitor adherence. However, this would require complex adaption of existing ring formulations.

1.12 Active pharmaceutical ingredient profiles

Nestorone

Nestorone (NES, also known as segesterone acetate), a hydrophilic molecule (log P = 2.9) with a molecular weight of 370.49 g/mol, is a 19-norprogesterone derivative with a progesterone-like hormone receptor binding profile which is only weakly active

when taken orally. When delivered by subcutaneous implant or by intravaginal ring, its potency is found to increase by 100-fold compared with oral administration [138]. NES works as a contraceptive to inhibit ovulation and thicken the cervical mucus, therefore preventing fertilization [139].

Recently, Population Council have received FDA approval for Annovera®, the first contraceptive vaginal ring that provides protection against unintended pregnancy for one year [140]. The ring, which provides release of 150 µg/day NES and 13 µg/day EE, is inserted for a period of 21 days and removed for 7 days for up to 13 cycles. The addition cure silicone elastomer IVR body contains two reservoir cores, one containing Nestorone and ethinyl estradiol (EE) and the other containing NES alone. NES/EE combination was found to be more effective at suppressing ovulation and controlling breakthrough bleeding than NES only [141].

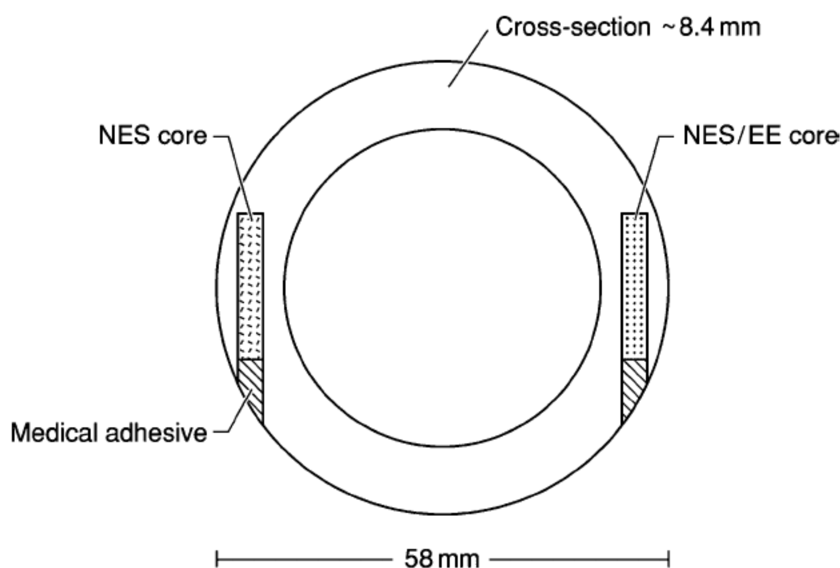


Figure 5. Schematic of NES/EE contraceptive ring showing location of the two drug-loaded cores. Images reproduced from Sivin *et al.* [96]

Although Annovera® is currently the only marketed product that contains NES, other NES products are being developed. For example, a contraceptive vaccine is being researched by the Sailor Research Group at the University of California. Biodegradable porous silicon microparticles that act as framework to deliver NES for up to 6 months are being explored, offering advantages such as allowing woman to have control over their contraceptive need as well as visiting clinics less frequently

[142]. Also, Population Council is currently investigating a contraceptive transdermal NES/estradiol gel for woman and a NES/testosterone gel for male contraception, both of which are currently in phase 2 clinical trials [143–145]. Early research shows that both gels are well tolerated with no adverse effects. NES is used in conjugation with estradiol to supress ovulation but with fewer side effects than with oral contraceptives while NES/testosterone gel supresses sperm production while minimising side effects such as lower libido, weight gain and acne.

MIV-150

MIV-150 is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which inhibits HIV-1 replication by blocking the reverse transcriptase enzyme from completing reverse transcription of the viral single-stranded RNA into DNA [146]. Although MIV-150 was originally developed by Medivir AB (Huddinge, Sweden), the Population Council has been given exclusive rights for its use under contractual agreement. MIV-150 is a hydrophobic molecule (log P = 3.5) will a molecular weight of 368.4 g/mol. Data from early phase 1 clinical trials showed that MIV-150 exhibited low both poor oral bioavailability and systemic absorption, properties that often bode well for vaginal administration [147]. *In vitro* studies have also revealed three important characteristics of MIV-150 [56]. First, it binds tightly to the HIV-RT enzyme and effectively inactivates clinical isolates of HIV at very low concentrations *in vitro*. Secondly, MIV-150 is effective at inactivating viruses that have developed resistance to other antiviral compounds, including NNRTIs, NRTIs and protease inhibitors. Thirdly, viral resistance to MIV-150 was found to develop slower compared to other marketed NNRTIs.

MIV-150 has previously been investigated in a number of experimental microbicide products, combination microbicide products, and multipurpose prevention technology products. A combination microbicide gel containing MIV-150 and carrageenan was found to better protect macaques against vaginal challenge with simian/human immunodeficiency virus compared with carrageenan alone; the combination gel required a tenfold lower dose to achieve EC_{50} values compared to carrageenan alone [56]. MIV-150 also effectively inactivates free virus and seminal fluid had no effect on the effectiveness of the combination microbicide. This combination had been

further evaluated by adding zinc acetate to the gel which was found to have potent activity against HIV/SHIV, HSV-2 and HPV [148]. Hsu *et al.* reported that the carrageenan gel containing MIV-150 and zinc acetate prevented SHIV-RT infection but only reduced HSV-2 by 20% when a single dose co-challenge was presented. This study demonstrated that MIV-150 can prevent immunodeficiency infection in the presence of other infectious microorganisms such as HSV-2, but also could reduce the likelihood of co-infection after a high dosage exposure.

Ugaonkar *et al.* have reported a novel IVR based on the same combination of actives as is the microbicide carrageenan gel containing MIV-150 and zinc acetate. MIV-150 and LNG are incorporated into an EVA-28 sheath that encapsulates a core containing ZA and CG [58]. Pores are drilled into the device to allow fluid access to the ZA/CG core. MIV-150 was released under non-sink conditions into 10 mL of 25 mM acetate buffer at pH 4.2. Under these non-sink conditions, MIV-150 crystallised on the IVR surface. MIV-150 was found to be stable throughout the study and released from the ring according to $t^{1/2}$ release kinetics. *In vitro* MIV-150 release of 5–7 µg/mL was reported for the IVRs, significantly above the 4 µg/mL target. In *in vivo* studies, Derby *et al.* reported that the IVR prevented SHIV-RT infection, reduced HSV-2 and provided adequate dosing of LNG to prevent cycling and also provide contraception. Based on experiments in mice, the CG component may also protect against HPV infection. No SHIV resistant viruses were detected after repeat exposure with the microbicide.

Figure 6. MPT vaginal ring reported by Population Council comprising of a MIV-150/LNG matrix ring with a zinc/carrageenan core. Image reproduced from Ugaonkar *et al.* [58].

Metronidazole

MET is an antibiotic with hydrophilic character (log P = –0.02) and a molecular weight of 171.156 g/mol. It is named by WHO as a key access antibiotic [110], and is commonly used in the treatment of bacterial vaginosis (BV), a vaginal infection caused an imbalance in the normal microbial balance in the vagina. The displacement of normal lactic acid-producing lactobacilli with unwanted anaerobic bacteria causes a rise in vaginal pH [20,21]. Current products for the treatment of BV include an oral tablet and a vaginal gel. An oral dose of 500 mg MET is prescribed twice daily for 5–7 days or MET gel (0.75%) at a dosage of 5 g per day can be prescribed for localised treatment [119]. Both dosage forms have been reported as having similar efficacy but with less side-effects reported in the use of localised treatment (e.g. nausea) [149,150].

MET has been used to treat infections such as skin, mouth and vaginal infections for more than 45 years and although rates of resistance are low there have been reported cases [119,151–157]. BV is commonly treated with MET. However, since no specific bacteria causes the infection, treatment with MET does not have good long-term outcomes, and recurrence is common. Over the last number of years, cure rates have decreased from 90% to 50–80% [158]. There are potentially two reasons for this decreasing cure rate, firstly, there are forms of bacteria that have become resistant to MET or secondly, the full infection is not being cured. The second reason seems more likely due to the fact many people stop taking antibiotics once their symptoms have subsided rather than finishing a treatment regime which will usually treat the infection.

Verstraete *et al.* have reported a polyurethane-based IVR for the treatment and prophylaxis of recurrent BV [159]. MET was incorporated into a hydrophilic TPU at two drug loadings (25% and 50% w/w) via hot melt extrusion and then IVRs manufactured using injection molding. IVR segments were placed into 3 mL of demineralised water and stored at room temperature for a period of 7 days with daily sampling and replenishment of media every 24 hr. The same quantities of MET were released the first 5 days of the study, suggesting saturation of the release medium and lack of sink conditions. The ring containing 25% w/w MET loading released all of the drug after 6 days, while the 50% w/w ring provided 50% after 7 days. Therefore, the lower loading might be useful for short treatment regimens, and the higher loading

more suitable for longer treatment, such as with recurrent BV. With the 3 mL volume used for testing, MET release provided concentrations ranging from 0.4–12.1 mg/mL, which is above the reported minimal inhibitory concentration [160–162].

1.13 Aims

In this project, the aim is to develop a new multipurpose prevention technology (MPT) platform to help address the global health burdens associated with sexually transmitted infections (e.g. HIV-1, HSV-2, HPV) and unmet need for contraception, which disproportionately affect young women in low and middle-income countries. The new MPT product concept is based around an innovative vaginal ring device in which drug-containing cores are physically inserted into and retained within a ring-shaped frame using 'interference fits'. Various model drugs will be used to demonstrate the concept: MIV-150 is an experimental antiretroviral drug owned by Population Council; Nestorone (NES) is a contraceptive progestin; metronidazole (MET) is an antibiotic commonly used to treat bacterial vaginosis; and lactic acid (LA) and its cyclic dimer form lactide (LT) can maintain protective vaginal pH and provide an anti-inflammatory effect.

The specific objectives are as follows:

I. Design, develop and validate ultra-performance liquid chromatography methods for all *in vitro* and content assay studies used throughout the project
II. Determine solubility of MIV-150, NES and MET in silicone at high temperatures alongside a low temperature solubility study involving silicone and thermoplastics.
III. Examine potential polymers for the manufacture of the ring body and determine their mechanical properties using various mechanical tests.
IV. Explore problems associated with the novel ring body such as reduced surface area and to determine an *in vitro* release medium to use throughout the project.
V. Perform *in vitro* release testing of silicone cores incorporated separately with the chosen APIs placed in the novel ring body.
VI. Perform *in vitro* release testing of novel ring body containing two types of API cores.

VII. Design a 28-day release study and subsequent two-month stability study on novel ring body containing three API cores.

VIII. Optimise novel ring body containing three API cores for a 72-day *in vitro* release study.

IX. Explore the use of lactide incorporated into silicone to maintain vaginal pH.

2

Materials and methods for sample analysis

2.1 Materials

DL-lactide (LT) (99%) was purchased from Alfa Aesar (Thermo Fisher Scientific, Lancashire); micronized DPV was supplied by S.A. Ajinomoto Omni Chem N.V. (Wetteren, Belgium); micronized NES and MIV-150 were supplied by Population Council (New York City, NY, US); and micronized MET was supplied by Farchemia Srl (Treviglio, Italy).

HPLC-grade acetonitrile, acetone, concentrated hydrochloride acid, phosphoric acid (85% w/w in water), sodium hydroxide, methyl red and Tween® 80 were purchased from Sigma-Aldrich (Gillingham, UK). Potassium phosphate and ammonium acetate was purchased from Honeywell (Bucharest, Romania). A Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK) was used to obtain HPLC-grade water.

Thermoplastic elastomers (Hytrel® HP-4056, HY; Desmopan 9370AU, DS; Vistamaxx TM 3000, VX; Estane- S8212, ES; Pebax- 4S335P01, PB) were gifted from School of Mechanical and Aerospace Engineering, at Queen's University, Belfast. EVA (17.5, 28 and 40% vinyl acetate grades) were obtained from Ateva (Edmonton, USA). TPU-87 was obtained from Lubrizol (Ohio, USA). Polyoxymethylene copolymer (POM; Hostaform MT24U01) was purchased from Ticona, UK (Telford, UK). Polypropylene (Luban HP 1151K, PP) was obtained from Orpic (Oman). Silicone tubing was obtained from Cole-Palmer (St. Neots, UK). DDU-4320 silicone elastomer kits were purchased from NuSil Technology LLC (Carpinteria, CA, US).

2.2 Methods

Ultra-performance liquid chromatography

Ultra-performance liquid chromatography was used provide quantitative and qualitative analysis for *in vitro* release studies, ring content studies, drug solubilities and drug stability assessments. A Waters Acquity UPLC system (Waters Corporation,

Dublin, Ireland) consisting of the following components was used for all UPLC analysis: binary solvent manager, sample manager, TUV detector.

2.3 UPLC method validation

The main purpose of a method validation is to demonstrate that the chosen method and parameters are fit for purpose in the identification and quantification of the selected analyte(s). A validation procedure consists of a number of different tests. All UPLC methods described in this thesis have underwent extensive industry standard validation and have passed all relevant specification criteria.

Linearity and Range

"The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample" [163]

For this analytical method, a range of at least five different concentrations are prepared and injected into the UPLC. The areas of these injections are then plotted against their concentration to ideally produce a linear plot. The success of this test is indicated by the equation of line. The desired value of the coefficient of determination is $R^2 = 1$. This indicates the data plotted is predicable to the equation of the line in the format $y=mx+c$.

"The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity." [163]

The range is usually determined from the linearity concentration. If an analytical method is proved to have pass criteria for precision, accuracy and linearity, the linear range is used as these values.

Accuracy

"The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found." [163]

An accuracy analytical procedure involves adding a solution of known concentration to a blank or placebo or to 'spike' a blank or placebo sample. This is calculated by percentage recovery of the spiked solution compared to a reference solution. The recovery depending on the type of test is usually stated at 90–110%. The International Conference on Harmonisation (ICH) guidelines recommend that no less that nine determinations over three different concentrations determined in the linearity range should be used to assess the accuracy. Accuracy samples are recommended to be spiked with at least 80%, 100% and 120% of desired drug concentration.

Specificity

"Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present." [163]

Specificity is a relativity quick validation test and can be completed along with other validation parameters or on its own. This procedure is usually achieved by injecting a sample of blank mobile phase, blank dissolution media or any diluent used in the test procedure and standard of concentration that will be used in the analytical test method and a placebo sample. This shows that there are no interfering peaks in any diluent, media, placebo or mobile phase that is used and the peak that is present in the standard is the analyte. Chromatographs are overlaid to show there are no other peaks at the retention time of the analyte in question.

Repeatability

"Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision." [163]

This is determined by assessing the RSD% between triplicate injections of all the accuracy injections of the same concentrations. This shows that samples results are repeatable.

Precision

"The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample

under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility." [163]

Precision is usually tested along with accuracy samples. This allows a simulation of actually samples and standards.

Intermediate Precision

"Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc." [163]

Intermediate precision is usually performed by two analysts running the same method, but on different days, using different equipment and different columns. This usually gets performed by one person completing the accuracy and the other the intermediate precision. During the intermediate precision, 100% sample concentrations are compared to 100 % samples from the accuracy. The standards are also compared. As there is no other machine available, or another analyst, this part of the validation will be done with a different column.

Reproducibility

"Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology)." [163]

Reproducibility will not be performed in this validation as there is no need for a collaborative study. Intermediate precision will be performed as within-laboratory variation. Reproducibility is not normally required if intermediate precision passes.

Limit of Detection

"The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value." [163]

Limit of detection is calculated determining the signal to noise ratio (S/N) of the peak in question. Usually the peak height in the linearity solution of 100%, is compared to the blank. A point is picked in the blank, usually the same width as the peak and the noise of the baseline is integrated. The signal from the peak then is divided by the signal of the noise. This is the signal to noise ratio. From there, a back calculation is

performed to assess at what concentration s/n ratio of 3 is achieved. Typically, this concentration is replicated and injected to prove that the back calculation was correct.

Limit of Quantification

"The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products."[163]

This is calculated the same way as LOD only the equation is S/N=10

Standard and Sample Stability

Although not specified in the United States Pharmacopeia (USP) or the Food and Drug Administration (FDA) regulations, stability of solutions is important. If solutions are found to have stability, less waste is produced as the solutions can be kept for as long it is proved they are stable. Extra sample/standard solution would be vialled and can be kept in ambient air temperature or refrigerated. These solutions are then injected periodically, T=1, 2, 3 days etc. until a suitable time is selected or the analyte area is deemed to have degraded. The RSD% between solutions at T=0 and T=x, where x is number of days, is deemed to be acceptable at RSD% ≤ 2.

2.4 UPLC methods for assay and *in vitro* release

Dapivirine (DPV)

Samples and standards were injected (2 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 25 °C and isocratic elution was performed using a mobile phase of 55 % 7.7 mM potassium phosphate buffer (pH 3.0) and 45% HPLC-grade acetonitrile (0.25 mL/min) with a run time of 3 min. DPV was detected using a wavelength of 210 nm and had a peak retention time of 1.53 min.

Metronidazole (MET)

Samples were injected (1 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 35 °C and isocratic elution was performed using a mobile phase of 30% HPLC-grade methanol and 70 % purified water (0.1 mL/min) with a run time of 3 min. MET was detected using a wavelength of 310 nm and had a peak retention time of 2.1 min.

Nestorone (NES)

Samples were injected (2 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 25 °C and isocratic elution was performed using a mobile phase of 40% 7.7 mM potassium phosphate buffer (pH 3.0) and 60% HPLC-grade acetonitrile (0.25 mL/min) with a run time of 3 min. NES was detected using a wavelength of 240 nm and had a peak retention time of 0.82 min.

MIV-150

Samples were injected (10 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 35 °C and isocratic elution was performed using a mobile phase of 50% 10 mM ammonium phosphate buffer (pH 5.0) and 50% HPLC-grade acetonitrile (0.5 mL/min) with a run time of 3 min. MIV-150 was detected using a wavelength of 260 nm and had a peak retention time of 0.701 min.

DL-lactide and lactic acid (LT and LA)

Samples were injected (10 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 35 °C and isocratic elution was performed using a mobile phase of 95% 7.7 mM potassium phosphate buffer (pH 3.0) and 5% HPLC-grade acetonitrile (0.2 mL/min) with a run time of 3 min. LT was detected using a wavelength of 210 nm. Peak retention time for lactic acid and lactide were 0.845 and 2.57 min, respectively

MIV-150 and NES

Samples were injected (5 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 40 °C and isocratic elution was performed using a mobile phase of 60% HPLC-grade acetonitrile and 40 % 7.7 mM potassium phosphate buffer (pH 3.0) (flow rate 0.25 mL/min, run time 3 min). MIV-150 and NES were detected using a wavelength of 240 nm and had peak retention times of 0.8 and 1.1 min, respectively.

MET and NES

Samples were injected (5 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 40 °C and isocratic elution was performed using a mobile phase of 40% HPLC-grade acetonitrile and 60% 7.7 mM potassium phosphate buffer (pH 3.0) (flow rate 0.25 mL/min, run time 3 min). MET and NES are detected using a wavelength of 240 nm and have a peak retention times of 0.7 and 1.7 min, respectively.

MIV-150 and MET

Samples were injected (5 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 40 °C and isocratic elution was performed using a mobile phase of 50% HPLC-grade acetonitrile and 50 % 10 mM ammonium acetate buffer (pH 5) (flow rate 0.5 mL/min, run time 3 min). MET and MIV-150 are detected using a wavelength of 260 nm and have a peak retention times of 0.3 and 0.7 min, respectively.

MIV-150, MET and NES

Samples were injected (5 µL) onto an Acquity UPLC BEK C18 column (2.1 mm x 50 mm, 1.7 µm particle size) fitted with a guard column. The column was held at 40 °C and isocratic elution was performed using a mobile phase of 50% HPLC-grade acetonitrile and 50 % 10 mM ammonium acetate buffer (pH 5) (flow rate 0.5 mL/min,

run time 3 min). MET, MIV-150 and NES were detected using a wavelength of 260 nm and had peak retention times of 0.3, 0.8 and 1.1 min, respectively.

2.5 Validation Parameters

All above API's have been validated as per parameters detailed in section 2.3. Details listed in Table 3.

Table 3. Validation parameters used for UPLC methods used in this thesis

***In vitro* release**						
API	**Range (µg/mL)**	**R^2 Value**	**Average % recovery**	**Average % RSD**	**LOD (µg/mL)**	**LOQ (µg/mL)**
DPV	0 – 600	1.0	97.7	0.1	0.01	0.03
MIV	0 – 100	1.0	101.3	0.5	2.1×10^{-3}	6.6×10^{-3}
NES	0 – 400	1.0	101.5	0.2	0.02	0.07
MET	0 – 1000	1.0	97.8	0.2	0.01	0.03
LT	0 – 1100	1.0	98.5	0.2	0.02	0.08
LA	0 – 1100	1.0	99.7	0.1	0.02	0.07
Content assay						
API	**Range (µg/mL)**	**R^2 Value**	**Average % recovery**	**Average % RSD**	**LOD (µg/mL)**	**LOQ (µg/mL)**
DPV	0 –30	1.0	97.7	0.1	0.01	0.03
MIV	0 – 55	1.0	102.1	0.3	2.2×10^{-3}	6.3×10^{-3}
NES	0 – 50	1.0	104.1	0.1	0.02	0.07
MET	0 – 500	1.0	103.6	0.2	0.01	0.03
LT	0 – 1100	1.0	98.2	0.2	0.02	0.08

3

Determination of drug solubility in silicone elastomer and other thermoplastics

3.1 Introduction

The release of drugs from diffusion-controlled polymeric drug delivery devices is dependent on two fundamental factors – the solubility of the drug in the polymer and the ability of the dissolved drug molecules to diffuse through the polymeric matrix. This is true for both matrix diffusion-controlled and membrane diffusion-controlled systems. For example, both the Higuchi matrix model (Equation 1a) and the membrane-controlled model (Equation 1b) illustrate how the rate of drug release per unit area (Q) from a polymeric matrix depends upon both the solubility (C_P) and diffusivity of the drug in the polymer (D_P) [164,165].

$$Q = \sqrt{D_P(2A - C_P)C_P t} \qquad \text{(a)}$$

$$Q = \frac{D_P C_P}{h_{sheath}} . t \qquad \text{(b)}$$

Equation 1. Equations showing the relationship between drug solubility and diffusivity in a polymer for a matrix (a) and reservoir-type device (b), where D_P is diffusivity of the drug in the polymer, C_P solubility of the drug in the polymer, Q is the cumulative amount of drug released per unit area of the device, A is the drug loading, h is the thickness of the membrane and t is the time.

Various methods to quantify drug solubility in polymers have been reported, including X-ray diffraction, thin film method, differential scanning calorimetry (DSC), dynamic mechanical analysis (DMA) and microscopy [77,166–169].

As part of early formulation development of NuvaRing®, Van Laarhoven *et al.* reported a thin film method to measure the solubility at 37 °C of etonogestrel and ethinyl estradiol in EVA copolymers [170]. EVA films were manufactured, immersed in saturated drug solutions and placed in an incubator to equilibrate (Figure 7). After 6 weeks, the films were removed from the solution, the APIs extracted using a suitable solvent, and the drugs quantified by HPLC.

Silicone oil has previously been used to model drug solubility in silicone elastomers. [77, 88, 90,171]. As with the thin polymer film method, excess drug is added to a low-viscosity silicone oil, after which the drug is extracted into a suitable solvent for HPLC analysis. This technique has previously been used to model the solubility of various drugs in a silicone elastomer matrix-type IVR and to determine the drug diffusion coefficients in the elastomer [77].

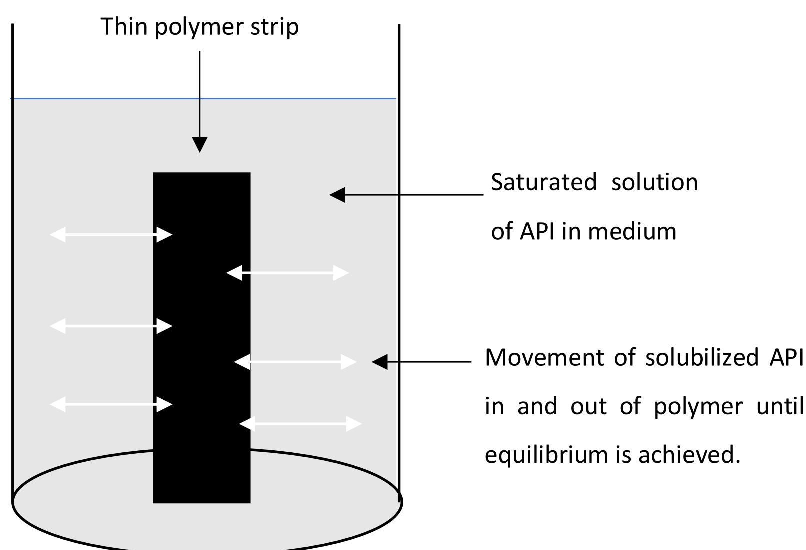


Figure 7. Depiction of thin film method; thin strip is submerged in a saturated solution of selected API.

DMA is a mechanical characterisation technique commonly used in the characterisation of viscoelastic materials. A novel application of DMA has previously been reported for measuring drug solubility in silicone elastomer systems [172]. In that study, drug-loaded silicone elastomer samples were shown to undergo a decrease in storage modulus value as the sample was heated through the drug melting temperature, attributed to melting of the drug within the silicone matrix. By plotting the extent of change in storage modulus as a function of the initial drug/excipient concentration, one can readily extrapolate to zero change in storage modulus, which corresponds to the solubility of the drug/excipient in the silicone elastomer at the melting temperature of the drug/excipient. Using this method, the DMA solubility value determined for the relatively low melting oxybutynin (59 °C) was similar to that previously measured in silicone oil. However, the silicone solubility values for other drugs and excipients were determined to be several orders of magnitude higher using the DMA method compared with the silicone oil method, mostly likely due to the fact that the DMA method measures solubility at the drug melting temperature

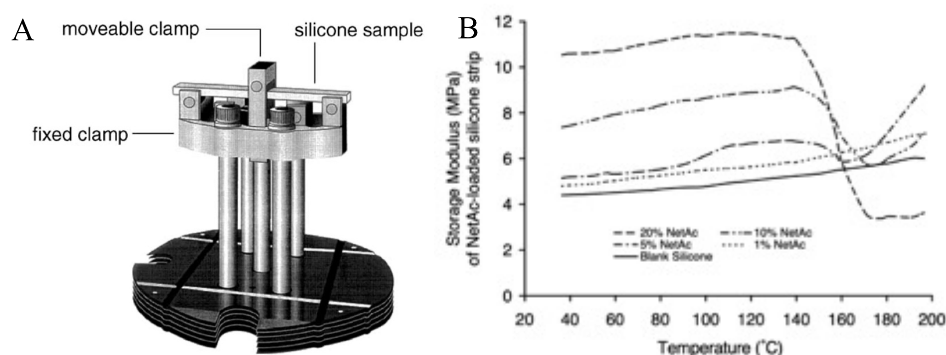


Figure 8. A – Three-point bend clamp used in DMA testing of polymer samples. B – Storage modulus of silicone elastomer sample loaded with norethisterone acetate as a function of drug concentration and temperature [172].

DSC has also been used to determine the solubility of oxybutynin – an anti-muscarinic drug used in the treatment of urinary incontinence – in silicone elastomer [77]. With this method, formulations having different drug concentrations were prepared, and the enthalpy of melting (ΔH) of the drug in the elastomer determined using DSC. After plotting the measured enthalpy values versus drug loading, the straight line was extrapolated to ΔH=0, which corresponded to a system in which no solid crystalline drug is present (i.e. the saturation solubility of the drug at its melting temperature). Gramaglia *et al.* reported use of a similar high speed DSC (HDSC) method to assess the solubility of metronidazole in a silicone elastomer [169]. Using a high heating rate of 400 °C/min, metronidazole solubility was calculated to be 2.16 mg/g, compared to 6.16 mg/mL when a heating rate of 20 °C/min was used. As faster heating rates reduces dissolution of the drug into the polymer, the results obtained using a higher heating rate are more comparable to values calculated at room temperature. Although DSC is useful for determining drug solubility in polymers, it must be emphasised that the solubility values measured are those at the melting point of the drug and will therefore be higher than the values normally measured at room or body temperature.

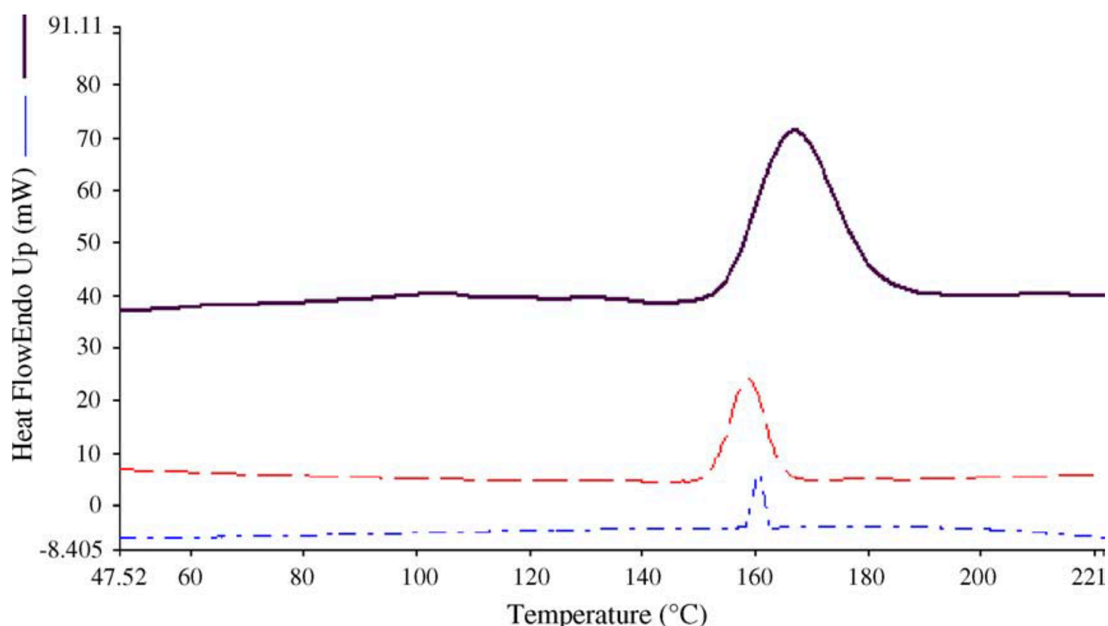


Figure 9. Melting endotherms observed for metronidazole (10%, w/w) in a silicone elastomer different heating rates (Top endotherm - 400 °C/min, middle 100 °C/min, bottom 20 °C/min) [169].

Here, the solubilities of MET, NES and MIV-150 have been measured in a silicone elastomer system using the DSC method, and in thermoplastics and silicone elastomer at 37 °C using a thin polymer film adapted from Van Laarhoven *et al.* [170].

3.2 Materials and methods

Materials

Materials for the methods described here are described in Chapter 2.

Preparation of API-loaded silicone films (DSC method)

DDU-4320 addition-cure silicone elastomer mixes (5.0 g) were prepared by adding equal amounts of Part A and Part B (and the required amount of API) to a sealed plastic container. The active silicone elastomer mixture was hand-mixed for 30 s and then mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixer™ DAC 150 FVZ-K, Hauschild, Germany) (30 s, 3000 rpm). The samples were cured at room temperature between two glass slabs with 2 x 0.2 mm shims in place for 24 hr and stored until analysis begun except for DL-lactide films that were cured for 10 min at 80 °C and stored until needed.

DSC experimental protocol

Thin DDU-4320 films loaded with API were analysed by DSC (TA Instruments 2920 modulated DSC) in standard heating ramp mode. Approximately 3–10 mg of each sample was accurately weighed into an aluminium pan and hermetically sealed. Samples were heated over a temperature range that included the API melting temperature (25 to 300 ºC) at a rate of 10 ºC per min alongside an empty reference pan. Helium was used as a purge gas (20 mL/min). For each sample, the following parameters were calculated by TA Universal Analysis software (TA Instruments, UK): drug melting onset temperature (ºC), drug melting peak temperature (ºC) and melting enthalpy (J/g). At least three replicates were used to calculate mean and standard deviation values for each sample. A blank DDU-4320 sample was prepared and analysed alongside samples to ensure no background thermal events where recorded.

Preparation of thin films

TPU-87, EVA-28, 40%, low density polyethylene (LDPE) and polyoxymethylene (POM) were placed on a non-stick sheet using 2 x 0.2 mm shims to control thickness. Another non-stick sheet was placed on top. Sheets were then placed in laboratory-scale injection molding machine heated to temperatures described in Table 4 and compressed. After 5 min, sheets where removed and left to cool. Thin sheets of polymer were cut to 1 x 3 cm strips and stored until testing commenced. Thin films were placed in a saturated solution of respective API in 10 mL of 0.2 % Tween solution. After T=1, 3 or 6 months, films were removed, washed lightly with deionised water, dried and placed in 50 mL acetone. Samples were placed in an orbital shaker at 37 °C. After 24 h, samples were removed and left to cool. A 1 mL sample was transferred to a 100 mL volumetric flask and made to volume with 1:1 solution of acetonitrile: water. Drug solubilities were then determined by reverse phases UPLC. (For UPLC methods, refer to chapter 2).

Table 4. Compression temperatures for the manufacture of thin films.

Polymer	Temperature (°C)
TPU-87	100
POM	175
EVA-28 %	50
LDPE	180
EVA-40 %	50

3.3 Results and discussion

DSC method

The DSC thermogram for the drug-free silicone elastomer film showed no thermal transitions over the temperature range 25–210 °C, in accordance with the literature [173]. Silicone rubbers do exhibit thermal transitions, albeit at much lower temperatures: glass transition –127 °C, exothermic crystallisation –102 °C, and endothermic melting at –43 °C. Thermograms of the supplied drug substances displayed a single endothermic melting transition – peak temperatures for MET, MIV-150 and NES were at 161 °C, 197 °C and 178 °C, respectively. These melt temperatures are consistent with literature values [174–176]. Representative DSC traces showing the endothermic melting transitions for the APIs incorporated into the silicone elastomer at various loadings (typically 2.5–25 % w/w) are presented in Figure 10. The melting onset and peak temperatures are reported in Table 5.

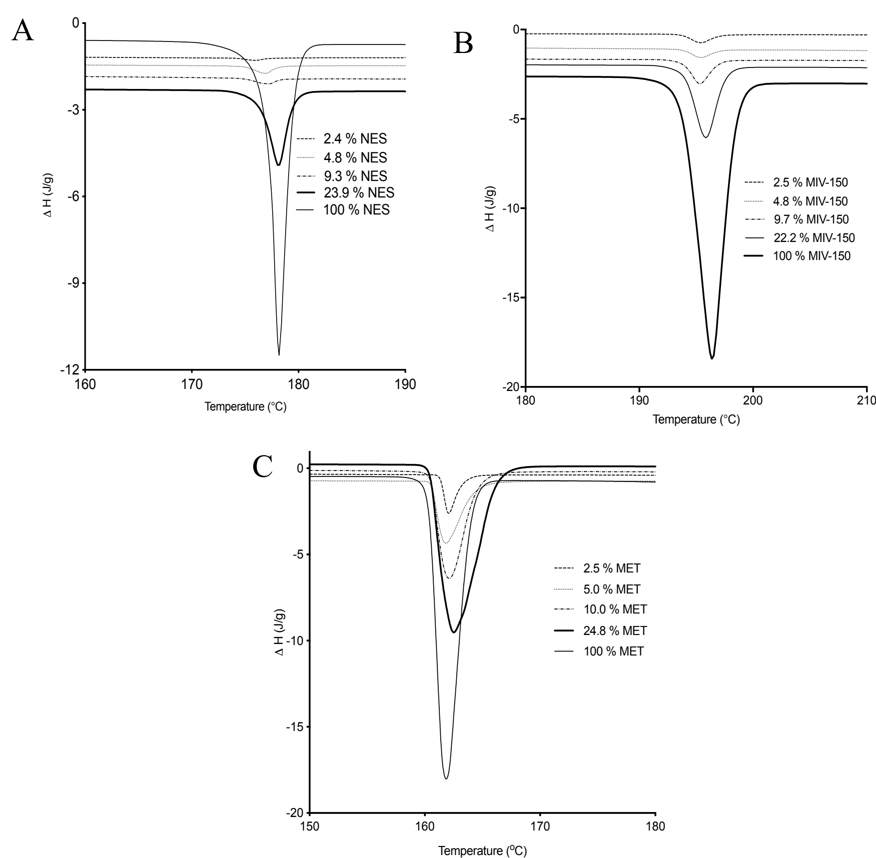


Figure 10. Representative DSC traces for silicone elastomer DDU-4320 samples containing different concentrations of NES (A), MIV-150 (B) and MET (C). All plots show the trend of increasing enthalpy with increasing drug loading.

Overall, the melt onset and peak temperatures did not vary with drug loading (RSD% < 0.7%). For each API, melting enthalpy increased with drug loading, indicating that the drugs are mostly present in the polymers in the solid crystalline state, right up until the drug melting temperature (Table 5).

Table 5 . DSC data for MET, MIV-150 and NES, both supplied drugs and incorporated into the DDU-4320 silicone elastomer system.

% w/w API content	Melt enthalpy Δ H (J/g)	Melt onset temp. (˚C)	Melt peak temp. (˚C)
Metronidazole			
2.5	3.48 ± 0.11	160.35 ± 0.04	161.08 ±0.10
5.0	7.94 ± 0.52	160.62 ± 0.15	161.90 ± 0.42
9.3	16.55 ± 1.01	160.52 ± 0.15	162.20 ± 0.42
23.9	43.15 ± 2.45	160.47 ± 0.12	162.99 ± 0.68
100.0	178.53 ± 1.40	160.83 ± 0.78	161.36 ± 0.84
	R^2 = 1.00	***y=1.78 x-1.05***	***Solubility = 0.59%***
MIV-150			
2.5	2.16 ± 0.13	193.73 ± 0.02	195.35 ± 0.12
4.8	2.38 ± 0.98	193.87 ± 0.20	195.33 ± 0.18
9.7	8.78 ± 1.58	193.80 ± 0.14	195.27 ± 0.08
22.2	15.80 ± 0.15	193.95 ± 0.02	195.83 ± 0.03
100.0	113.59 ± 4.88	193.92 ± 0.17	196.51 ± 0.38
	R^2 = 0.94	***y=0.68 x-0.07***	***Solubility = 0.10%***
Nestorone®			
2.4	0.18 ± 0.07	174.50 ± 0.42	176.13 ± 0.29
5.2	1.28 ± 0.35	174.95 ± 0.27	176.78 ± 0.10
9.3	6.58 ± 0.46	174.91 ± 0.15	177.14 ± 0.10
23.9	15.25 ± 0.40	176.40 ± 0.07	178.15 ± 0.17
100	58.61 ± 3.86	177.09 ± 0.13	178.13 ± 0.04

By plotting the values of the melting enthalpies against the initial drug loadings (w/w %) and applying linear regression, extrapolation of the best-fit line to the x-axis intercept allows estimation of the solubility of the APIs in DDU-4320 (Figure 11). R^2 values and equations for linear regression are presented in Figure 12. MET had the greatest solubility in DDU-4320 (0.59%) followed by NES and MIV-150 (0.42 and 0.10%, respectively). These solubility values generally correlate with the hydrophobicity (log P values) of the APIs (Figure 12).Solubility data for Nestorone® and MIV-150 in a silicone elastomer have not previously been reported.

The solubility of metronidazole (MET loadings 1–10 %w/w) in an addition-cure silicone elastomer has been measured previously using DSC (20 °C/min) and HDSC

methods (400 °C/min) [169]. With DSC, the solubility value was 0.6% w/w and HDSC was 0.216 % (w/w). This former DSC value is the same as that reported here using a scan speed of 10 °C/min. The HDSC value is substantially lower due to higher heating rates reducing the extent of dissolution of MET in the silicone elastomer.

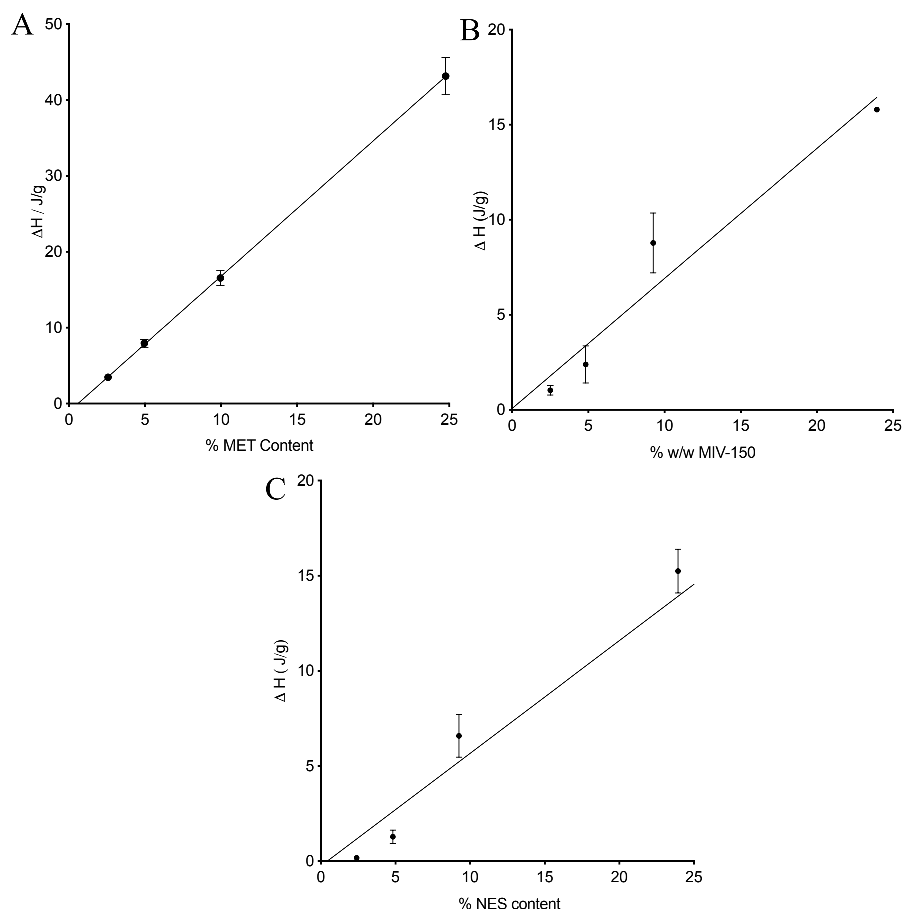


Figure 11. Graphs showing melting enthaply vs % loading for MET (A), MIV-150 (B) and NES (C) incorporated into the silicone elastomer DDU-4320.

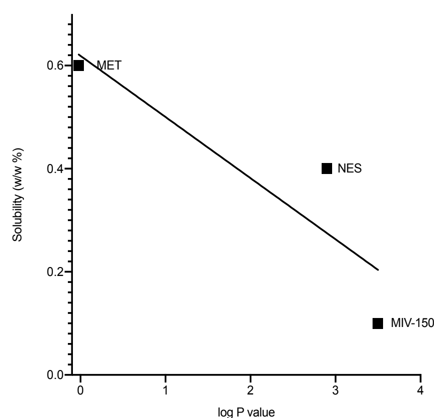


Figure 12. Graphs showing % w/w solubility in DDU-4320 versus log P value for MET, MIV-150 and NES. (R^2 = 0.79, best fit line (y= -0.12 x + 0.62).

Thin film method

Van Laarhoven *et al.* previously reported use of 0.2 mm thin films for measurement of etonogestrel and ethinyl estradiol solubility in EVA copolymers. Here, thin films having 0.4 mm thickness were used. Sampling timepoints (T=1, 3 and 6 months) were therefore extended to account for potentially longer equilibrium times. Percentage w/w API uptake into the thin films (Table 6) shows the rank order of the APIs in each polymer, with MIV-150 being the most hydrophobic of the three of APIs. This is not surprising as these results show that it was the least soluble API in the range of polymers (apart from the TPU polymer). NES, the second most hydrophobic, was second most soluble, and the hydrophilic MET was the most soluble. MIV-150 and NES showed solubility values similar at room temperature across the range of polymers. For example, both APIs show a particular affinity for the hydrophobic TPU, consistent with previous use of this TPU material in drug delivery of hydrophobic drugs [177]. This thin film method tended to produce much lower uptake values for the APIs compared to the DSC solubility testing, as by design, the thin film method used much lower temperatures than DSC. Equilibrium was defined as no change in solubility results between two timepoints therefore the only polymer to fully achieve equilibrium with all three APIs was the silicone elastomer.

POM, LDPE and PP materials showed limited uptake of MIV-150 and NES, presumably due to their relatively high crystallinity (which afford these polymers their mechanical strength and chemical resistance) [178–180]. These polymers were selected as potential frame components for the novel vaginal ring. PP has been reported as having less chemical resistance than LDPE which corresponds to results reported in Table 6 [181]. For example, MET had a higher solubility value in PP than LDPE.

Overall, this data highlights that flexible polymers such as silicone, TPU and EVA are more suited for use a core materials due to a larger API uptake than the more rigid polymers. The rigid polymers are considered more suitable for the construction of the non-medicated frame since less API uptake was observed, especially for MIV-150 and NES. Although silicone elastomers could potentially be used for frame components, drug ingression into the frame would have to be considered.

Table 6. Drug uptake (%w/w) of APIs in silicone and thermoplastic thin films at various timepoints (n=4).

Polymer /API	MIV-150 (% w/w)			NES (% w/w)			MET (% w/w)		
	T=1	T=3	T=6	T=1	T=3	T=6	T=1	T=3	T=6
Silicone	0.01 ± 0.00	0.01 ± 0.00	0.01 ±0.00*	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00*	0.33 ± 0.11	0.39 ± 0.08	0.39 ± 0.02*
TPU	0.05 ± 0.01	0.11 ± 0.00	0.15 ± 0.03	0.01 ± 0.00	0.03 ± 0.00	0.14 ± 0.01	0.03 ± 0.02	0.05 ± 0.01	0.13 ± 0.04
EVA-28	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00*	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.18 ± 0.03
EVA-40	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.11 ± 0.02
POM	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00*	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.40 ± 0.02	0.82 ± 0.03
LDPE	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00*	0.00 ± 0.00	0.11 ± 0.01	0.15 ± 0.01
PP	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00*	0.12 ± 0.00	0.12 ± 0.01	0.99 ± 0.12

* represents equilibrium achieved. This was apparent when results from T=3 and T=6 were the same. As this experiment ended at T=6 M, an accurate solubility determination was not achieved. The only polymer to achieve equilibrium for all three APIs was the silicone elastomer.

3.4 Conclusions

This study highlighted the importance of polymer choice for the novel modular vaginal ring, not only for the rods themselves but for the ring body. Solubility of MET, NES and MIV-150 was assessed using a DSC method with MET having the highest solubility in silicone at 0.59% w/w compared to MIV-150 (0.10% w/w). The disadvantage of this method is that the solubility is determined at the drug melting point rather than at a more clinically relevant temperature.

The thin film method involved immersion of polymers being submerged in a saturated solution over a predetermined time frame (T = 1, 3 and 6 months). These polymers were then analysed for API content at their scheduled timepoint. These results provided API uptake data not only for silicone that had been previously tested via the DSC method but also for thermoplastics. As with DSC results, there was the same trend of increased w/w % solubility in the polymers with increasing log P value across all the polymers. More rigid polymers such as POM, LDPE and PP showed most resistance to hydrophobic API intake compared to the more flexible polymers such as silicone, TPU, and EVA. The evidence from this study suggests that the more rigid polymers would be best suited being used as a non-medicated frame and the more flexible polymers as the insertable cores.

4

Preliminary *in vitro* release testing of a new modular vaginal ring

4.1 Introduction

When a new API is considered for formulation as a vaginal ring, a range of preliminary studies are usually conducted to demonstrate compatibility of the drug with the ring excipients and the practicality of the overall formulation strategy. For silicone elastomer vaginal rings, the primary concerns are to ensure: (i) the drug molecule does not inhibit the silicone elastomer curing reaction, (ii) the drug is capable of permeating the silicone elastomer, and (iii) the selected medium is suitable for solubilisation of released drug. Selection of the release medium is therefore critical; the drug needs to have appreciable solubility in the release medium in order to permit release.

All of the active pharmaceutical ingredients (APIs) being considered in this project have previously been incorporated into and released from various conventional designs of silicone elastomer intravaginal ring [58,59, 96, 139,159], although not in the combinations proposed here. Therefore, the APIs are known to be compatible with silicone elastomers, allowing ease of incorporation, no cure inhibition, and measurable drug release. However, selection of an appropriate release medium and volume of medium have not previously been reported. In this chapter, a range of release media will be assessed for the various drug molecules.

***In vitro* release methods for intravaginal rings**

Currently, there are no compendial methods for *in vitro* release testing of intravaginal rings (IVRs). Instead, the various marketed vaginal rings are tested for *in vitro* release using different release media and apparatus (Table 7) [198]. For example, the contraceptive vaginal ring NuvaRing® is suspended via a nylon thread in a glass flask containing 200 mL of water stirred at 750 rpm by a magnetic stirrer. Annovera®, a one-year contraceptive ring that is similarly suspended by a nylon string, is tested using 400 mL water as the release medium and is shaken in a linear orbital incubator at 100 rpm. The progesterone-releasing rings Progering® and Fertiring® are tested for *in vitro* release using a flow-through dissolution method (similar to USP Apparatus IV) comprising a peristaltic pump to provide constant flow of isotonic saline (4 L/day) through a cell containing the ring.

In addition to differences in choice of apparatus, stirring speeds and methods for ring placement in flasks, a variety of different types and volumes of release media are also used for *in vitro* release testing (Table 7). These include unbuffered simulated vaginal fluid (SVF), solvent/water mixtures, buffered media and surfactant solutions. Fluid volumes typically range from 100–500 mL, with the primary objective to maintain sink conditions. Sink conditions require that the drug concentration in the release medium never exceeds 1/10 (sometimes 1/5) of the saturation concentration, thereby mimicking the sink afforded by the fluid, tissue and systemic compartment *in vivo*.

SVF is the most physiologically relevant release medium [199]. Its composition is primarily intended to mimic the chemical composition, pH and osmolarity – but not the viscosity – of vaginal fluid. However, as with other simple aqueous buffer systems, SVF generally does not have sufficient solubilising capacity to provide sink conditions at practical volumes for *in vitro* release testing of poorly water-soluble drugs [200,201]. Historically, this issue of poor solubility has been resolved using isopropanol/water mixtures or by using aqueous media with surfactants [32, 43, 46,47, 62, 85, 109,202,203]. For example, Boyd *et al.* used a 1:1 v/v isopropanol+water mixture for *in vitro* testing of a dapivirine+levonorgestrel vaginal ring for the purpose of screening and comparing different formulations during preclinical development [89]. Regulatory agencies struggle with use of solvent+water systems; they are not preferred while an aqueous media incorporating surfactants or other solubilising agents are favoured.

Controlling surface area in drug delivery devices

Controlling surface area is essential in the manufacture of oral dosage forms as it impacts dissolution rates [204,205]. Control of vaginal ring surface area has also been reported as a means of modulating drug release. For example, the Population Council reported testing different pore sizes in an multi-purpose prevention technology (MPT) ring containing a zinc acetate and carrageenan gel surrounded by a matrix of LNG and MIV-150 [58]. The different pore sizes allowed release rates of these highly hydrophilic components to be controlled. McBride *et al.* have also reported adjusting the number of orifices in an exposed core type ring to modulate the release of the HIV CCR5 inhibitor 5P12-RANTES [54]. Welsh *et al.* reported using an additive

manufacturing technique that uses droplet deposition modelling to create IVRs having different infill densities (and thus different surface areas) to increase the release of DPV [177]. It was reported that lower infill densities for the IVR resulted in higher DPV release compared to a traditional injection molded (IM) IVR. This decrease in infill density resulted in a substantial increase in surface area (IM (100%) surface area – 1,974 mm^2 compared to 19,152 mm^2 for an infill density of 50%. This increase in surface area allowed a 50% infill to release 14 times that of a traditionally IM IVR over the 29-day period. For the new modular ring designs being developed in this project, insertion of the drug-loaded rods into the polymer ring frames will significantly reduce the surface area of the rod in contact with the release medium, which in turn should lead to a reduction in the drug release rate compared to drug release from the rod itself.

The main aims of this chapter are to develop *in vitro* release test methods that may be broadly applicable to all of the APIs used in this thesis and to assess the effect of surface area on drug release from both matrix and reservoir type rods. Here, three preliminary *in vitro* release experiments were carried out over a 10-day period to assess drug release a silicone elastomer. The first experiment investigated DPV release from matrix-type vaginal ring segments in different release media. The hypothesis was that a simple aqueous medium comprising water+Tween 80 would be just as suitable a release medium as isopropanol+water and SVF+Tween 80 for testing *in vitro* dapivirine release. Based on the data generated, a release medium will be selected for all future *in vitro* release studies in this project.

The second and third experiments assessed the impact of reducing the surface area available for drug dissolution. In the second, the effect on *in vitro* release of insertion of the drug-loaded rod into a basic prototype ring body was compared with rods alone. Although this basic prototype ring body is far from what the actual ring frame will look like, it will provide preliminary data on the impact of drug release. Third, a study was completed to assess the contribution of reservoir rod end effects on drug release rate.

Table 7. Descriptions of marketed vaginal rings and details of *in vitro* testing methods. All *in vitro* tests are performed at 37 °C.

Vaginal ring	Active agent(s) (loading/release rate)	Apparatus	Medium, †	Volume†	Speed†
Estring® [206]	17β-estradiol (2 mg / 7.5 μg/day)	linear shaking water bath or shaking incubator / dissolution medium changed periodically	0.9% saline	250 mL	60 or 130 rpm
NuvaRing®[98, 170,207,208]	etonogestrel (11.7 mg / 120 μg/day) ethinyl estradiol (2.7 mg / 15 μg/day)	incubator with magnetic bar stirring / ring suspended in flask with nylon string	water	200 mL	750 rpm
Femring® [209]	17β-estradiol-3-acetate (12.4, 24.8 mg / 50, 100 μg/day)	shaking orbital incubator / ring suspended by thread / dissolution medium replaced daily	0.9% w/w saline 0.133 or 1.0% w/v benzalkonium chloride	500 mL	rpm not specified
Progering®[210–212]	progesterone (2074 mg / ~10 mg/day)	constant-flow release system comprising peristaltic pump and ring suspended in flask with nylon string	isotonic saline	250 mL	4 L/day
Fertiring®	progesterone (1000 mg / ~10 mg/day)	constant-flow release system comprising peristaltic pump and ring suspended in flask with nylon string	isotonic saline	250 mL	4 L/day
Ornibel®/ Myring™ [213]	etonogestrel (11.0 mg / 120 μg/day) ethinyl estradiol (3.47 mg / 15 μg/day)	shaking incubator	sodium acetate solution (25 mM, pH 4.2) + 0.05% Solutol HS-15	100 mL	60 rpm
Annovera® [128,214]	Nestorone® (103 mg / 150 μg/day) ethinyl estradiol (17.4 mg / 15 μg/day)	linear shaking water bath (1 inch) / ring suspended in flask with nylon string	water	400 mL	100 rpm
DPV ring [215,216]	dapivirine (25 mg / 2600–180 μg/day in IPA/water / 350–100 μg/day in SVF/Tween 80®)	orbital shaking incubator (25 mm) / screw-cap glass flasks	IPA/water (1:1 v/v) or SVF/0.2% Tween 80®	100 mL weekdays, 200 mL weekend	60 rpm

4.2 Materials and methods

Materials for the work described in this chapter are presented in Chapter 2.

Manufacture of matrix-type silicone cores

Matrix-type silicone elastomer vaginal rings (overall diameter 56.7 mm; cross-sectional diameter 7.8 mm) were manufactured using a laboratory-scale injection molding machine fitted with a custom stainless-steel ring mold assembly. Separate 25 g Part A and Part B premixes of the DDU-4320 addition-cure silicone elastomer systems were prepared by adding weighed quantities of selected API into a screw-cap polypropylene container followed by addition of the silicone part. The premixes were first-hand-mixed (30 s) and then mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixer® DAC 150 FVZ-K, Hauschild, Germany) (60 s, 3000 rpm). A and B premixes were combined in a 1:1 ratio. This mix was then transferred into a polypropylene SEMCO® injection cartridge designed for manual injection. Rings were manufactured by manually injecting the active mix into the heated ring mold assembly using an 80°C mold temperature and 180 s cure time. Rings were removed from the heated mold, deflashed as needed, and stored at ambient temperature until further testing.

Manufacture of reservoir-type silicone cores

Reservoir-type cores were prepared as for matrix-type cores except the API-loaded silicone was injected into a length of silicone tubing (chapter 5, 1.5 mm wall thickness; 5 mm luminal diameter). Cores were left overnight at room temperature to cure.

***In vitro* release testing**

On Day 0, devices were placed individually into 100 mL glass bottles containing 50 mL of selected medium and stored in an orbital shaking incubator (Unitron HT Infors; 37 °C, 60 rpm, 25 mm orbital throw). After 24 ± 0.25 hr, the release medium was sampled and replaced with a fresh 25 mL of solution, and the sample retained for

UPLC analysis. Sampling and 25 mL replacement of the release medium was performed daily out to Day 10, except on Fridays when, after sampling, the flask was replenished with a 50 mL volume of release medium and no further replacement or sampling performed until the following Monday. Samples were analysed by UPLC according to the methods described in Chapter 2.

4.3 Results and discussion

Evaluation of different release media for *in vitro* release testing of vaginal rings containing dapivirine using ring segments

Overall, the trend in DPV release from the ring segments across the various release media was: IPA/water > 0.2% Tween > SVF+2%Tween (Figure 13) reflecting the solubility of DPV in the various media and consistent with solubility data (Table 8). That the 0.2% Tween medium provides greater DPV release than the SVF+Tween medium may be due to the fact the SVF medium already contains various dissolved species (e.g. salts, glycerol, glucose, acetic acid, etc.)

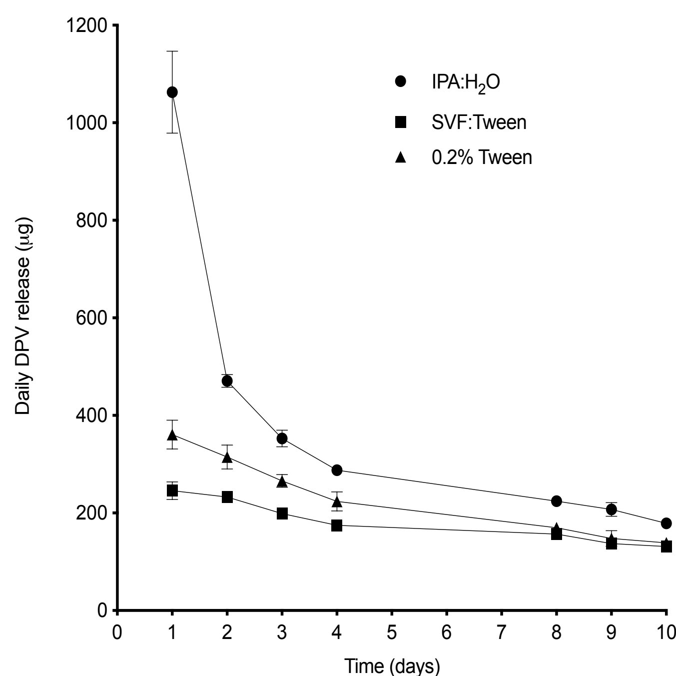


Figure 13. Mean daily release of DPV loading (2.5 % w/w) silicone ring segments placed into medium of IPA:H_20, SVF: Tween or 0.2% Tween vs. time in various media over a period of 10 days. Error bars represent mean ± standard deviation of four replicates; error bars were often smaller than the plot symbols.

Table 8. Solubility of DPV in IPA+$H_2$0, SVF+0.2% Tween, and 0.2% Tween.

Medium	DPV solubility
IPA/water	531.01 ± 8.98
SVF/Tween	17.11 ± 0.51
0.2 % Tween	29.26 ± 2.42

A relatively large day 1 DPV burst (1017 µg) was observed with the IPA/water medium compared to that for Tween-only and SVF+Tween media (361 µg and 246 µg, respectively). This burst release is characteristic of matrix-type vaginal rings and is due to the rapid dissolution and release of solid drug at or close to the surface of the ring segment [77,217]. After the initial burst, daily DPV release declined steadily over the 10-day period, attributed to the inward-moving drug depletion zone that occurs following drug release from a matrix-type device [218]. By Day 10, daily DPV release had decreased to 179, 139 and 131 µg for the three media.

Boyd *et al*. have previously reported a day 1 DPV release of ~6000 µg into IPA+H_2O (1:1) release medium for a 200 mg DPV matrix-type vaginal ring, which has the equivalent DPV loading per volume as the quarter-ring segments used in this study. Nel *et al.* reported a mean *in vivo* release rate (25 mg DPV ring) of ~140 µg/day based on residual content after 28-day use [99]. Although this mean value assumes a constant daily release rate (which is not the case for a matrix-type ring), the value does correlate more closely with those measured for the aqueous media rather than the IPA+H_2O medium, suggesting that the aqueous media are more biorelevant.

Cumulative DPV release vs. root time plots were used to assess degree of correlation with root time release kinetics for the three media (Figure 14). The total mean cumulative release after 10 days release was 3507, 1625 and 2070 µg for IPA+H_2O, SVF+Tween and 0.2% Tween media, respectively. Mean DPV release rates, obtained by linear regression analysis of the cumulative release vs. root time data, were 1129, 626, and 783 µg/day $^{0.5}$ for the IPA+H_2O, SVF+Tween and Tween-only release media, respectively (Table 8). Linear correlation coefficients (R^2) close to unity are indicative of root time kinetics, as anticipated for a matrix-type permeation-controlled drug delivery system in which a significant fraction of the drug loading is present in the solid state above the solubility limit.

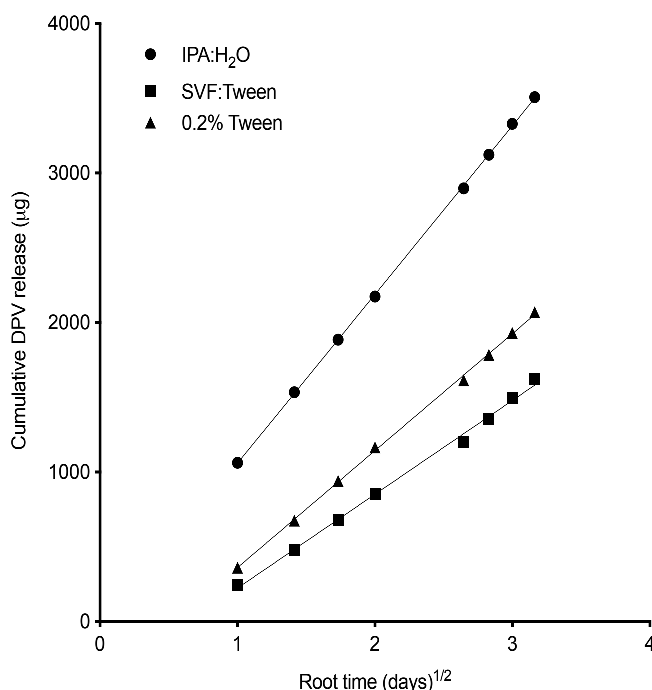


Figure 14. Cumulative release of DPV loaded (2.5 % w/w) silicone ring segments placed into medium of IPA:H20, SVF: Tween or 0.2% Tween vs root time**.**

The lag times, calculated by extrapolation of the linear regression line (Figure 14) to its x-axis intercept, are significantly different between the release media (Table 9), again reflecting the differences in DPV solubility in these media. The lag time for the solvent IPA+H_2O system, which offers the highest solubility for DPV, was almost 10-fold lower than those measured for Tween-based media.

Table 9. Linear regression analysis for cumulative DPV (µg) vs root time (day ½) release in various media.

Release medium	**R^2**	**Lag time (day ½)**	**Release rate (µg/ day ½)**
IPA: H_2O	1.00	0.06	1129
SVF: 0.2% Tween® 80	1.00	0.64	627.7
0.2% Tween® 80	1.00	0.54	782.5

Although regulatory bodies permit the use of organic solvents for *in vitro* release testing of drug products containing highly lipophilic drugs, there is a concerted effort to encourage use of more biologically relevant release media [215,219–221]. Historically, IPA+H_2O media has been used for *in vitro* release testing of DPV-releasing vaginal rings [47, 68, 89, 202, 215,216, 218,220–222]. However, this study demonstrates that aqueous surfactant media are also useful. In particular, a simple

aqueous Tween 80 solution provided similar release to that of an SVF+Tween medium, thereby offering greater efficiency in laboratory settings by eliminating the time needed to prepare SVF solutions. For this reason, all future release studies as part of this project will make use of 0.2% Tween® 80 in water adjusted to pH 4.2.

In vitro drug release from silicone matrix rods

Silicone elastomer rods containing 2.5 % w/w of each API (MET, NES MIV-150, DPV) were prepared for *in vitro* release testing. Half of the rods were placed in a prototype 'skeleton'. This skeleton is intended to model how a matrix rod would fit into the novel IVR design.

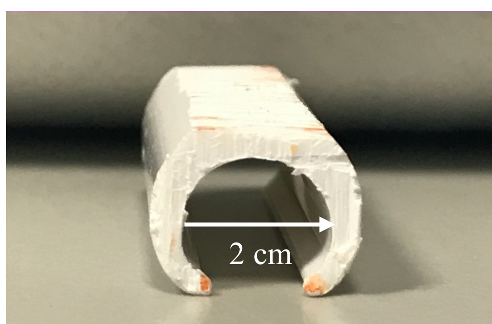


Figure 15. Prototype skeleton section to house matrix-type drug loaded rods.

Cumulative release vs. root time plots are presented in Figure 16. The trend in release was NES > MET > DPV ≈ MIV-150 for rods with or without a skeleton frame. NES, a hydrophobic API, released the highest amount of the drug over the study suggesting the need in future formulations to reduce and better control the amount of NES released (target dose 150 μg/day [128]). MET showed higher release than DPV and MIV-150, which was expected due to its hydrophilicity. This data shows that API loading will have to increase in order to achieve target therapeutic dosage (MET target ~ 37.5 mg/day [223], MIV-150 ~ 150 μg/day [69]). When the rods are not placed in a skeleton, DPV release was greater than MIV-150 which suggests that it could be more challenging to achieve a high target release rate of MIV-150 in future formulations [69,224].

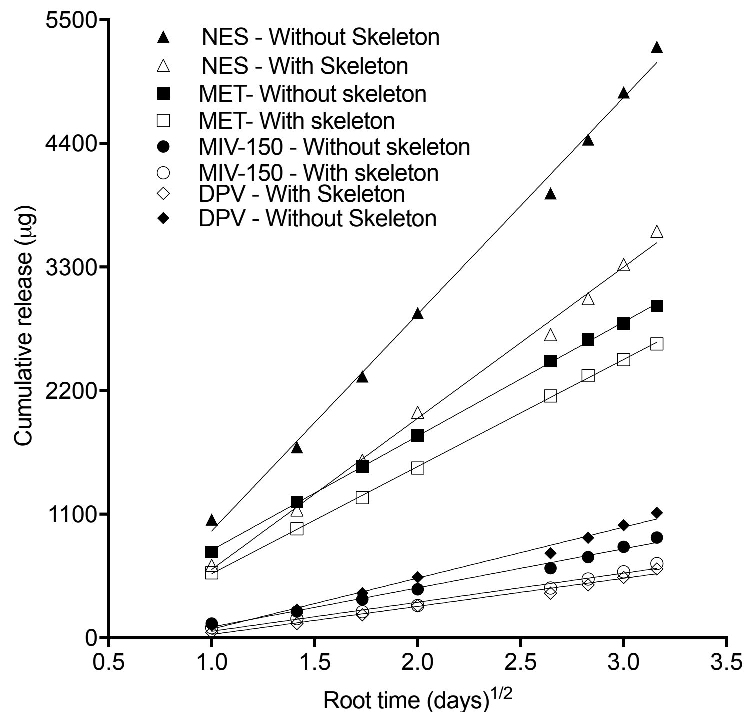


Figure 16. Cumulative release versus root time profiles for NES, MET, DPV, MIV-150 (2.5 % w/w) silicone ring segments (n=4) with and without skeleton in 0.2 % Tween.

Linear regression analysis provided correlation coefficients close to unity (Table 10), indicative of a permeation-controlled drug release mechanism obeying root time kinetics. The data also clearly demonstrates that insertion of the matrix rods into prototype ring skeletons considerably reduces the measured drug release rates (by 44.9%, 25.7%, 30.2%, 6% for DPV, MIV-150, NES and MET, respectively) due to a reduction in the surface area of the rod exposed to the release medium. It appears that the extent of reduction in release correlates to some extent with drug hydrophilicity; with smallest decrease (6%) was measured for the hydrophilic MET (log P = –0.02) and the largest (44.9%) for hydrophobic DPV (log P = 5.3). MET, due to its hydrophilicity is much more likely to diffuse from the core into any media that it comes into contact with it than DPV (hydrophobic) especially since these samples do not have a perfect inference fit. Placement of a matrix rod in the prototype ring skeleton theoretically reduces the surface area of core exposure by ~70%. However, the measured release rates are significantly higher than expected, suggesting that the release medium may ingress into the interfacial space between the matrix core and the skeleton due to less than perfect inference fit. However, in these experiments, the skeleton casing is only an early prototype and was not custom designed for use with these rods. Custom ring skeletons are manufactured and tested later.

Table 10. Summary API release data (Figure 16. Cumulative release versus root time profiles for NES, MET, DPV, MIV-150 (2.5 % w/w) silicone ring segments (n=4) with and without skeleton in 0.2 % Tween.) following linear regression analysis of cumulative release vs time graphs for skeleton and non-skeleton samples.

Device type	R^2 value of linear regression line	Surface area of matrix core (%)	Release rate (µg/day $^{1/2}$)	Lag time (day $^{1/2}$)
DPV- With skeleton	0.98	29.23	250	0.89
DPV- Without skeleton	0.99	100.0	453	0.83
MIV-150 - With skeleton	0.98	29.23	259	0.77
MIV-150 - Without skeleton	0.99	100.0	348	0.72
NES - With skeleton	1.00	29.23	1346	0.55
NES - Without skeleton	1.00	100.0	1929	0.51
MET - With skeleton	1.00	29.23	953	0.40
MET - Without skeleton	1.00	100.0	1015	0.23

The effects of end-effects on reservoir silicone rods

A study was conducted to investigate the impact of exposed ends of reservoir rods on *in vitro* release. For this purpose, only MET and DPV were selected, representing a hydrophilic and hydrophobic drug, respectively. Reservoir-type rods (length 6 cm, 1.5 mm wall thickness, 5 mm luminal diameter) having a 2.5 % w/w drug loading were manufactured using PVC tubing. The rods and then cut into lengths of either 1, 3 or 6 cm (Figure 17). *In vitro* experiments were then performed to compare release across a single 6 cm rod, two 3 cm rods, and six 1 cm rods; different number of rods were used to demonstrate an increase in available surface area would allow increased drug release.

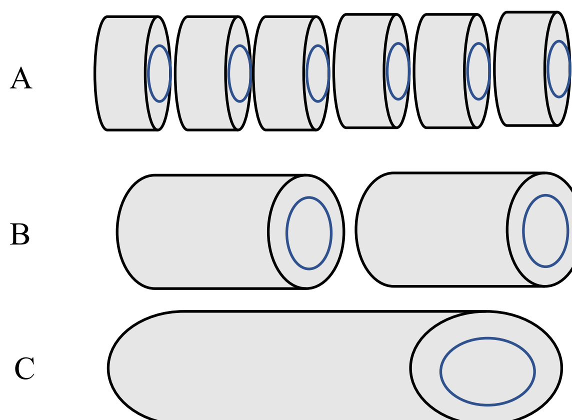


Figure 17. Configuration of rods used in end-effects study. A) 6 x 1 cm rods B) 2 x 3 cm rods and C) 1 x 6 cm rod. Total length of each sample (n=4) is 6 cm.

Daily and cumulative release graphs for the reservoir devices are presented in Figure 18 and Figure 19. Generally, the trend in the amount of drug released correlated with the total surface area of the test devices: A > B > C. The MET devices all showed a pronounced burst release on Day 1; the DPV C device also showed a small burst. Burst aside, all of the devices displayed near constant release until, except for MET A rods which displayed a more conventional matrix-type release profile.

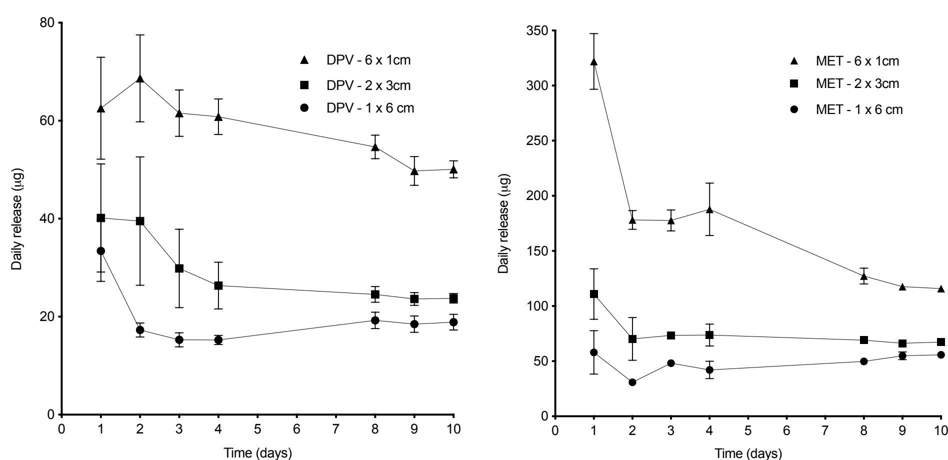


Figure 18. Daily release vs. time graph showing release of DPV and MET over 10 days from rod combinations having a combined length of 6 cm in 0.2% Tween. Error bars in graph represent ± standard deviation of four replicates; some error bars were smaller than the plot symbols.

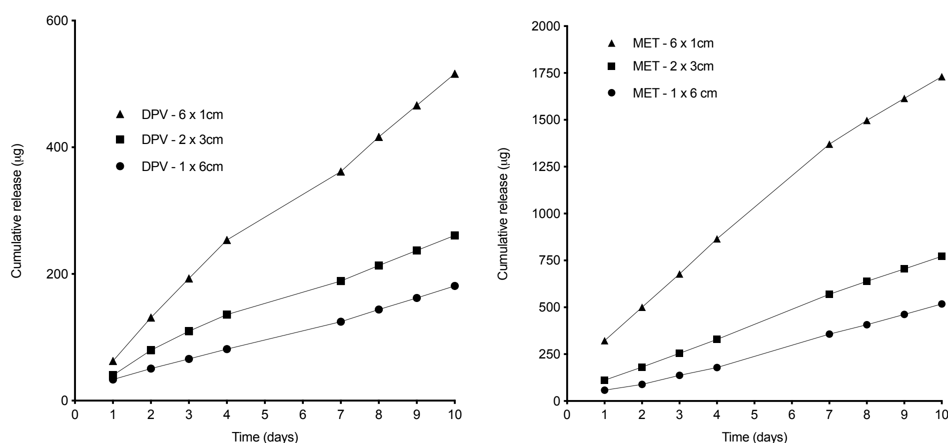


Figure 19. Cumulative release of DPV and MET over 10 days from rod combinations having a combined length of 6 cm in a 0.2 % Tween solution (n=4).

There is a clear correlation between the amount of drug released and the number of exposed rod ends (Figure 19). Increasing the number of rod ends increased the overall surface area (Table 11) exposed to the release medium, resulting in increased drug release. This data is useful in the context of the proposed modular vaginal ring concept, since the drug release can be readily modulated through judicious selection of the length and number of the drug modules.

There are two potential mechanisms for drug release from these rods – drug may be released directly from the rod ends (as with a diffusion-controlled matrix-type system) or through the silicone membrane (as for membrane-controlled diffusion). The data demonstrates that both drug release pathways are operating.

Table 11. Device characteristics and cumulative release characteristics of DPV and MET reservoir rods investigated as part of the end-effect study. SA = surface area

Rod length and number	Total surface area (mm^2)	Surface area of matrix core (%)	Release rate of DPV (µg/day)	Release rate of MET (µg/day)
1 x 6 cm	251.3	15.6	16.0	52.6
2 x 3 cm	351.9	22.3	23.0	74.7
6 x 1 cm	754.0	31.3	48.0	159.1

4.4 Conclusion

Based on data generated in preliminary *in vitro* release experiments, it was concluded that a simple 0.2 % v/v Tween 80 (pH 4.2) would serve as a practical release medium for the purposes of future experiments throughout this research project. Although the extent of drug release into SVF+Tween medium was similar, the long preparation times required for preparation of SVF was considered a disadvantage. Although release of DPV into 0.2 % v/v Tween 80 was significantly less that that into the traditional 1:1 IPA+water medium, it is widely acknowledged that the solvent/water system overestimates release *in vivo*.

Using a prototype ‘skeleton’ section, it was shown that in *vitro* drug release was generally significantly decreased when rods were fitted into the skeleton due to a reduction in the surface area of rod exposed to the release medium. A general trend of decreasing drug release was evident in the following order, NES > MET > DPV ≈ MIV-150 with both rods housed in a skeleton frame and matrix-type rods. Although this is a prototype ring section, it provided initial drug release data that is important to take into consideration when developing drug loadings in future studies. For example, the fast release of NES, which will potentially need to be controlled due to its high release.

By preparing reservoir-type drug-loaded rods of different lengths, it was demonstrated how rod end-effects contributed to drug release. There was trend of increasing drug release for both the hydrophilic MET and hydrophobic DPV with increased surface area. As drug can be directly released from the exposed matrix-type ends and through the silicone membrane, this approach will be considered in future formulations.

Overall, the preliminary studies described in this chapter determined that a 0.2 % Tween solution will be used as *in vitro* release medium for all future studies and discussed issues that will have to be addressed for the novel ring design. These issues, such as reduced surface area of the exposed rod to the release medium and ends of cut rods providing another path for drug release, will be a major factor in achieving sustained and targeted drug release for this novel ring design, not only for the ring body itself but for formulating drug loaded rods.

5

Mechanical testing of polymer frames for use in a modular vaginal ring

5.1 Introduction

The modular vaginal ring concept described in this thesis comprises two components – one or more drug-releasing modules and a polymer frame into which the modules can be easily inserted and retained. Since the polymer frame will likely contribute significantly to the overall mechanical properties and performance of a fully constructed ring device, it is necessary to evaluate the mechanical properties of the frames themselves.

The mechanical characteristics of vaginal rings are critical to their clinical performance, including (i) ease of insertion and removal, (ii) comfort during use, and (iii) ability to be retained in in the vagina. These mechanical characteristics are largely determined by three key factors – the type of polymer, the grade of polymer, and the overall dimensions of the ring. For example, silicone elastomer rings (e.g. Femring®) are generally larger than rings manufactured from EVA copolymer (e.g. NuvaRing®), since the type of silicone elastomers are usually softer than most EVA materials.

Currently, only three different types of polymers are used for manufacturing marketed rings, silicone, ethylene vinyl acetate (EVA) copolymers, and thermoplastic polyurethanes (TPU). These polymers exhibit a range of different physical and mechanical properties depending on the polymer composition. Of these three polymers, only silicones and EVAs are in direct contact with vaginal tissue. TPU, although included in the manufacture of Ornibel®, is only used to fabricate the inner drug-loaded core. However, TPU has been widely reported in a number of experimental antiretroviral-releasing rings that do contact vaginal tissue [48, 64,182–184]. There is also limited reported literature on other polymeric materials for use in vaginal ring manufacture, including polycaprolactone and various hydrogels [185–189].

Silicone elastomers are synthetic, chemically crosslinked, non-biodegradable, highly elastomeric, thermosetting polymer systems [190]. Their physical and mechanical characteristics that can be adjusted by varying the concentration of the components, for example, mechanical fillers [191]. With a large number of suppliers offering

different grades, silicone elastomers are supplied having a wide range of viscosities, cure temperature, shore hardness and mechanical properties.

Different types of silicone elastomers are available, such as liquid silicone rubbers (LSRs), room-temperature vulcanised (RTV) silicone rubbers, silicone elastomer dispersions (in solvents), and high consistency silicone rubbers (HCRs). For use in medical and drug delivery applications, grades are selected conforming to USP Class VI / ISO 10993 specifications. LSRs and RTVs are the most common types of silicone rubbers for manufacture of vaginal rings as they are capable of being injection molded. HCRs are also available but have a much higher viscosity than that of LSRs and RTVs. Estring® is the only marketed vaginal ring manufactured from an HCR (Silastic® Q7-4735). Silicone elastomer dispersions are used for fabrication of the shell component of breast implants.

Different cure chemistries are also possible with silicone elastomers, the most common of which – at least for ring manufacture – are condensation-cure and addition-cure systems. Condensation-cure systems (used for Femring® and the cores of Annovera™) use a tin catalyst and due to their unique chemistry can be cured rapidly at temperatures lower than 100 °C. This makes these systems particularly suitable for drugs that are heat sensitive. However, the tin catalyst can be easily poisoned by certain chemical functional groups, including amine groups. Addition-cure silicone elastomer systems (used in the manufacture of Estring®, Progering®, Fertiring® and the body of Annovera™) cure at significantly higher temperatures (120–180 °C) but tend to be compatible with a wider range of chemical functional groups. Other cure chemistries for silicone elastomers include peroxide and UV cure systems. However, due to the higher potential for drug degradation and lack of medical/drug delivery grades, they have not been widely used.

Thermoplastic EVAs, like silicone elastomers, have a long history of use as drug delivery products providing controlled and sustained release [192]. Vaginal ring products such as NuvaRing® and the subdermal contraceptive implants Implanon®/Nexplanon® were developed following the success of the ocular implant Ocusert® (1974) and the intrauterine device Progestasert® (1976) [193]. EVAs are hydrophobic, non-biodegradable, thermoplastic copolymers manufactured by free

radical polymerisation of ethylene (ethene) and vinyl acetate. Vinyl acetate content ranges between 1–40 % with properties such as thermal, mechanical and drug permeability dependent on this content [192]. EVAs are generally stiffer than silicone elastomers such that EVA vaginal ring products tend to have much thinner cross-sectional diameters (4–4.5 mm). NuvaRing® is the only vaginal ring product on the market assembled fully from EVA. The ring is created by co-extruding a drug-free sheath of 9% vinyl acetate EVA over a drug-loaded core prepared from 28% vinyl acetate [87].

Polyurethanes (TPUs) are thermoplastic polymers suitable for injection molding, extrusion and 3D-printing of drug delivery devices [177]. They are beginning to emerge as useful materials for fabrication of vaginal rings, particularly given the broad range of properties available by manipulating their chemistries (adjusting the soft and hard segments) [48, 159, 182,189]. The drug-loaded core component of the combination contraceptive vaginal ring Ornibel® contains a TPU material. Like EVAs, rings made from TPUs are generally thinner than those made from silicones due to their increased stiffness of the polymer.

While it is widely understood that both the overall dimensions and the nature of the polymeric materials used in vaginal ring construction contribute to the final mechanical properties, there has been only limited discussion around mechanical testing methods in the literature [194]. Most new products are designed with similar mechanical performance specifications to existing marketed rings. Mechanical tests (Table 12) results are dependent on various factors such as ring dimensions, drug loading, type of polymer used and manufacturing technique [177,194]. These mechanical tests are designed to be clinically relevant and demonstrate mechanical reliability to regulatory authorities [195].

Currently, there are no official standard test methods for the mechanical testing of IVRs, although McCoy *et al.* have recently reported a detailed testing regime [194]. The test methods were based upon two existing standards: the ISO 8009:2014 standard for testing mechanical contraceptives and ASTM D2240-15, Standard Test Method for Rubber Property – Durometer Hardness [196,197]. Test methods are described in Table 12. The number of samples per test reflects the ISO standard for diaphragms

which is n=13. These tests provide a variety of assessments that reflect both quality control of rings and the forces experienced in clinical use.

Table 12. Description and purpose of mechanical testing methods commonly used for IVRs.

Test method	Purpose	Action	Time period/Cycles
Durometer hardness	Quality control for IVR polymers	Measure Shore A or M value on IVR	Four measurements per ring. N=13 rings per formulation
1000-cycle	Quality control to ensure mechanical strength and durability.	Compression to 25 % original OD. Measure recovery of ring.	1000 compressions. N=13 rings per formulation
5 mm compression	Small compression used to compare numerous rings	Compress 5 mm. Record force.	Six compressions per ring. N=13 rings per formulation
28-day deformation	Mimics compression during clinical use	Compress to 25 % original OD. Measure recovery of ring.	Static 28-day study. N=13 rings per formulation
Elongation to break	Reflects removals from the vagina	Ring stretched until fracture achieved. Record maximum extension.	N=13 rings per formulation
Compression to 25% of original OD	Mimics forces experienced by the IVR in clinical use	Compress to 25% of original OD. Record force.	N=13 rings per formulation
Twist during compression	Adapted from industry standard for diaphragm devices	Compression to 25 % original OD. Measure twist.	N=13 rings per formulation. Rings tested before and after 1000-cycle compression.

In this study, six different polymers considered potentially useful for construction of the ring frame were tested for their mechanical properties: EVA-17.5%, LDPE, Pebax®, Estane® , Hytrel®, Desmopan® and Vistamaxx™ (Table 13). Thin polymer sheets were first prepared and tested for compression. Subsequently, a single-sized injection mold was designed and fabricated, and prototype polymeric frames were prepared by injection molding and assessed for mechanical strength using compression test methods adapted from those described by McCoy *et al.* [194]. Finally, mechanical testing was also performed with prototype ring frames having silicone rods inserted to assess the mechanical strength of a fully constructed ring device.

Table 13. Description, properties and previous uses of the various polymer materials used to fabricate the ring frames.

Polymer Name	Polymer type	Properties	Uses	Drug delivery devices
EVA-17.5 %	Ethylene vinyl acetate (% denotes vinyl acetate content)	Flexible, transparent	Orthotics, mouthguards, foam sheets, shoes	NuvaRing ®, Progestasert®, Nexplanon®, Ocusert^{t}®,
LDPE	Thermoplastic made from monomer ethylene	Good chemical resistance, flexible and tough	Work surfaces, plastic wraps, playground slides, containers	Polymer frame of Mirena
Pebax®	Thermoplastic elastomer made of polyether and polyamide	Lightweight, water resistance, flexible	Injected parts, silent gears, transmission belts	None
Estane®	Thermoplastic polyurethane	UV resistant, good melt strength, high clarity	Rubber replacement for outdoor shoes, tubing 3D printing	None
Hytrel®	Polyester elastomer	Flexible, chemical resistant, durable, strong	Cable insulation. Food contact materials, polymers for oil and gas	None
Desmopan®	Thermoplastic polyurethanes (TPU)	Flexible, hydrolysis and microbe resistance, high impact strength	Roof lining, seals, membranes, films, rigid/flexible composite systems; sport shoe soles	None
Vistamaxx™	Isotactic propylene repeats units with random ethylene distribution	Tough, good elasticity, good chemical resistance, soft	Adhesives, sealants, building and construction, compression packing, packaging	None

5.2 Materials and methods

Materials

See Chapter 2 for a description of the materials used in this chapter.

Manufacture of ring frames

Ring frames were manufactured from different thermoplastics using a Babyplast 6/10 P horizontal injection molding machine (Molteno LC, ITA) fitted with a custom stainless steel ring mold assembly. Drug free pellets were dried as per manufacturer recommendation and loaded separately into the machine's hopper. Ring frames were manufactured by injecting molten thermoplastic into the custom mold using 100 bar clamping pressure, 70 bar injection pressure and various temperature and polymerisation conditions (Table 14). Rings were removed from the molds, deflashed (where necessary) and stored at ambient temperature until testing.

Table 14. Temperature profiles used for injection molding of thermoplastic ring frames.

Condition for injections molding	VX	DS	HY	ES	EVA-17.5%	PB
Plastification unit temperature (°C)	190	190	190	190	190	220
Injection chamber temperature (°C)	195	200	195	200	195	230
Nozzle temperature (°C)	195	200	195	200	195	230
Fixed mold temperature (°C)	30	30	30	30	30	30
Polymerisation time (secs)	60	25	25	25	25	25

Mechanical testing of ring frames

Ring frames (cross-sectional diameter 5.78 mm, outer diameter 52 mm) and underwent compression testing using a TA.XT plus Texture Analyser (Stable Micro Systems, Surrey, UK).

5 mm compression

IVR frames were tested using the compression mode, with a target distance of 5.00 mm and a test speed of 2 mm/s. Six compression cycles were completed, with the maximum mean force value being recorded for the last five cycles.

Compression to 50% outer diameter

IVR frames were tested using the compression mode, with a target distance of 20 mm and a test speed of 10 mm/s. Six compression cycles were completed, with the maximum mean force value being recorded for the last five cycles.

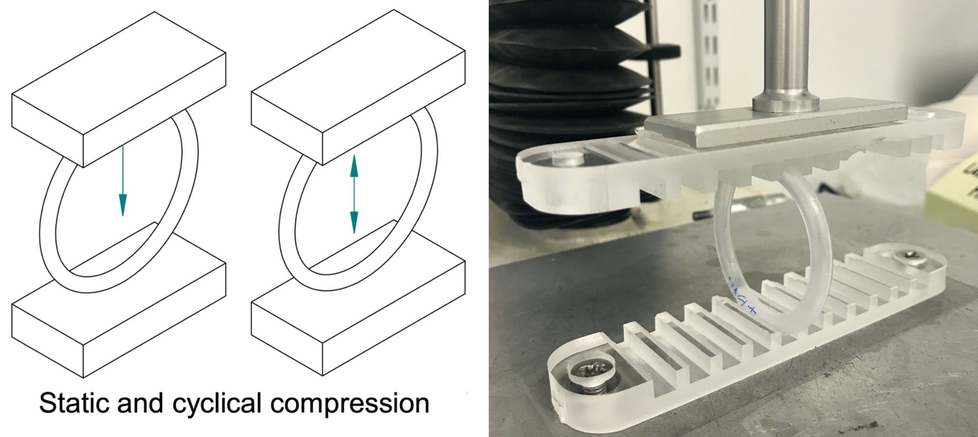


Figure 20. Pictorial depiction of static and cyclical compression cycle experienced by polymer ring frames.

28-day static compression

Each polymer ring frame (n=4) was individually placed in a compartment of a custom aluminium compression jig and secured with a Perspex® cover (Figure 21). A central single screw was inserted into each compartment and the ring compressed to 50 ± 5% of its OD for a 28-day period. Each ring was then removed and allowed to recover for a period of 30 s before the percentage recovery of the original ring diameter was measured using a custom ring recovery gauge. The ring recovery gauge ensured consistent and accurate measurement of recovery.

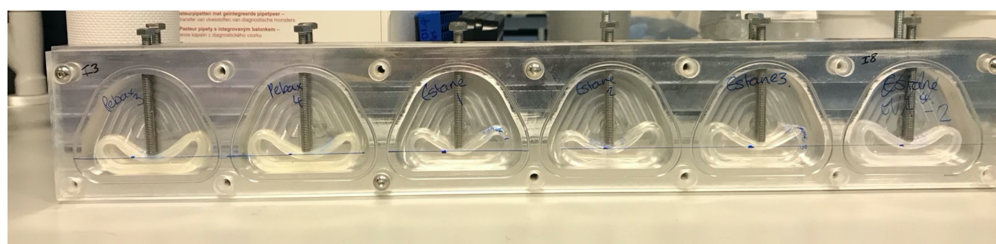


Figure 21. Custom aluminium compression jig for 28-day static compression study.

5.3 Results and discussion

5 mm compression of ring frame, with and without a full core inserted

The trend in the mean force required to compress the ring frames to 5 mm – both with and without a full silicone elastomer core inserted – was: DS < VX < HY < EVA-17.5% < PB < ES (Figure 22). Both of these materials are TPUs. ES possesses a higher ratio of hard to soft segments compared to DS making DS more flexible, as which is evident in Figure 22. Mean force required to compress polymer rings frames to 5 mm: A – frames only; B – frames with silicone core inserted. Error bars represent ± 1 standard deviations of n=4 replicates..

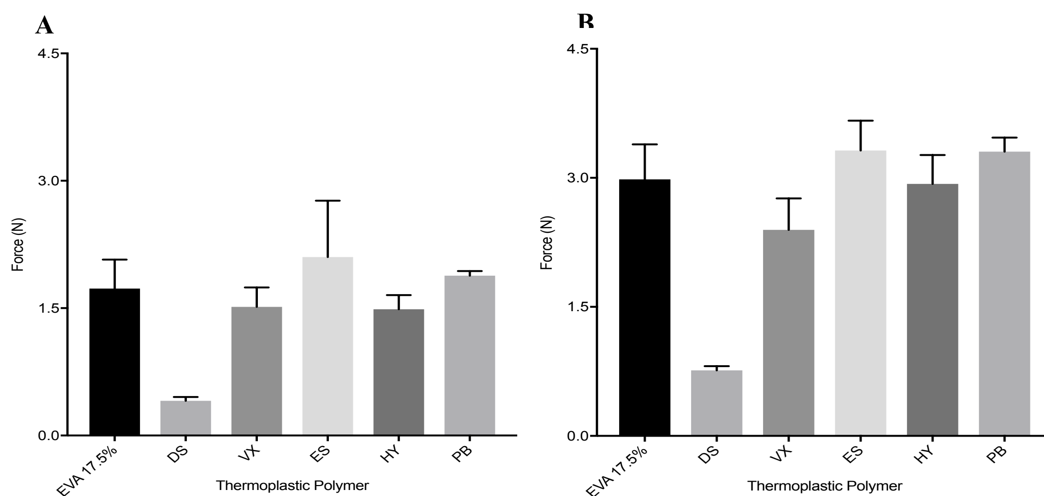


Figure 22. Mean force required to compress polymer rings frames to 5 mm: A – frames only; B – frames with silicone core inserted. Error bars represent ± 1 standard deviations of n=4 replicates.

Comparing Figure 22 A and B (A = frame only, B = frame with a full 4 mm silicone core), there is an increase in force needed to compress the frame with a core inserted compared to an empty frame. On average, an extra 42 % (SD ± 5 %) increase in force is needed to compress the filled ring frames compared to the empty frames. This is as expected, since the added silicone core will add resistance and strength to the ring frame. Although the ring frame is intended to accommodate multiple cores, this experiment was limited to a single full core to allow direct comparison between ring frames.

Using One-way ANOVA and Tukey's Multiple Comparisons test, it was calculated that there was a statistically significant difference ($p < 0.05$) between EVA 17.5% and DS and VX; DS and VX, ES, HY and PB; VX and ES, HY and PB.

Compression of ring frame to 50% of their outer diameter with and without cores

The mean forces required to compress ring frames to 50 % of their outer diameter (OD) with and without a full silicone core are presented in Figure 23. As with the 5 mm compression, the force required to compress the ring frames with cores inserted was increased compared to frames without cores. On average there was an 44% increase of force when a silicone core was inserted into the ring frame. This is similar to an increase in force seen in the 5 mm compression with and without the core (42%). This suggests there is no added mechanical resistance for a deeper compression.

McCoy *et al.* have previously reported compressing various IVRs to 25 % of their original OD [194]. However, in that study, most of the rings were manufactured from silicone elastomer; since thermoplastic rings are prone to deformation during compression (i.e. do not fully recover to 100 % OD), a 50 % compression was chosen for this study.

The mechanical characteristics of thermoplastic rings fitted with a silicone core were compared with those of commercial rings reported by Johnson *et al.* [48]. While jigs and test methods used by Johnson *et al.* differ from that used here (in both cases custom jigs are used and Johnson *et al*. used a test speed of 1 mm/s compared to a test speed here of 10 mm/s), it is the only reported incidence of a 50 % OD compression. Femring, Estring and NuvaRing IVRs required approximately a force of 4, 9 and 2.25 N, respectively. All ring frames required less force to compress than commercially manufactured rings such as Femring® and Estring®, while DS was the only ring frame to require less force than NuvaRing®. These findings, while preliminary, suggest that this set of polymers have similar mechanical compressional strength comparable to that of polymers used for commercial manufactured IVRs.

Using One-way ANOVA and Tukey's Multiple Comparisons test, it was calculated that there was a statistically significant difference ($p < 0.05$) between all samples apart from EVA 17.5% and PB.

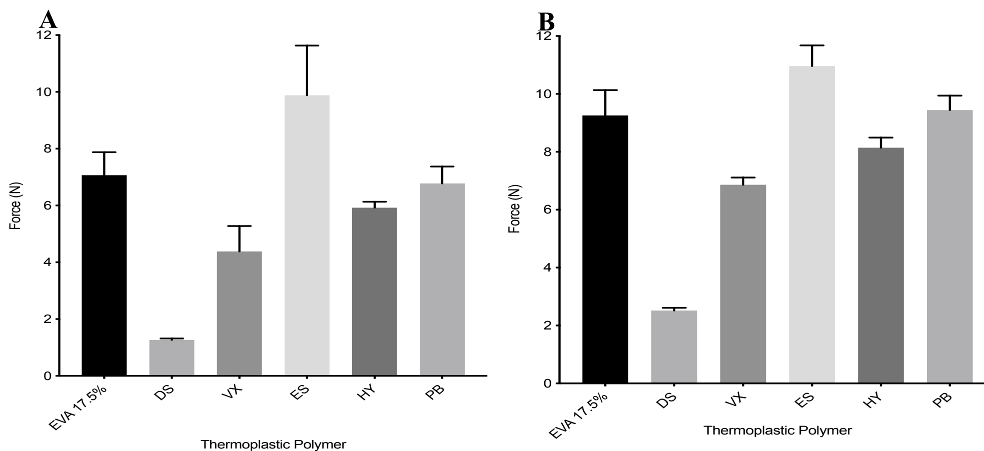


Figure 23. Mean force required to compress polymer ring frames to 50% of their original outer diameter A – frames only; B – frames with silicone core inserted. Error bars represent ± 1 standard deviations of n=4 replicates.

28-day static compression of ring frames

Mean recovery values for samples (n=4) compressed to 50 % of their outer diameter for 28 days were determined using a customised ring gauge (Figure 24). Static compression results are broadly similar to previous mechanical testing results for both a 5 mm and 50 % OD compression. ES showed the worst recovery after the 28 days, followed by PB and then EVA-17.5%, consistent with an increased force needed to compress these ring frames in both 5 mm and to 50% OD. DS and HY had recovery values between 70–80 %, while VX recovered to 80–90% of its original OD.

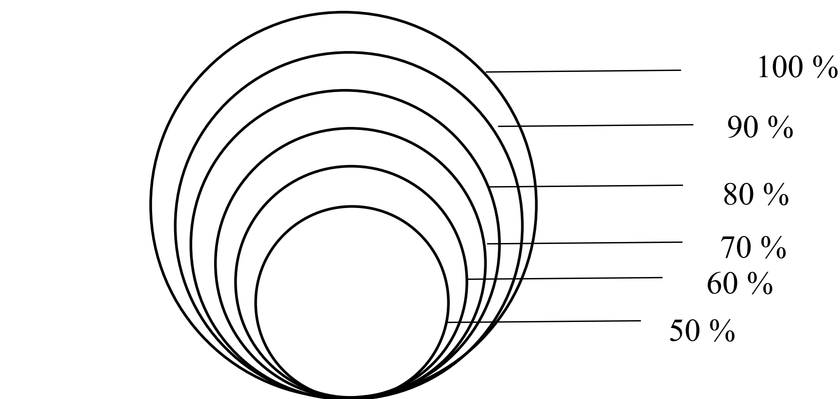


Figure 24. Customised ring gauge chart to document recovery values for a 28-day static compression.

28-day static compression of the EVA vaginal ring Nuvaring® has previously been reported by McCoy *et al.* [194]. Nuvaring® consists of a drug loaded EVA-28 % core co-extruded with an EVA-9% sheath. Nuvaring® recovered to 50–60% of its original OD. The EVA ring tested in this study contained a 17.5% VA content but was only compressed to 50% original OD rather than to 25% reported by McCoy *et al.,* which is likely the cause of the different reported values.

Table 15. Average recovery values for a 28-day static compression testing on ring frames (n=4).

Polymer ring frame	Recovery valve (%)
PB	50 – 60
VX	80 – 90
DS	70 – 80
HY	70 – 80
ES	> 50
EVA-17.5%	60 – 70

5.4 Conclusion

Novel vaginal ring frames were manufactured from a range of six different polymers and their mechanical properties assessed using three difference mechanical testing methods. A small 5 mm compression on the polymer frames with a silicone core inserted and the frame only resulted in a clear order of mechanical strength, DS < VX < HY < EVA-17.5% < PB < ES, with ES requiring the most force to compress. This order was similar to that of the results obtained in a compression to 50 % original outer diameter. At the end of a static 28-day compression study, recovery values resulted in VX, DS and HY showing best recovery values. Moving forward in this thesis, VX, HY and DS will be used as potential polymers for the novel ring frame.

6

In vitro release study of drug loaded matrix-type cores placed in thermoplastic ring frames

6.1 Introduction

This chapter focuses on incorporation manufacture and preliminary *in vitro* release testing of prototype rings for an MPT ring, potentially allowing for rapid assembly of different ring configurations by permitting insertion of one or more individual drug modules into a thermoplastic IVR frame, thereby permitting co-release and independent control of actives having very different physicochemical properties and target release rates. Part of this study was also to explore ingress of drug into the prototype polymer frames. The incorporation of dyes into drug delivery systems and devices can be helpful in many aspects of drug product development, including gaining insights into the mechanisms of drug release and visualising drug distribution (both *in vivo* and *in vitro*) [225–228]. Dyes have also been used as a visual indicator in formulating drug delivery devices, either by placing the drug delivery system into a dye-containing solution dye or incorporating dye into the device itself [79,229]. For example, Morrow *et al.* used methylene blue dye to assess the extent of uptake of aqueous release medium into a protein-releasing silicone elastomer vaginal ring formulation containing large concentrations of water-swelling excipients [79].

Firstly, a study was performed using dye-loaded silicone elastomer rods which were manufactured and inserted into the ring frames to provide a visual indication of the extent to which a hydrophobic drug might permeate into the ring frame. Methyl red, an azo dye compound having a log P value of 3.3 and a molecular weight of 269.3 g/mol, was selected as it has similar physicochemical characteristics to MIV-150 (log P 3.5) [230]. After an *in vitro* release study was conducted to investigate the impact on release of insertion of silicone elastomer rods containing MET, NES and MIV-150 into prototype ring frames manufactured using three different polymers – Vistamaxx™ (VX), a propylene-based elastomer; Desmopan® (DS), a hydrophobic polyurethane; and Hydtrel® (HY), a semi-crystalline polyester. The potential for drug permeation from the rods into the polymeric ring frames was also assessed after completion of *in vitro* release via a solvent extraction method.

Figure 25. Computer-aided design (CAD) drawings showing the prototype modular ring design.

6.2 Materials and methods

Materials for the work described in this chapter are presented in Chapter 2

Manufacture of drug loaded silicone cores

Drug loaded (2.5% w/w NES, MIV-150 and MET) silicone elastomer cores (overall diameter 52 mm; cross-sectional diameter 4 mm) were manufactured using a laboratory-scale injection molding machine fitted with a custom stainless-steel ring mold assembly. Separate 25 g Part A and Part B premixes of the DDU-4320 addition-cure silicone elastomer systems were prepared by adding weighed quantities of selected API into a screw-cap polypropylene container followed by addition of the silicone part. The premixes were first-hand-mixed (30 s) and then mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixer® DAC 150 FVZ-K, Hauschild, Germany) (60 s, 3000 rpm). A and B premixes were combined in a 1:1 ratio. Specifically, 25 g of each premix were added to a screw-cap polypropylene container to a final weight of 50 g and hand-mixed for 30 s and then DAC mixed (60 s at 3000 rpm). This mix was then transferred into a polypropylene SEMCO® injection cartridge designed for manual injection. Rings were manufactured by manually injecting the active mix into the heated ring mold assembly using an 80°C mold temperature and 180 s cure time. Rings were removed from the heated mold, deflashed as needed, and stored at ambient temperature until further testing.

Manufacture of ring frames

Ring frames where manufactured as described in Chapter 4. Briefly, drug free pellets were dried as per manufacturer recommendation and loaded separately into the

machine's hopper. Ring frames were manufactured by injecting molten thermoplastic into the custom mold conditions described (Table 16). Rings were removed from mold, deflashed (where necessary) and stored at ambient temperature until testing took place.

Table 16. Temperature profiles for thermoplastics used for injection molding ring frames

Condition for injections molding	VX	DS	HY
Plastification unit temperature (°C)	190	190	190
Injection chamber temperature (°C)	195	200	195
Nozzle temperature (°C)	195	200	195
Fixed mold temperature (°C)	30	30	30
Solidification time (secs)	60	25	25

In vitro release testing

On Day 0, rings (n=4) with segments inserted in a selected configuration (Figure 26) were placed individually into 250 mL glass bottles containing 100 mL of release medium (0.2 % Tween adjusted to pH 4.2) and stored in an orbital shaking incubator (Unitron HT Infors; 37 °C, 60 rpm, 25 mm orbital throw). After 24 ± 0.25 hr, the release medium was sampled and replaced with a fresh 50 mL of the respective media, and the samples retained for UPLC analysis. Thereafter, sampling and 50 mL replacement of the release medium was performed daily out to Day 10, except on Fridays when, after sampling, the flask was replenished with a 100 mL volume of release medium and no further replacement or sampling performed until the following Monday. Samples were analysed by UPLC according to the methods described in Chapter 2.

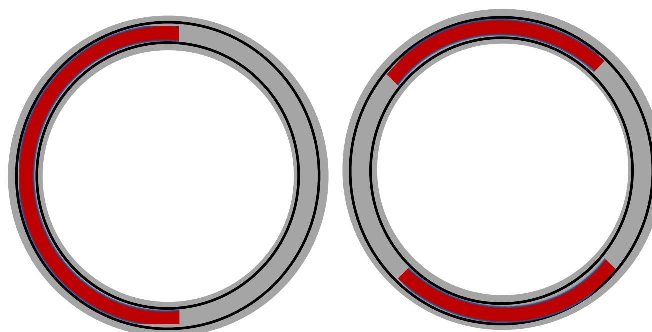


Figure 26. Diagram showing the two different configurations of API-loaded silicone cores (red area) in the thermoplastic ring frames.

Content assay for ring frame and cores

Content assays were performed to accurately quantify the total amount of drug loading in the ring cores and to determine the extent of drug leaching into the thermoplastic frame. Samples (n=3) were weighted, cut into 1 cm pieces and placed in 250 mL glass bottle with selected media. Acetone was used as the extraction solvent for content assay of ring cores and for the VX and HY rings frames. Since acetone dissolved the DS ring frames, ethanol was used as the extraction solvent instead. Extraction flasks were stored in an orbital shaking incubator (Unitron HT Infors; 37 °C, 60 rpm, 25 mm orbital throw) for 24 hr. After 24 ± 0.25 hr, samples were removed and left to cool to room temperature. Extraction samples were diluted as per Table 17 and analysed as per UPLC method described in Chapter 2.

Table 17. Extraction volume and dilution factors used for the content assay of thermoplastic ring frames and silicone cores.

Section for content assay	Volume of extraction media mL	Dilution factor
Full thermoplastic ring frame	25	0.25 mL in 10 mL
Half thermoplastic ring frame	10	0.25 mL in 10 mL
Quarter thermoplastic ring frame	10	0.1 mL in 5 mL

Dye penetration study

Dye-loaded silicone rings were manufactured similar to drug loaded cores except instead of adding API, a 2.5% w/w loading of methyl red was added to the silicone elastomer. Rings were cut in half and stored until testing commenced. On Day 0, the devices (n=4 per formulation) were placed individually into 250 mL sealed glass flasks containing 200 mL of release medium (0.2 % Tween adjusted to pH 4.2) and the flasks placed into a shaking orbital shaking incubator (Unitron HT Infors; 37 °C, 60 rpm, 25 mm orbital throw). After 24 ± 0.25 hr, the medium was sampled and replaced with 100 mL of fresh medium. Thereafter, sampling and 100 mL replacement of the medium was performed daily out to Day 10, except on Fridays when, after sampling, the flask was replenished with a 200 mL volume of medium and no further replacement or sampling performed until the following Monday. The devices were removed periodically from the medium and photographed.

6.3 Results and discussion

Visual representation of drug (dye) ingress into polymer frames

Initial photographs (Day 0) of rings were taken to allow for comparison on subsequent days of the study (Figure 27). Both DS and VX polymers produced colourless ring frames, and the HY polymer produced an off-white/yellow-tinted ring frame. Methyl red loaded cores of 4 mm cross-sectional diameter were inserted into the rings. The dark red loaded cores showed good contrast against the thermoplastic ring frames on Day 0.

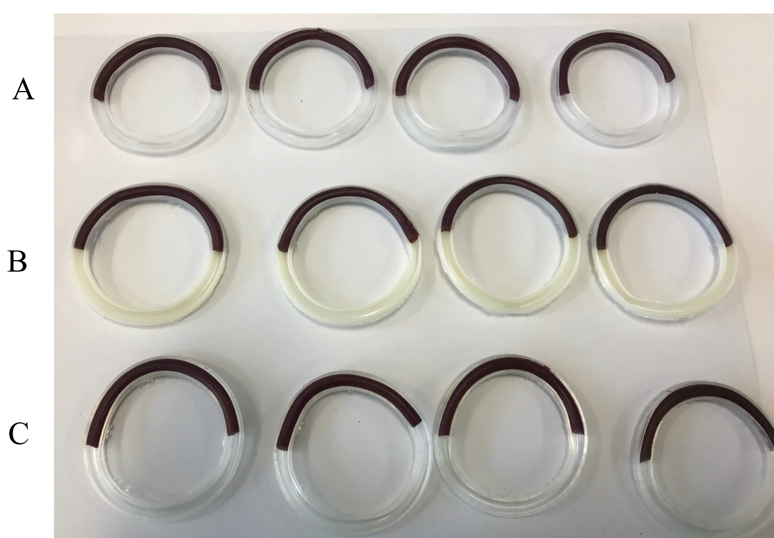


Figure 27. Thermoplastic ring frames (A – Vistamaxx™, B – Hydtrel®, C – Desmopan®) fitted with methyl red loaded (2.5% w/w) silicone ½ core.

Before changing the medium on Day 1, rings were removed and photographed. The media was tinted red due to release of dye from the silicone core (photo not shown). After 24 hr (Figure 28), the VX ring frame shows no colourisation, while both the HY and DS ring frames showed significant penetration of the methyl red dye. With the HY ring, the ring frame immediately surrounding the core and were more heavily coloured the section of ring frame that did not include the core; a clear red-coloured section is apparent close to the core ends.

On Days 2, 3 and 4, ring frames were removed from the release media and photographed. Once again, the release media were observed to be coloured due to

methyl red release. The extent of colourisation of the ring frames after T= 4 days is evident, with DS and HY showing the greatest uptake of dye (Figure 29).

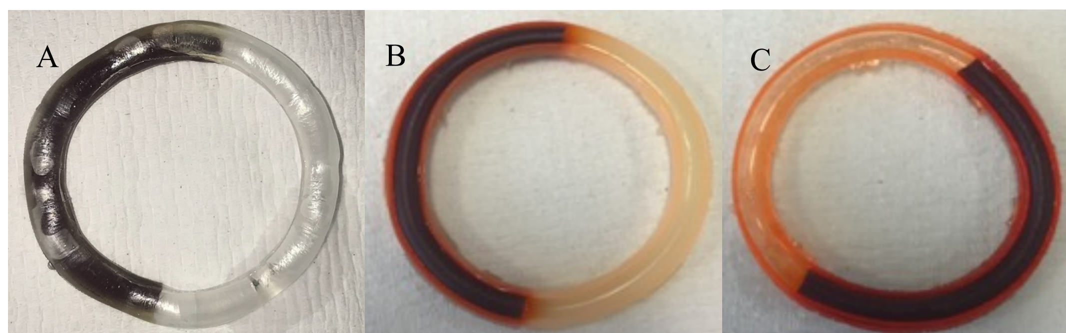


Figure 28. Vistamaxx™ (A), Hydtrel® (B) and Desmopan® (C) rings showing the extent of methyl red penetration from the cores into the frames after 24 hr placement in the *in vitro* release medium.

After 10 days, the rings were removed from the release media, dried, and photographed with cores inserted and removed. The extent of colourisation is evident (Figure 30 & Figure 31) with the Day 0 rings presented as controls. Figure 30 clearly shows that the methyl red dye completely penetrated into those sections of the DS and HY ring frames, but not those of VM which did not initially contain a methyl red core. Visually, the extent of penetration into the DS and HY ring frames appeared similar. There are two possible mechanisms accounting for this observation: (i) the methyl red is diffusing from the core into the DS and HY ring frames, and subsequently diffusing throughout the entire volume of the ring frame; (ii) the methyl red is releasing from the cores into the release medium, and subsequently diffusing back into the ring DS and HY ring frames.

When silicone core were removed (Figure 31), the VX and HY ring frames – but not the DS frames – clearly show darker discolouration due to methyl red penetration at those sections of the ring frame that contained the core. The extent of discolouration of the VX ring frames was very limited, though, compared to the very extensive discolouration of the HY rings.

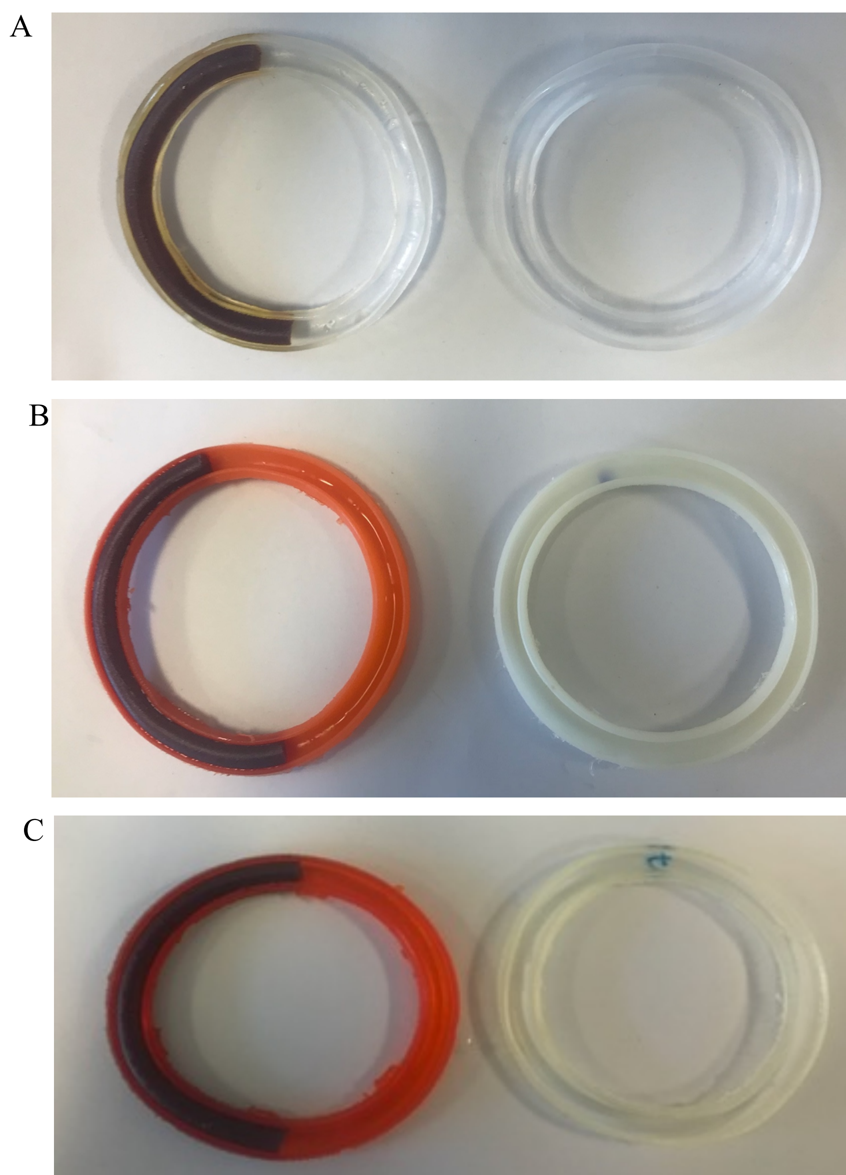


Figure 29. Vistamaxx™ (A), Hydtrel® (B) and Desmopan® (C) rings showing the extent of methyl red penetration from the cores into the frames after 4 days placement in the *in vitro* release medium.

The data obtained here with methyl red sheds further light on the *in vitro* release and content assay data obtained previously. First, it is clear that the dye is capable of penetrating the polymeric frame directly from the core. Therefore, the physical interference fit does not appear to be an obstacle to drug penetration into the frame. Second, once the dye has penetrated the frame, the dye is capable of diffusing relatively quickly throughout the frame volume. Third, the dye is effectively released from the rings into the release medium, as evidenced by the coloured media. And fourth, the dye appears to be able to penetrate into the frame from the release medium.

A further study was performed to measure the extent of swelling of the empty polymer ring frames when immersed in the release medium. A mean weight gain (n=4) of 0.6, 1.5 and 1.7 % for the HY, VX and DS polymers, respectively, was recorded (HY < VX < DS). This rank order contrasts with that observed for dye penetration (VX < HY < DS), although DS does show the highest values for both measurements.

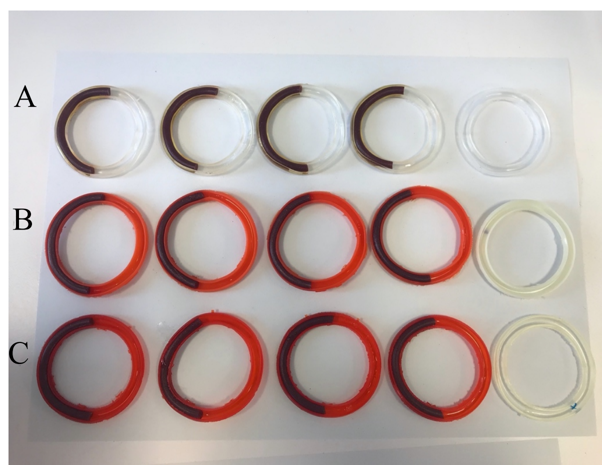


Figure 30. Vistamaxx™ (A), Hydtrel® (B) and Desmopan® (C) rings with dye cores inserted on Day 10 of the study. A comparison with control rings can be seen at the right-hand side of picture.

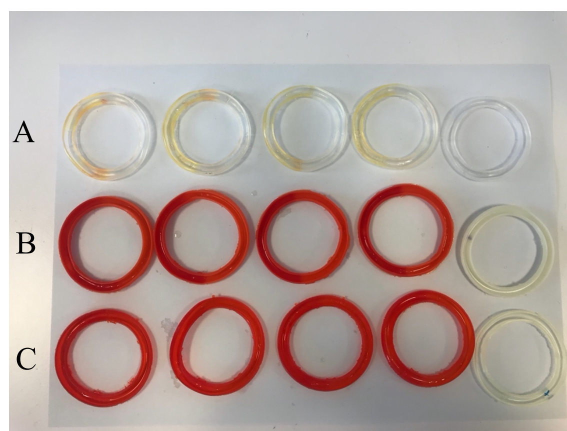


Figure 31. Vistamaxx™ (A), Hydtrel® (B) and Desmopan® (C) rings with dye cores removed on Day 10 of the study. A comparison with control rings can be seen at the right-hand side of picture.

In vitro release of MET, NES and MIV-150 from drug-loaded cores

Metronidazole

Mean daily and cumulative release versus time plots for MET are presented in Figure 32 A and D, respectively. Overall, Day 1 MET release ranged from 1330–2209 μg/day and release amounts declined steadily throughout the experiment (Day 10 range: 470–591). The overall trends observed in daily release were similar across the formulations

(Figure 28A). The cumulative release plots (Figure 28D) better highlight the differences in release, and indicate total MET release ranging between 7 and 10 mg.

Unsurprisingly, MET release was highest with the fully exposed control silicone elastomer cores with no ring frame, at least during the early period of release; mean Day 1 release was similar for the single ½ core and the two ¼ cores (2389 and 2362 μg, respectively). By Day 10, cumulative MET release from these control cores was close to 10 mg, the highest values obtained.

In general, MET release from cores inserted into ring frames was lower than that for the control cores, although not as low as anticipated based on the extent to which the surface area of exposure to the release medium was reduced. For both DS and HY, their flexible material allowed a secure fit around the rod, causing for minor differences between samples, however, VX due to the long solidifying time needed to solidify the material in the mold, even after 50 sec, the material is malleable and shrinks. Although this was tried to keep to a minimum shrinkage of the sides occurred causing an unevenness in fit and coverage of surface area causing the difference in release amounts.

Over the 10-day *in vitro* release study, daily release values become lower, consistent with a matrix-type vaginal ring behaviour due to depletion of the drug layer on the surface being available. However, cumulative graphs (and root time, not shown here) do not fit a straight line indicating that these devices do not corresponding with matrix-type release characteristics. This is due to the nature of the thermoplastic ring frame. Although a small section of the surface is exposed, most of the core is contained in the frame, providing a type of reservoir. This means this frame presents its own type of release kinetic, not matrix nor reservoir but is somewhere in between.
A target release of 37.5 mg/day was set for MET to provide therapeutic levels for indications of bacterial vaginosis compared to a recommended dose of MetroGel [231]. In all cases, this target was not achieved; however, these studies were carried out at a 2.5% w/w loading, and future studies will involve a much higher loading of MET. The release experiments provided sink conditions for MET (solubility in 0.2% Tween, pH 4.2 is 8.72 mg/mL).

Using One-way ANOVA and Tukey's Multiple Comparisons test, it was calculated that there was a statistically significant difference ($p < 0.05$) in the initial release (day 1) of MET release from DS in any combination and VX and HY. While there was no statistical different between VX and HY. This was the same at Day 10 also. In regard to the single ½ core and the two ¼ cores, there was no significant difference ($p > 0.05$) in overall cumulative release.

MIV-150

Mean daily and cumulative release versus time plots for MIV-150 are presented in Figure 28 B and E, respectively. MIV-150 release on Day 1 ranged from 18–93 µg/day and had declined to 16–56 µg/day by day 10. Figure 28E (cumulative release graph) better depicts the different in release around the different formulations.

Similar to MET, MIV-150 drug release was highest with the fully exposed cores than cores in the ring frame throughout the study. Day 1 release for single ½ core and the two ¼ cores was similar while there was a 10% different by day 10 (55.8 and 50.5 respectively).

Overall MIV-150 release from cores inserted into the ring frames was reduced greatly compared to control cores. There was a large reduction in drug release between cores inserted into the VX frame and the HY and DS frames. Clearly thermoplastic frame polymer choice has a greater influence on reduction of release rate rather than reduction in surface area as previously hypothesised. This substantial difference in Day 10 cumulative release differences was not as significant during *in vitro* release studies using MET cores due to MET being a highly hydrophilic drug. MIV-150 is a highly hydrophobic drug and HY and DS polymers are hydrophobic thermoplastics. It is theorized that the frames have the potential to uptake drug once it has been released from the core, either from the release medium itself or from the cores that are in constant contact with the frame throughout the study.

A target release of 150 µg/day was set for MIV-150 to provide therapeutic levels for protection against the acquisition of HIV-1. In all cases, the therapeutic target was not met, with the control core -only samples providing the highest release of 91 and 93 µg

for 1 x ½ and 2 x ¼ cores respectively. Again, because these studies were carried out at a 2.5% w/w loading and not in sink conditions (MIV-150 solubility in 0.2 % Tween pH 4.2 is 1.73 µg/mL) which allowed preliminary results to be obtained, there is the opportunity to substantially increase drug loading of the silicone rods and to potentially achieve sink conditions.

Using One-way ANOVA and Tukey's Multiple Comparisons test, it was calculated that there no statistically significant difference ($p > 0.05$) in the initial release (day 1) of MIV-150 release from any ring frame in any combination. By Day 10 the only combination that had no statistically significant difference ($p > 0.05$) was MIV-150 release from DS and HY. In regard to the single ½ core and the two ¼ cores, there was no significant difference in overall cumulative release.

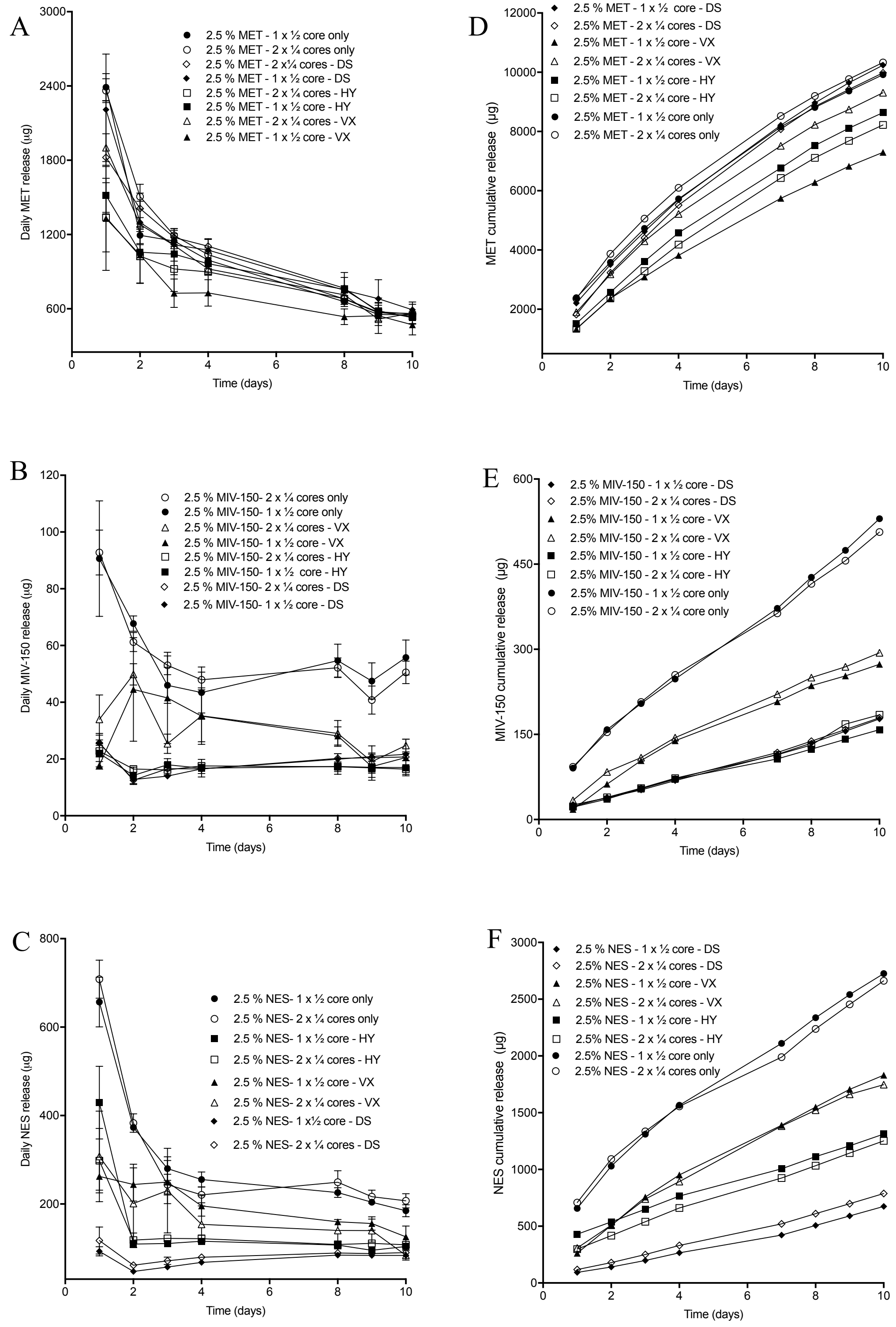


Figure 32. Graphs A, B and C – daily release of a 2.5% w/w MET, MIV-150 and NES silicone rod respectively in two configurations in a selected thermoplastic frame in 0.2 % Tween including a control sample of the core alone vs time (days) for a period of 10 days. Graphs D, E and G – cumulative release of the 2.5% w/w in two configurations in a selected thermoplastic frame including a control sample of the core alone vs time (days) for a period of 10 days in 0.2% Tween.

Nestorone

Mean daily and cumulative release versus time plots are presented in Figure 28 C and F, respectively for NES release. Overall, Day 1 NES release ranged from 93–708 µg/day. By Day 10, daily release had decline to 84–207 µg/day. Across all formulations there was an observable trend in daily release. (Figure 28 C). Figure 3F highlights the differences in release across the formulations with total NES release ranging between 674–2725 µg/day. As with the previous formulations containing MIV-150 and MET, the control samples containing NES showed highest release with a Day 1 mean release value of 656 and 708 µg/day for single ½ core and the two ¼ cores. In general, daily release of NES loaded cores inserted into the thermoplastic ring frames had a reduced release rate compared to control cores. NES release was also modulated depending on which thermoplastic ring frame was used. NES is a hydrophobic drug; however, it is less hydrophobic than MIV-150. As with MIV-150, NES uptake into the selected polymer is expected and potentially can explain the reduction in release amount over the course of the study. However reduced surface area will also play a part in the reduction of release amount, further studies to explore the extend of this were carried out.

A target release of 150 µg/day for a was set for NES to provide therapeutic levels for protection against unwanted pregnancy. Therapeutic targets were met for 9 days for both types of cores being inserted into the VX ring frame. A loading of 2.5% w/w NES loading was used in this study so that results could be comparable with previous formulations (Chapter 4). For future studies, NES release will be manipulated in terms of core length and possibly a sheath to provide sustained release of NES at a therapeutic level.

Assessment of drug uptake after *in vitro* release study.

After completion of *in vitro* release testing, content assay was performed to determine the amount of drug present in the frame at the end of the 10-day study. This study was designed to contain three different sample types from each of the three thermoplastic rings; the full ring of the chosen thermoplastic, each half of the half core configuration of the thermoplastic rings and each quarter of the quarter core configuration. Due to

the limited number of rings, this was carried out as n=1 and is not deemed to be scientifically accurate due to the number of replicates. However, the experiment was conducted to gain an overview of drug ingress into the different polymer rings.

When the full polymer ring frame was assessed, all three thermoplastic frames showed uptake of MET over the 10-day study with VX having the highest drug ingress (Table 18). The greatest NES ingress into the polymer ring frame was observed for the HY polymer, while the greatest MIV-150 ingress was into DS. These results are similar to those reported in Chapter 3, where greatest ingress of API was noted for the most flexible polymers.

Table 18. API content (% w/w) determined from full thermoplastic ring frame content assay.

Thermoplastic frame/API content % w/w	MET	MIV-150	NES
VX	0.14	0.00	0.00
HY	0.06	0.01	0.11
DS	0.01	0.02	0.02

The remaining ring frames were cut into segments for quantification of uptake of API. During this analysis, a trend emerged across the samples. MIV-150 and NES did not permeate the VX samples, while MET had the highest penetration into VX. In sections of the ring frame that housed the core (half or quarter) for the study there was a higher level of API present than the empty section of the frame. API was also detected in the empty sections of the ring frame. This could have been caused by close contact of the end of the cores with the empty frame or the released drug in the media has penetrated into the ring frame.

Although this study only evaluated one ring for each type, it showed drug uptake into the polymer rings, not only in the section of the frame housing the core but in free sections also. This suggests that API is penetrating into the polymer from the core and also potentially from the medium itself.

Using One-way ANOVA and Tukey's Multiple Comparisons test, it was calculated that there a statistically significant difference ($p < 0.05$) in the initial release (day 1) of NES release from any ring frame in any combination. This was the same from

cumulative release on Day 10. In regard to the single ½ core and the two ¼ cores, there was no significant difference in overall cumulative release.

6.4 Conclusions

A dye study was conducted to assess drug permeation into the ring frame using a visual aid. Half-length dye-loaded cores were placed into the ring frame and an *in vitro* release study was carried out over a period of 10 days. Methyl red was used as a small-molecule, hydrophobic model drug compound to visually assess drug penetration into and subsequent diffusion within the ring frame. The study clearly demonstrated that the hydrophobic methyl red dye (simulating hydrophobic APIs) are capable of penetrating all three thermoplastic ring frames directly from the core, although the extent of penetration depends upon the polymer. This observation confirms that the interference fit used to hold the core in the ring frame does not act as an obstacle to drug penetration into the frame.

The *in vitro* release study showed how different thermoplastic materials used for ring frame manufacture influenced the release of different APIs. For example, a VX frame showed the least reduction in release of the three frames and very little drug uptake was observed. However, when VX was injection molded deformations in the ring where seen. These deformations did not provide a secure fit with the silicone cores. These studies suggest that drug uptake has the potential to impact drug release. Furthermore, following on from previous dye study, it suggests that drug penetration into the frame will likely play a role in modulating the drug release characteristics.

Based on the data, HY was selected as the preferred polymer ring frame for use in future studies, since it performed well in mechanical testes (Chapter 5) and provided good *in vitro* release tests for the three APIs. HY did not completely prevent drug/dye ingression into the ring frame during the *in vitro* release, and this will need to be considered for future studies.

7

In vitro release testing of a novel MPT vaginal ring

7.1 Introduction

The fundamental premise behind multi-purpose prevention technologies (MPT) is to address women's multiple sexual and reproductive health issues with use of a single product. Some of most recent innovations in MPTs have focused on vaginal ring products that release multiple active pharmaceutical ingredients (API) [49, 59, 63,64, 89,232,233]. For example, with the dapivirine (DPV) ring having successfully gained approval by the European Medicines Agency for its use in HIV prevention, a second-generation ring containing both DPV and a contraceptive drug would be an obvious next step. Towards this goal, Boyd *et al.* have reported both a 60-day matrix ring and a 90-day reservoir vaginal ring containing DPV and the progestin contraceptive levonorgestrel (LNG) (Figure 34 A and B) [89].

While the current prototypes of the DPV+LNG MPT silicone elastomer rings provide simultaneous release of both APIs close to target release rates, other drug combinations of potential interest will likely be a greater challenge. Both DPV and LNG are small poorly water-soluble molecules that have similar permeation characteristics in silicone elastomer. Also, the absence of any serious chemical interactions between these drugs has allowed them to be incorporated together in a simple matrix-type ring formulation. However, this strategy would likely prove more difficult for combinations of APIs which have different physicochemical properties and/or dosing regimens. Designing a simple matrix-type (monolithic) MPT vaginal ring to simultaneously release both an antiretroviral and a contraceptive steroid is itself hugely challenging, since each drug has unique physicochemical properties and target release rates; it is difficult to optimise the ring device for one drug without also impacting the other.

Typically, contraceptive progestins are released in low microgram per day doses; for example, the levonorgestrel (LNG) intrauterine system releases around 20 μg/day. By comparison, antiretroviral drugs administered vaginally for HIV prevention generally require at least hundreds of micrograms per day; for example, the DPV ring releases about 4 mg over 28 days, equivalent to ~145 μg per day, and efforts are underway to increase this rate to further enhance protection against HIV infection [89,234]. As a

consequence, complex ring designs are often required to successfully formulate each drug component and achieve the desired release rate. For example, Clark *et al.* have reported a segmented dual-reservoir MPT vaginal ring (IVR) releasing tenofovir (TFV) and LNG; the device comprises two partial ring segments welded together to form a full ring [64] (Figure 34C). TFV is an established antiretroviral drug known for its safety, stability and use in pre-expose prophylaxis (PrEP) regimes, while LNG is a long-established steroid compound used in various contraceptive products, including a number of long-acting contraceptive implants (e.g. Mirena®, Skyla®, Jadelle®, Norplant®). Simultaneous release over weeks or months of both TFV and LNG from a monolithic IVR would be very difficult given the different molecular properties of these drugs (TFV is hydrophilic and LNG hydrophobic) and the divergent release targets (TFV ~10 mg/day; LNG ~ 20 μg/day) [64]. The dual-segmented reservoir-type thermoplastic polyurethane (TPU) ring was developed in an attempt to overcome these difficulties (Figure 34C). A hydrophilic TPU segment was used to formulate TFV within the ring, allowing a relatively high drug loading to be achieved and, as a result, producing high release rates. By comparison, a hydrophobic TPU segment was used to formulate LNG within the ring. By changing the length of these segments, the release rates of TFV and LNG could be easily modulated. Blocking caps were also fitted at the ends of each segments to limit diffusion of TFV and LNG between the segments. This segmented ring, although arguably overly complex in terms of its design, demonstrated clinically relevant release and represents a novel formulation approach to co-delivery of two physiochemically-diverse APIs over 90 days.

Baum *et al.* have reported a different approach for simultaneous administration of up to ten different APIs from a single IVR (Figure 33D). The so-called 'pod-type ring' comprises numerous polymer-coated drug-loaded pods embedded into a silicone elastomer ring holder [63]. This novel approach allows drug release from each pod to be controlled independently, such that APIs with different physiochemical properties can be co-released. Standard compressed tablets containing each drug are first coated with polylactic acid. These pods are then individually placed into cavities within the silicone elastomer ring, each cavity exposed to the outside via a narrow delivery

channel. The thickness of the polymer coating and the diameter of the delivery channel serve to control drug release.

Another version of the novel pod-type IVR has been reported by Moss *et al.* [49]. The pharmacokinetics of this pod ring has been reported in an ovine model for the delivery of five different drugs – TFV, nevirapine (NVP) and saquinavir (SQV) are antiretrovirals of different mechanistic classes, while etonogestrel (ETG) and estradiol (E2) are contraceptive hormones. Measured *in vivo* concentrations in sheep of the antiretroviral compounds correlated with release rates for an effective device while ETG and E2 release was comparable with the NuvaRing® release rates. This proof of principle study demonstrated how the pod ring has the flexibility to control the rate and duration of drug release by modulating the number of pods and the thickness of the polymer membrane.

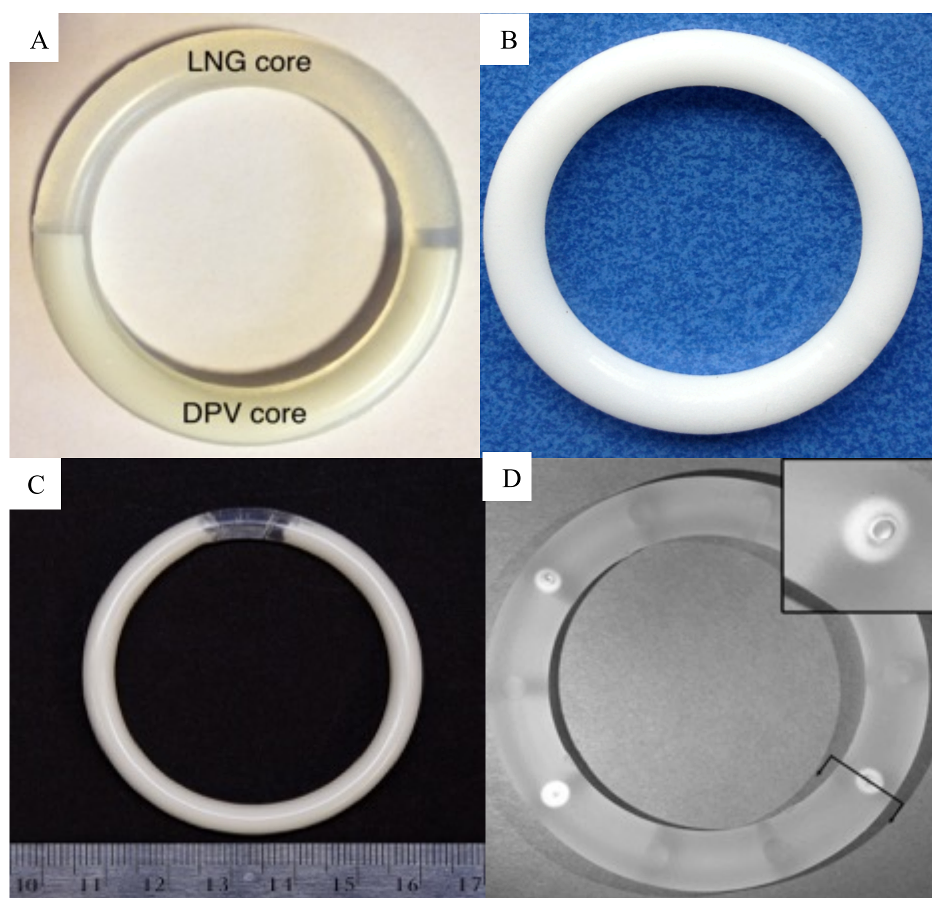


Figure 33. New concepts in MPT vaginal ring devices. A and B – DPV+LNG reservoir and matrix vaginal rings, respectively, reported by Boyd *et al.* [89] C - segmented dual-reservoir MPT IVR releasing tenofovir (TFV) and levonorgestrel reported by Clark *et al.* [64]. D – 'pod-type ring' reported by Baum *et al.* [49].

Population Council has reported a novel MPT IVR aimed at preventing HIV-1, HSV-2, HPV and unintended pregnancy (Figure 34) [58]. The device has characteristics of both matrix and reservoir-type rings, comprising a core containing compressed powdered zinc acetate (ZA) and carrageenan (CG) and a surrounding ethylene vinyl acetate (EVA-28) matrix loaded with the experimental antiretroviral compound MIV-150 and the contraceptive steroid LNG. In this way, the hydrophilic actives are located in the core and the hydrophobic actives in the sheath. Finally, channels (pores) of various sizes extend through the matrix to expose the underlying powder core. This ring design is similar to that of the pod-type cores described previously. *In vitro* release studies using rings designed for testing in macaques showed steady state release of CG and ZA while *in vivo* LNG levels demonstrated adequate levels for contraception. Notwithstanding complications with the manufacture of larger sized rings for woman, this novel ring demonstrates an innovative way of combating the difficulties of releasing both hydrophilic and hydrophobic molecules from IVRs.

Figure 34. MPT vaginal ring reported by Population Council comprising of a MIV-150/LNG matrix ring with a zinc/carrageenan core.

In this chapter, a new design concept for an MPT ring is described which potentially allows for rapid assembly of different ring configurations by inserting multiple individual drug cores into a thermoplastic IVR frame (Figure 35). As with the previous innovative MPT designs, this approach should permit co-release and independent control of actives having very different physicochemical properties and target release rates. This chapter will also explore the stability of an MPT ring containing three different actives.

Figure 35. Computer-aided design (CAD) drawings showing the prototype modular ring design.

7.2 Materials and methods

Materials

See chapter 2 for details of the materials used in this chapter.

Manufacture of silicone cores

Drug loaded silicone elastomer cores (overall diameter 52 mm; cross-sectional diameter 4 mm) were manufactured using a laboratory-scale injection molding machine fitted with a custom stainless-steel ring mold assembly (Table 19). Separate 25 g Part A and Part B premixes of the DDU-4320 addition-cure silicone elastomer systems were prepared by adding weighed quantities of selected API into a screw-cap polypropylene container followed by addition of the silicone part. The premixes were first-hand-mixed (30 s) and then mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixer® DAC 150 FVZ-K, Hauschild, Germany) (60 s, 3000 rpm). A and B premixes were combined in a 1:1 ratio. Specifically, 25 g of each premix were added to a screw-cap polypropylene container to a final weight of 50 g and hand-mixed for 30 s and then DAC mixed (60 s at 3000 rpm). This mix was then transferred into a polypropylene SEMCO® injection cartridge designed for manual injection. Rings were manufactured by manually injecting the active mix into the heated ring mold assembly using an 80°C mold temperature and 180 s cure time. Rings were removed from the heated mold, deflashed as needed, and stored at ambient temperature until further testing.

Reservoir-type cores were prepared as per matrix-type cores except the active mix was injected into a length of silicone tubing (external diameter 4 mm; internal diameter 2 mm; wall thickness 1 mm). Injected tubes were left at room temperature to cure overnight. Cores were cut to 13.5 cm (herein after referred to as three-quarter core segments), 7.5 cm (half core segments) or 3.75 cm (quarter core segments) before inserting into the ring frame.

Table 19. Ring formulations with core configurations for use in *in vitro* release study. M – matrix-type core; R – reservoir-type core (with 1 mm membrane).

	MET			**MIV-150**			**NES**		
	Core length	Core Type	Loading (w/w %)	Core length	Core Type	Loading (w/w %)	Core size	Core type	Loading (w/w %)
Ring 1	-	-	-	¾	M	25	¼	M	0.8
Ring 2	½	M	25	-	-	-	½	R	2.5
Ring 3	¼	M	25	¾	M	25	-	-	-
Ring 4	½	M	25	¼	M	25	¼	M	0.8

Manufacture of ring frames

Ring frames were manufactured from Hytrel® (a thermoplastic copolyester elastomer) using a Babyplast 6/10 P horizontal injection molding machine fitted with a custom stainless-steel ring mold assembly. Hytrel® pellets were dried as per manufacturer recommendation and loaded into the machine hopper. Ring frames were manufactured by injecting molten Hytrel® into the custom mold using 100 bar clamping pressure, 70 bar injection pressure and the temperature and cooling conditions listed in Table 20. Rings where removed from the mold, deflashed (where necessary) and stored at ambient temperature until testing was performed.

Table 20. Injection molding conditions used in the manufacture of Hytrel® ring frames.

Condition for injections molding	**HY**
Plastification unit temperature (°C)	190
Injection chamber temperature (°C)	195
Nozzle temperature (°C)	195
Fixed mold temperature (°C)	30
Cooling time (sec)	25

In vitro release testing

On Day 0, fully constructed rings (n=4) with cores inserted were individually placed into glass bottles containing 1 L of release medium (0.2 % Tween adjusted to pH 4.2) and stored in an orbital shaking incubator (Unitron HT Infors; 37 °C, 60 rpm, 25 mm orbital throw). After 24 ± 0.25 hr, the release medium was sampled and replaced with a fresh 500 mL of the respective media and the samples retained for UPLC analysis. Thereafter, sampling and 500 mL replacement of the release medium was performed daily out to Day 29, except on Fridays when, after sampling, the flask was replenished with a 1 L volume of release medium and no further replacement or sampling performed until the following Monday. Samples were analysed by UPLC.

Stability study

For the stability study, samples of formulation Ring 4 were stored under ICH accelerated stability test guidelines (in a humidity chamber at 40 ± 2 °C/75 ± 5% RH) [235] in two orientations for 1 month:

- Device A (n=4) – rods placed in the ring frame and stored as one product.
- Device B (n=4) – rods stored separately from each other and ring frame.

After 1 month, rings were removed from chamber, equilibrated to room temperature, and then placed on *in vitro* release testing.

Mechanical Testing

Ring 4 samples (Device A and B, n=4 each) were tested in compression mode, using compression distances of 5, 10, 15 and 20 mm and a test speed of 10 mm/s. Six compression cycles at each distance were completed, with the maximum mean force value being recorded for the last five cycles of each distance.

7.3 Results and discussion

Ring 1

Ring 1 comprised a ¾ length silicone matrix core containing 25 % w/w MIV-150 and a ¼ core containing 0.8 % w/w NES. These drug loadings were chosen using a custom loading calculator developed by the group. The daily and cumulative release graphs are presented in Figure 36. Although both cores are matrices, an initial burst was only observed for NES. Release of NES peaked at day 1 with a mean value of 218 µg/day. MIV-150 release, however, peaked at day 24 with a release of 100 µg/day, with day 1 release of just 77 µg.

Although daily NES release declined with time, an increase in release was observed on days 15 and 24. Although the cores themselves are of matrix-type design, when placed into the ring frame it acts as a reservoir device decreasing release rate. These anomalies are potentially due to a slight protrusion of the core from the frame. This would allow the medium to gain access to areas usually protected by the frame.

NES release rate was targeted at 150 µg/day based on therapeutic levels previously reported for protection against unwanted pregnancy [96]. This target was achieved from day 1–3, with subsequent release ranging from 73–134 µg/day. Again, due to the nature of the modular ring design, target drug release could be easily achieved by increasing the NES loading or increasing the core length.

A target release rate of 150 µg/day was also set for MIV-150 to provide therapeutic levels for protection against the acquisition of HIV-1. According to Singer *et al.*, similar release values were reported for silicone rings tested in macaques [174]. A 58% reduction in the acquisition of simian/HIV reverse transcriptase (SHIV-RT) was seen *in vivo* results that corresponded to a daily range of 11–33 µg/ml *in vitro* release from a 50 mg loaded MIV-150 silicone elastomer ring. In that study, partition-controlled kinetics were determined for the 50 mg macaque ring based on a plot of cumulative percentage MIV-150 release vs. time (Q_a time $^{0.90}$, $R^2 > 0.983$).

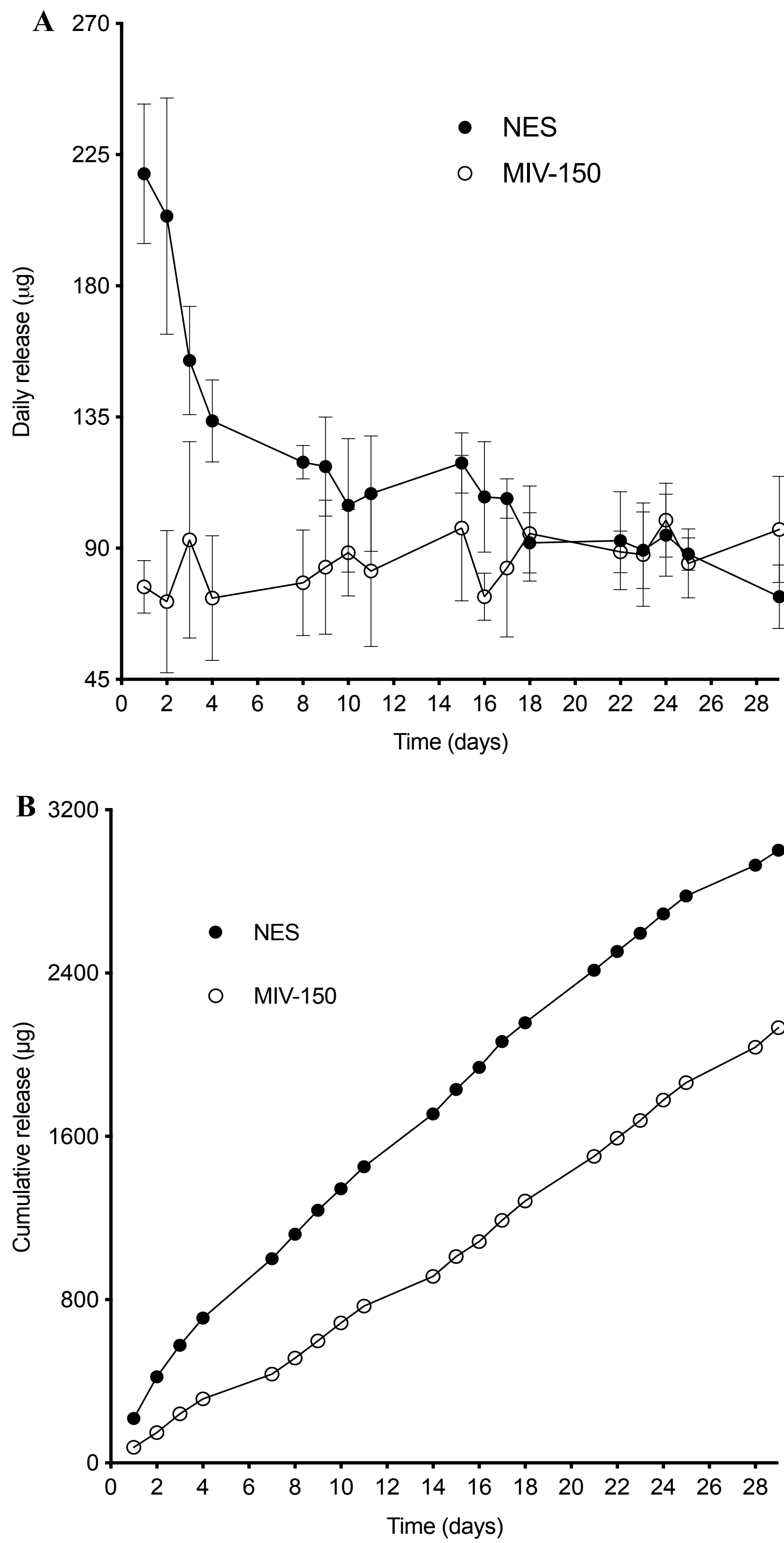


Figure 36. Daily (A) and cumulative (B) release (mean ± SD, n=4) vs time plots over 29 days for NES and MIV-150 silicone cores placed into a thermoplastic frame.

Mathematical modelling

Both NES and MIV-150 cumulative daily release amounts were modelled according to zero order, Higuchi and Korsmeyer–Peppas models. The resulting graphs are presented in Figure 37 and the kinetic parameters summarised in Table 22.

<u>*Zero order model*</u>

Zero order drug release kinetics describe systems where the drug release rate is diffusion controlled and constant with respect to time. The zero-order rate equation is presented in Equation 2, where Q_t is the cumulative amount of drug release at time t, and K is the zero-order release constant. The model requires a plot of cumulative drug release vs. time and linear regression applied [236].

Equation 2. Zero order release kinetics

$$Q_t = K_0 t$$

<u>*Higuchi model*</u>

For matrix-type vaginal rings, drug release is also diffusion controlled. However, the release kinetics are complicated by the development of a receding boundary layer resulting in longer diffusional pathways for drug molecules as time elapses. Most commonly, drug release kinetics from matrix-type rings have been modelled using the Higuchi equation [88,89]. However, the Higuchi equation is only applicable for planar matrices rather than the torus shape of vaginal rings. Helbling *et al*. have adapted the Higuchi model to provide a more concise model for vaginal rings where data is plotted as cumulative drug release vs the square root of time and linear regression applied (Equation 3) [237].

Equation 3. Higuchi model release kinetics as described by Helbling *et al.* [237].

$$Q = A\sqrt{D(2C - C_s)C_s t}$$

<u>*Korsmeyer–Peppas model*</u>

Korsmeyer *et al*. originally described a simple relationship to describe drug release from a polymeric device. However the model was later adapted to interpret the mechanism of drug release for the first 60%, and became known as the Korsmeyer–

Peppas model [238,239]. M_t/M_∞ is the fraction of drug release at time *t,* k is the release rate constant and *n* is the release exponent. Data is plotted in log form with log M_t/M_∞ vs log *t* (Equation 4). When linear regression is applied, the slope of the line allows the value of the release exponent *n* to be calculated, which is indicative of the release mechanisms for cylinder shaped matrices (Table 21).

Equation 4. Korsmeyer-Peppas model

$$\log\left(\frac{M_t}{M_\infty}\right) = \log k + nlogt$$

Table 21. Interpretation of *n* values for Korsmeyer-Peppas model

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.45	Fickian diffusion	$t^{-0.5}$
0.45 < n < 0.89	Non -Fickian transport	t^{n-1}
0.89	Case II transport (anomalous)	Zero order release
Higher than 0.89	Super case II transport	t^{n-1}

When the NES *in vitro* release data is modelled using the zero order and Higuchi models, it more closely follows zero order kinetics. Zero order release kinetics are common for release of poorly water-soluble APIs, such as NES, from silicone elastomers. However, there was also a high correlation with the Higuchi model, suggesting that release may well be dependent on root time kinetics. In fact, the Korsmeyer-Peppas model suggests an anomalous (non-Fickian) transport, with the *n* value being closer to zero order release than $t^{-0.5}$. As mathematical modelling showed no definite release mechanism, and NES was released under sink conditions, we can assume that drug release is mostly controlled by the root time kinetics.

MIV-150, when modelled using the Higuchi model produced a R^2 value of 0.93 compared to 0.98 for zero order kinetic model, again attributable to its low aqueous solubility. Korsmeyer-Peppas model suggests Super case II drug transport mechanism, typical for poorly water-soluble drugs. These results provide a good basis for further development of an MPT vaginal ring for sustained release of NES and MIV-150. The versatility of this ring means that drug loading a core length can be separately manipulated to achieve the desired drug release rates.

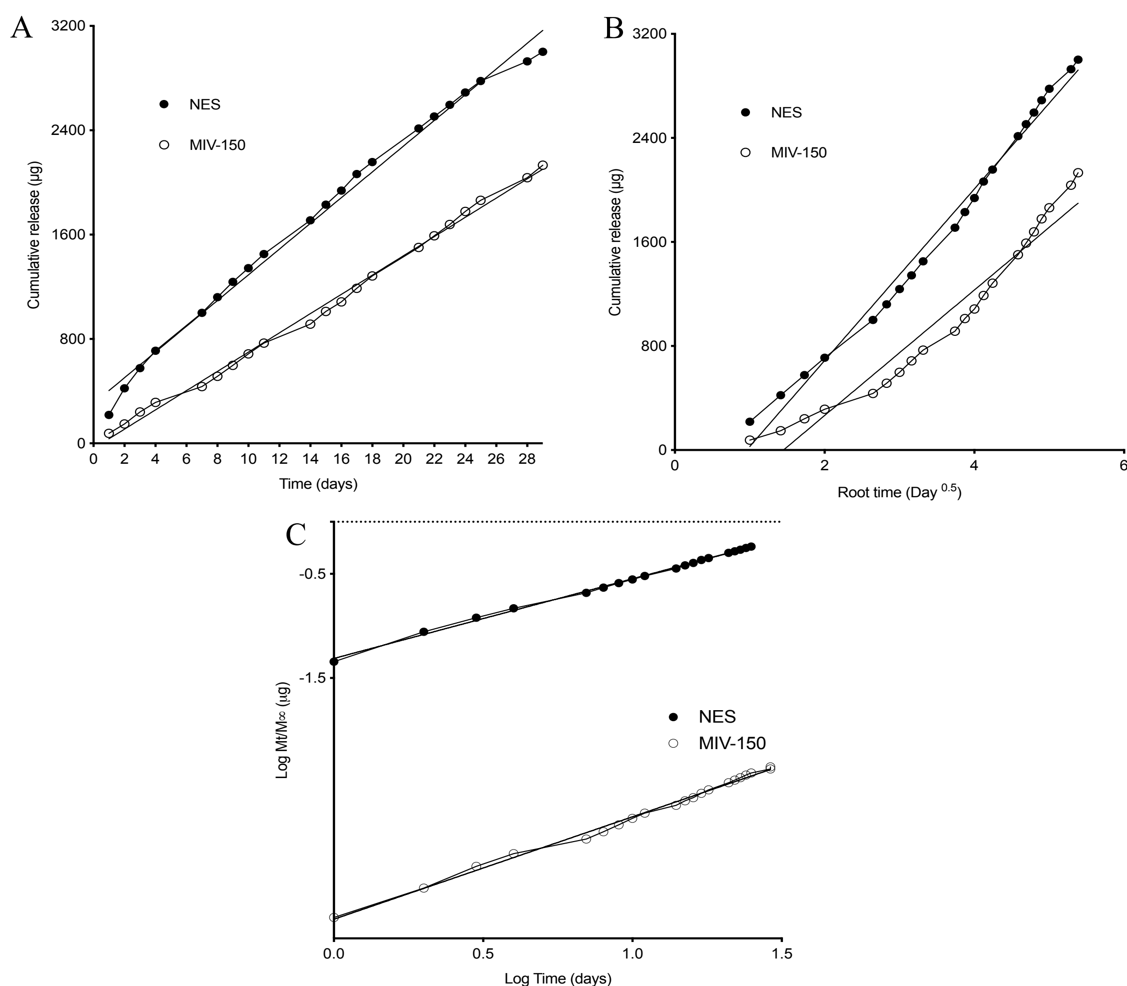


Figure 37. Graphs depicting mathematical modelling of cumulative release of NES and MIV-150 from silicone cores placed into a thermoplastic frame for a period of 29 days with linear regression applied. A) Cumulative release vs time (zero order model), B) Cumulative release vs root time (Higuchi model) and C) Log cumulative release vs log time (Korsmeyer–Peppas)

Table 22. Summary of mathematical modelling results

Model		**NES**	**MIV-150**
Zero Order	Release rate µg/day	98.62	73.83
	R^2	0.99	0.98
Higuchi	Release rate µg/day$^{0.5}$	659.9	482.5
	R^2	0.98	0.93
Korsmeyer–Peppas	Release exponent (n)	0.76	0.98
	Drug transport mechanism	Anomalous transport	Super case II transport
	Rate as a function of time	t^{n-1}	t^{n-1}

Ring 2

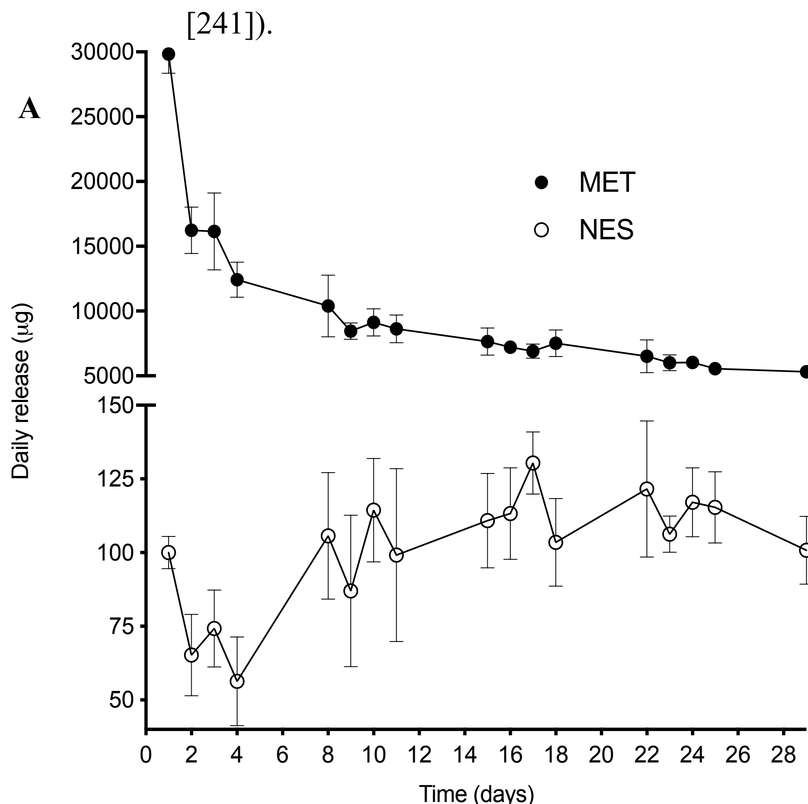
Ring 2 comprised a ½ length silicone matrix core containing 25 % w/w MET and a ½ length silicone reservoir core containing 2.5% w/w NES (internal diameter 2 mm, wall thickness 1 mm). These drug loadings were chosen using a custom loading calculator developed by the group, particularly NES. Ring 1 used a 0.8% loading in a matrix core, however for this reservoir core, the drug loading was decided to be raised to 2.5% due to the slower release associated with a reservoir core.

Daily and cumulative *in vitro* release graphs are presented in
Figure 38 A and B, respectively. A first day burst release was observed for both drugs – 29,827 and 100 µg for MET and NES, respectively. The MET burst was followed by a steady decline in daily release over the study period. By day 29, daily release was reduced to 5305 µg. However, NES release did not follow this pattern. NES release was variable, ranging from 53–130 µg/day. These fluctuations are attributed to two things. First, the NES contained in the centre of the core had likely not reached equilibrium with the sheath, since manufacture of the cores was performed a short time before the start of *in vitro* release. Following initial manufacture of all reservoir-type drug delivery systems, the drug slowly permeates into the drug-free membrane until, after several days/weeks, equilibrium solubility has been reached. This process can be accelerated by storing the devices at elevated temperature, although this may also lead to an increase in the burst effect. Second, the ends of the NES cores were not sealed before insertion into the ring frame, such that the drug at the ends of the core was exposed directly to the release medium. Once the NES at or close to the core ends has depleted, the daily release rate declines – as expected – and subsequent drug release is afforded primarily by drug permeation through the rate controlling membrane.

Mean NES release of 150 µg/day has been reported previously from a two-core silicone elastomer reservoir-type ring containing NES and EE [96]. This 150 µg/day target was not achieved with this formulation. However, the data produced will be useful for next generations of this ring. NES drug release in this formulation is permeation-controlled, allowing release rates to be easily manipulated by altering wall thickness and core length. It is also important to note that similar release values were

achieved from Ring 1. Comparing NES release for Rings 1 and 2 show how drug release can be readily modulated by using different designs and materials (matrix-type and reservoir).

Metrogel® is a common vaginal gel product used for treatment of BV. The gel contains 0.75% w/v MET, and each 5 g daily application administers a dose of 37.5 mg MET [223]. By comparison, Ring 2 offered release of ~30 mg MET on day 1 with release rates declining thereafter over the 28-day test period. By increasing the MET loading in the core or increasing the core length, daily MET release could be further increased. Since MET release from the MPT ring is below the levels required for efficacy, alternative strategies might be needed to make this ring concept work. For example, it might be possible to provide an initial vaginal gel dose of Metrogel® followed by ring device offering maintenance release of MET. There is already a precedent for this approach; some market treatments for vaginal indications provide an initial high drug dose followed by a maintenance dose (e.g. Canesten Thrush Duo for treatment for thrush) [240]. The oral capsule is used to treat the site of infection internally and the external cream soothes itching. Along with this formulation there would also be the option of providing a MET tablet to either take orally (like the thrush treatment previously mentioned) or a vaginal suppository (current BV treatment – a 500 g tinidazole [241]).



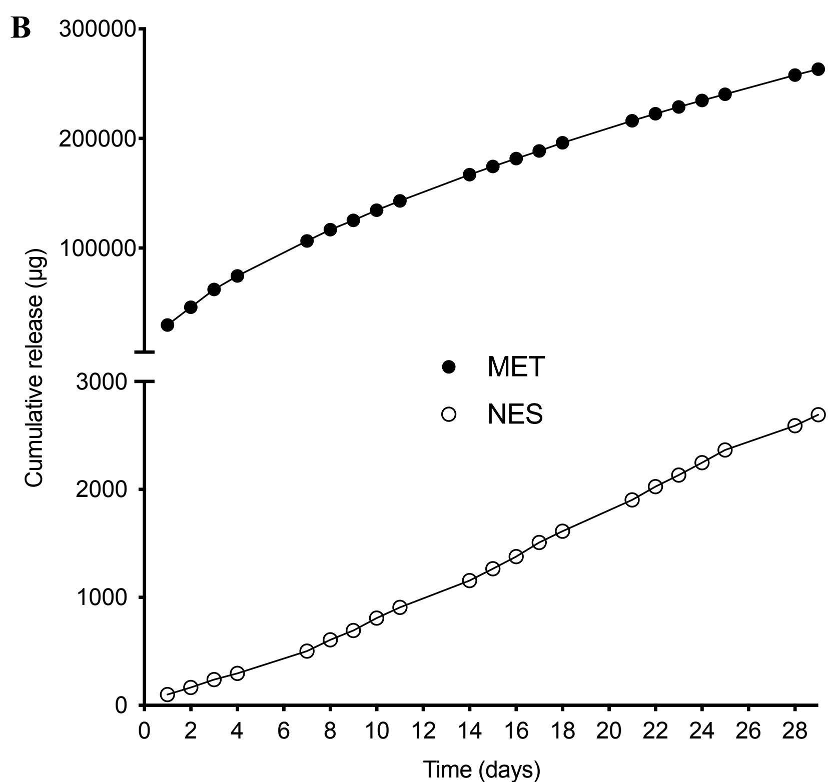


Figure 38. Daily (A) and cumulative (B) release (mean ± SD, n=4) vs time graphs over 29 days for MET and NES from silicone cores placed into a thermoplastic frame (days).

Mathematical modelling

Both NES and MET cumulative daily release amounts were modelled by the Zero Order, Higuchi and Korsmeyer–Peppas models. The graphs are presented in Figure 40 and summary results in Table 23.

When the NES *in vitro* release data is modelled using the zero order and Higuchi models, it closely obeys zero order kinetics. Zero order kinetics are commonly observed with reservoir devices and are due to permeation control exerted by the rate-controlling membrane. The parameters derived from the Korsmeyer-Peppas model (Table 23) suggests Super Case-II transport.

MET, when modelled using Higuchi model produces a R^2 value of 0.97 compared to R2 =0.95 with the zero order model. This is typical of drugs that are highly soluble in the release media such as MET and have a high API loading (25% w/w). Since Korsmeyer-Peppas is only applicable for the first 60% of drug release, the data were only modelled from days 1–17. This result indicates an anomalous transport

mechanism (n=0.66) which is closer to Fickian diffusion than zero order, again attributable to highly soluble drugs.

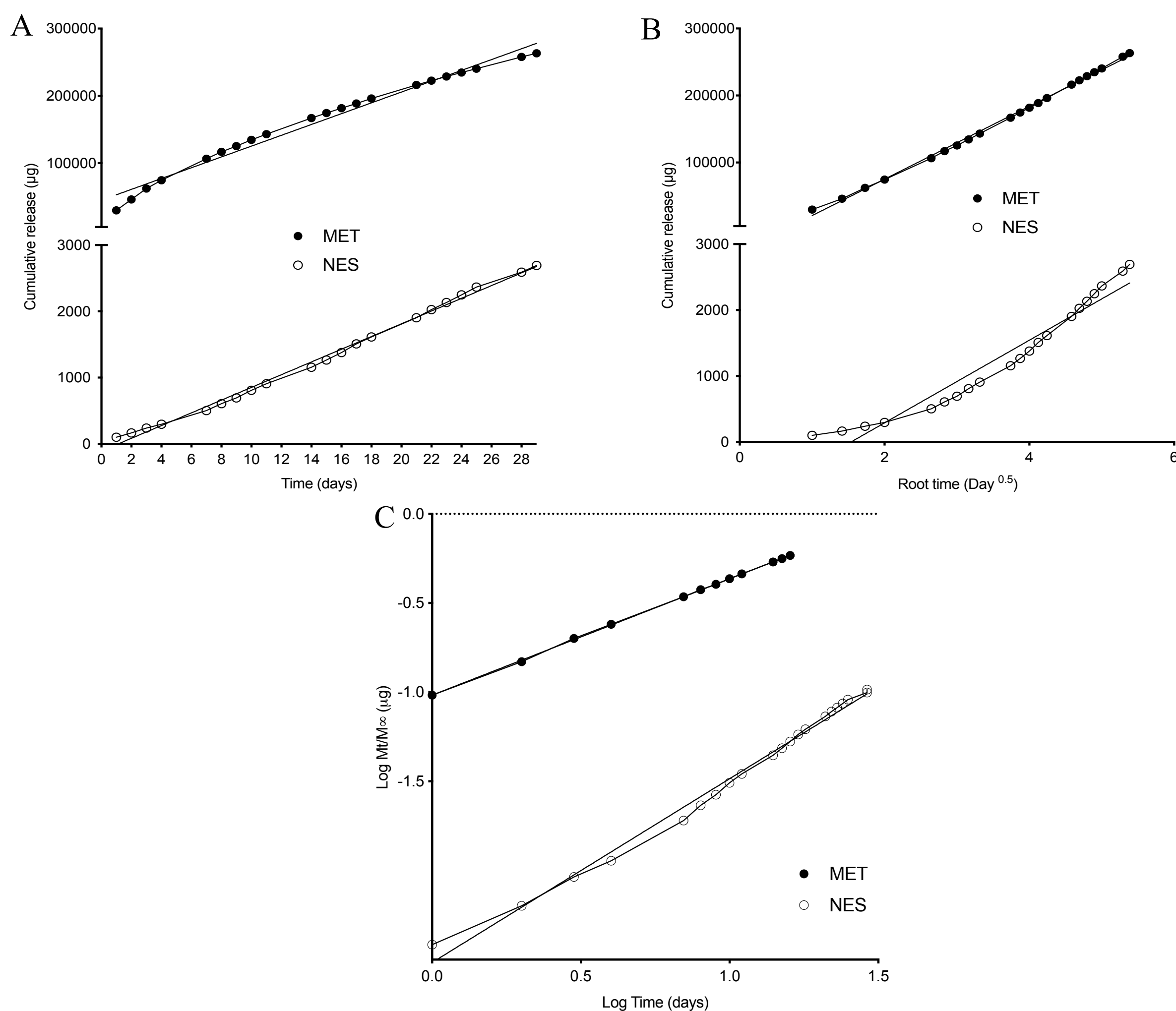


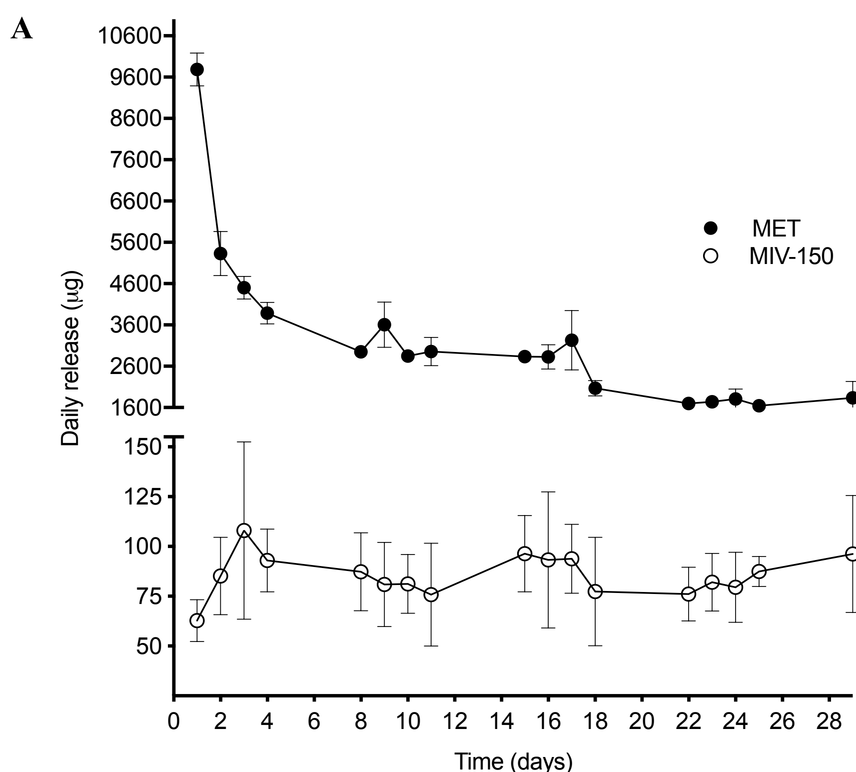
Figure 39. Mathematical modelling of cumulative release of NES and MET from silicone cores placed into a thermoplastic frame for a period of 29 days with linear regression applied. A) Cumulative release vs time (Zero Order model), B) Cumulative release vs root time (Higuchi model) and C) Log cumulative release vs log time (Korsmeyer–Peppas).

Table 23. Summary of mathematical modelling results

Model		**MET**	**NES**
Zero Order	Release rate µg/day	8038	96.01
	R^2	0.95	0.98
Higuchi	Release rate µg/day$^{0.5}$	54359	626.4
	R^2	0.97	0.93
Korsmeyer–Peppas	Release exponent (n)	0.65	1.03
	Drug transport mechanism	Anomalous transport	Super Case-II transport
	Rate as a function of time	t^{n-1}	t^{n-1}

Ring 3

Ring 3 comprised two matrix-type silicone elastomer cores, one a ¾ length core containing 25% w/w MIV-150 and the other a ¼ core containing 25% w/w MET. Daily and cumulative release versus time graphs are presented in Figure 40. Daily release from Ring 3 showed a characteristic burst release of MET on day 1 of 9.8 mg. By day 3, this had halved to 4.5 mg and after day 29 daily release ranged from 1.8–3.6 mg. The reduced release rate and the 68% overall reduction in cumulative release are consistent with reduction in length of the MET core compared to Ring 2. This formulation demonstrates that MET release can be readily manipulated from a silicone core of this loading by increasing and decreasing core length. Unlike MET, MIV-150 showed an initial lag, with day 1 of 63 µg, increasing to 108 µg on day 3, and then maintaining a near constant release rate ranging between 76–96 µg (day 15 mean of 96 µg) for the remainder of the study. This is comparable to the MIV-150 release rate observed with Ring 1. Although the MIV-150 core length in these rings was the same, MIV-150 was co-released with different APIs in Ring X, suggesting its release rate is not affected by the additional incorporation of NES and MET.



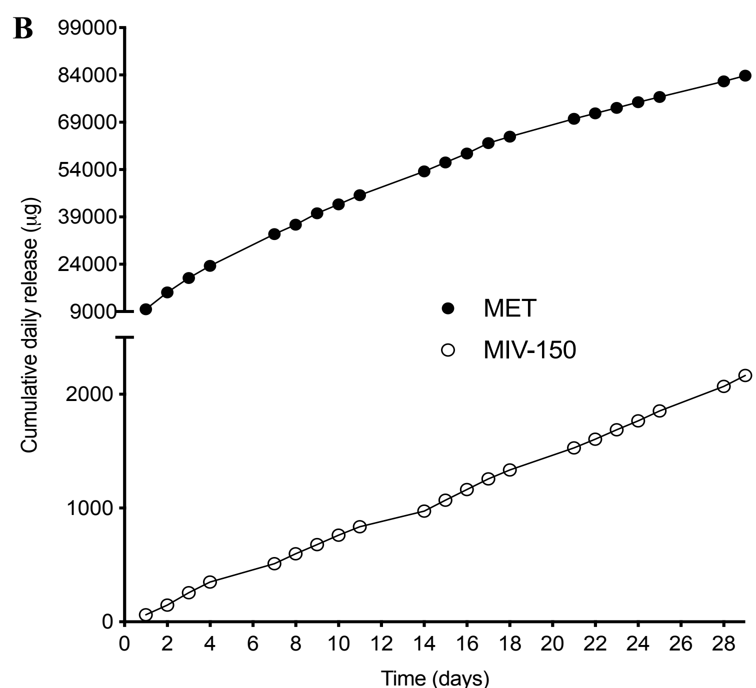


Figure 40. Daily (A) and cumulative (B) release (mean ± SD, n=4) vs time profiles for MET and MIV-150 from silicone cores placed into a thermoplastic frame vs time (days) for a period of 29 days.

Mathematical modelling

Both MIV-150 and MET cumulative daily release amounts were modelled by the zero order, Higuchi and Korsmeyer–Peppas models. The graphs are presented in Figure 41 and summary results in Table 24. As with Ring 2, MET *in vitro* release data more closely follows the Higuchi model than zero order (R^2 = 0.98 and 0.96 respectively), indicating root time kinetics consistent with permeation-controlled release from a non-biodegradable matrix loaded with a large quantity of solid drug. MIV-150, when modelled using Higuchi model produced a R^2 value of 0.93 compared to 0.96 for zero order kinetic model, again attributable to its low aqueous solubility and possible permeation controlled release. This data is comparable to that modelled for Ring 1.

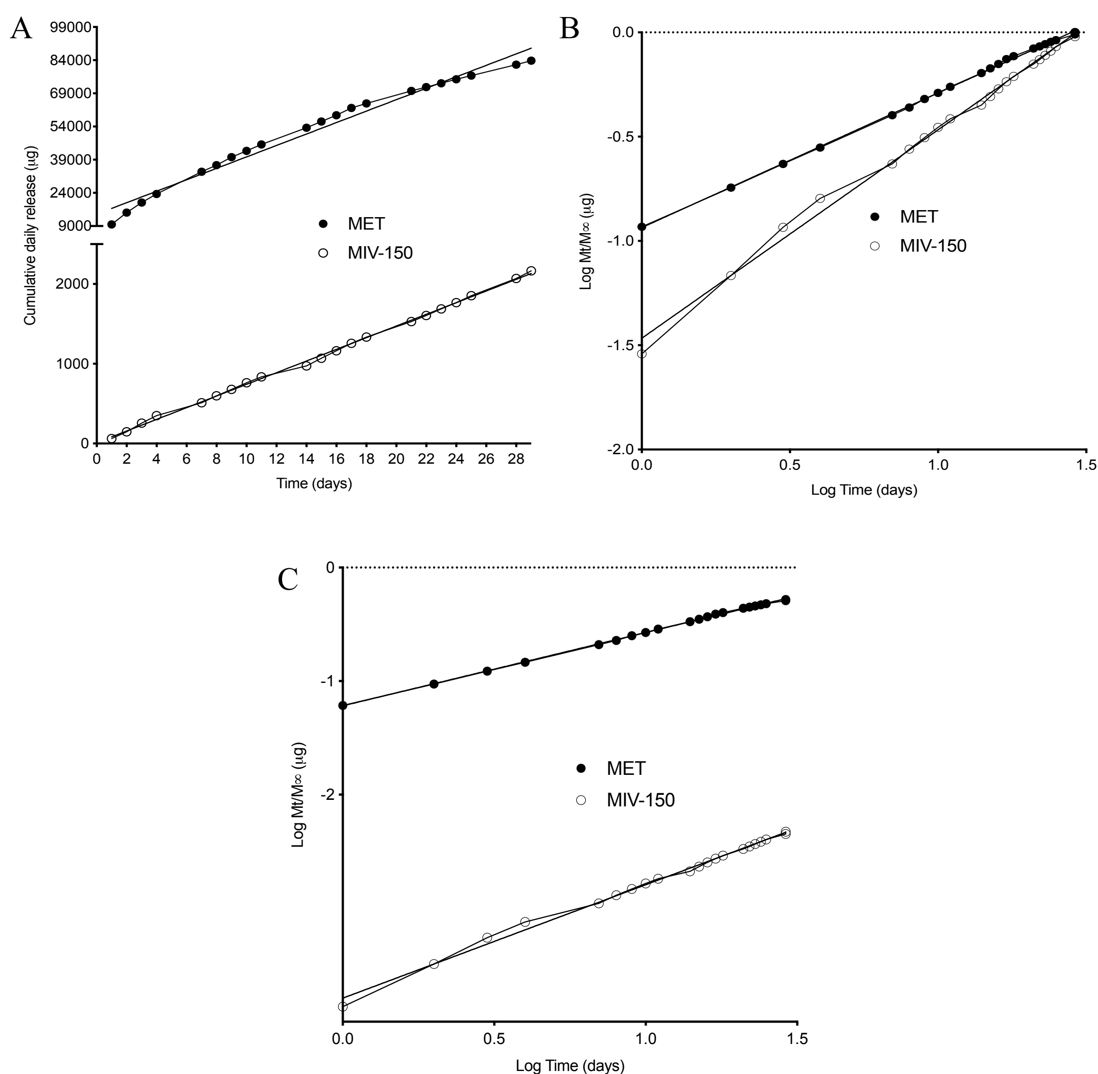


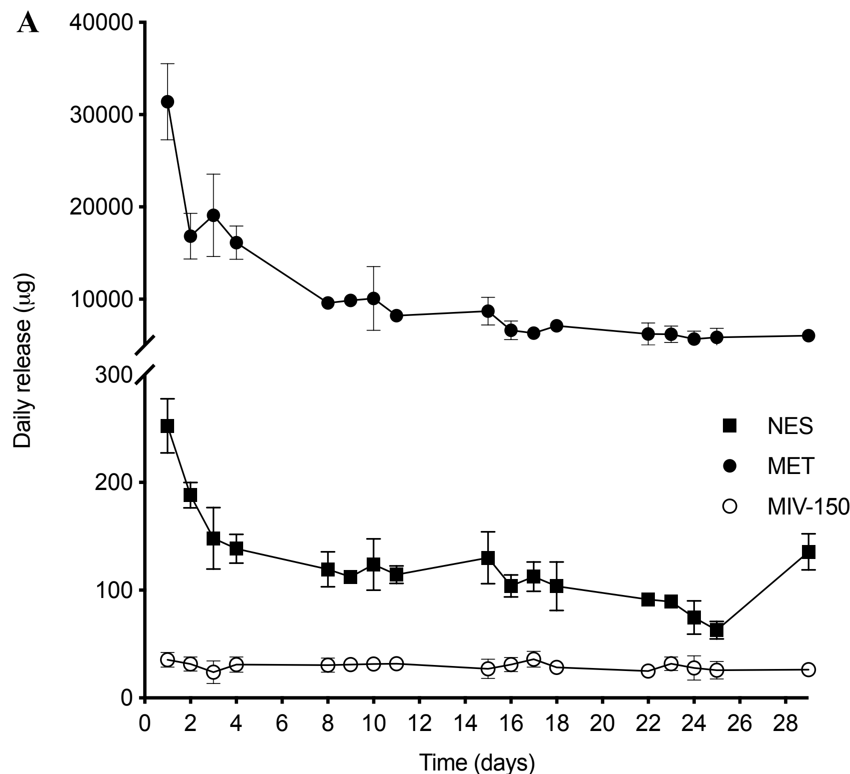
Figure 41. Graphs depicting mathematical modelling of cumulative release of MET and MIV-150 from silicone cores placed into a thermoplastic frame for a period of 29 days with linear regression applied. A) Cumulative release vs time (Zero Order model), B) Log cumulative release vs log time (Korsmeyer–Peppas) and C) Cumulative release vs root time (Higuchi model))

Table 24. Summary of mathematical modelling results

Model		**MET**	**MIV-150**
Zero Order	Release rate µg/day	2587	73.14
	R^2	0.96	0.96
Higuchi	Release rate µg/day$^{0.5}$	17516	481.7
	R^2	0.98	0.93
Korsmeyer–Peppas	Release exponent (n)	0.65	1.0
	Drug transport mechanism	Anomalous transport	Super Case-II transport
	Rate as a function of time	t^{n-1}	t^{n-1}

Ring 4

Ring 4 contained three matrix-type silicone elastomer cores – a ¼ length core containing 25% w/w MIV-150, a ¼ length core containing 0.8% w/w/ NES, and a ½ core containing 25% w/w MET. Daily and cumulative release versus time graphs are presented in Figure 42. Daily release from Ring 4 showed a characteristic burst release of MET on day 1 of 31 mg with this reducing to had nearly half (16 mg) by day 4. Between day 7 and 29 release ranged from 6.1–9.5 mg. This range is comparable to Ring 2 MET release. NES also showed a burst release on day 1 (253 µg) with daily release ranging from 63–139 µg/day. This release is comparable with that measured for Ring 1. MIV-150 provided no burst release; instead, a constant release rate in the range 26–35 µg/day was maintained throughout the study. As this ring only contained a ¼ length core of 25% w/w MIV-150, compared to the ¾ length core used in Rings 1 and 3, it is not surprising that total cumulative release was reduced by around 65%.



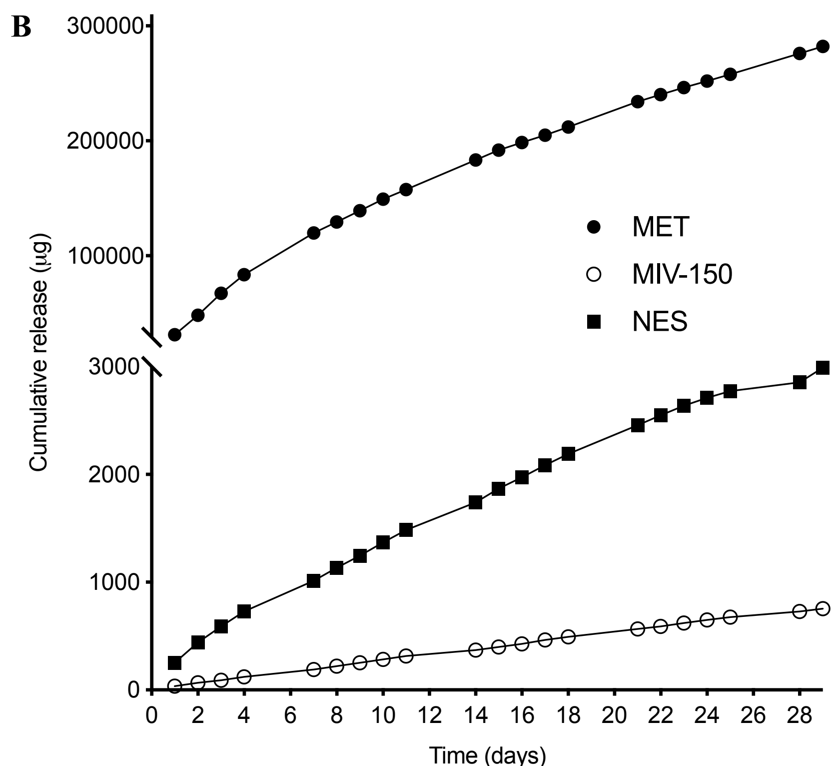


Figure 42. Daily (A) and cumulative (B) release (mean ± SD, n=4) vs time profiles for MET, NES and MIV-150 from silicone cores placed into a thermoplastic frame vs time (days) for a period of 29 days.

Mathematical modelling

Cumulative daily release amounts for all APIs in this ring were modelled by the Zero Order, Higuchi and Korsmeyer–Peppas models. The graphs can be seen in Figure 43 and summarised results can be seen in Table 25. Equations used for modelling can be seen in the previous section. As with Ring 2 and 3, MET *in vitro* release data more closely follows Higuchi model than zero order (R^2 = 0.97 and 0.95 respectively). This data is again a reaffirmation that release of MET is square root of time dependant. This is also consistent with MET being very water soluble and the core itself having a high drug loading (25 % w/w). The release exponent for Korsmeyer–Peppas model is also consistent across Ring 2, 3 and 4 (Anomalous transport, t^{n-1}).

As with Ring 1 and 3, when MIV-150 release from Ring 4 was modelled using Higuchi model, the R^2 value was 0.94 compared to 0.97 for zero order kinetic model. The release exponent for Korsmeyer–Peppas model is also consistent across Ring 1, 3 and 4 (Super Case-II transport, t^{n-1}), again attributable to the low aqueous solubility of

MIV-150 and possible permeation-controlled release due to the MIV-150 being placed in a frame.

When the NES *in vitro* release data from Ring 4 is modelled using the zero order and Higuchi models, it closely favours zero order kinetics (R^2 = 0.99 and 0.98 respectively). The Korsmeyer-Peppas model suggests a Super Case-II transport which also correlates with a zero-order release mechanism which is consistent with mathematical modelling data compiled NES release from Ring 1 and 2.

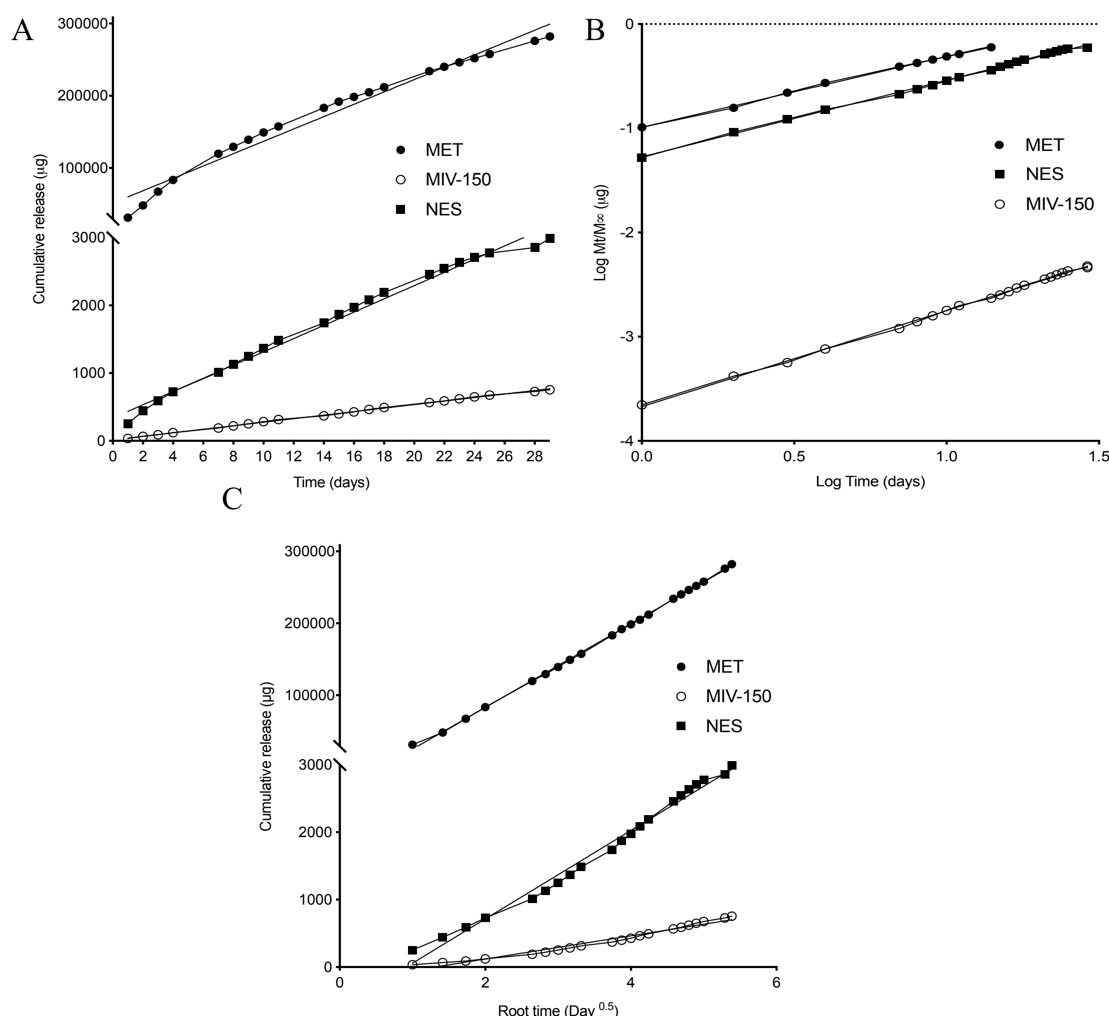


Figure 43. Graphs depicting mathematical modelling of cumulative release of MET, NES and MIV-150 from silicone cores placed into a thermoplastic frame for a period of 29 days with linear regression applied. A) Cumulative release vs time (Zero Order model), B) Log cumulative release vs log time (Korsmeyer–Peppas) and C) Cumulative release vs root time (Higuchi model)

Table 25. Summary of mathematical modelling results

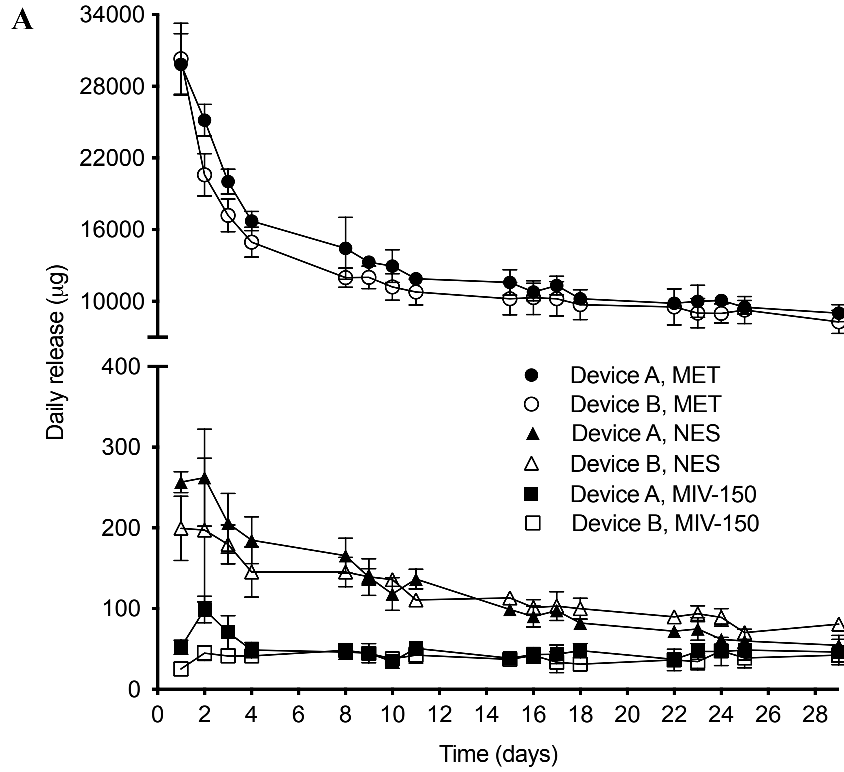
Model		MET	MIV-150	NES
Zero Order	Release rate µg/day	8548	26.01	97.73
	R^2	0.95	0.97	0.99
Higuchi	Release rate µg/day $^{0.5}$	58085	171.9	655.4
	R^2	0.97	0.94	0.98
Korsmeyer–Peppas	Release exponent (n)	0.68	0.92	0.74
	Drug transport mechanism	Anomalous transport	Super Case-II transport	Anomalous transport
	Rate as a function of time	t^{n-1}	t^{n-1}	t^{n-1}

Accelerated stability study of Ring 4

In vitro release

In vitro release of stability study of Ring 4 included eight samples; Device A (n=4), cores were placed inside the ring frame and placed on stability and Device B (n=4), cores stored separately from ring frame. Although all cores were matrices, only MET (both Device A and B) provided the expected initial burst release (Figure 44 A). MET release peaked at 29.8 and 30.3 mg/day for Device A and B respectively. From day 15 of the study, MET release ranged from 8.3–11.6 mg/day from both sample sets. NES peaked at day 2 for Device A and B with a release of 262 and 200 µg/day for Device A and B respectively. From day 4, release ranged from 54.7–166 µg/day for both sample sets. Similar to NES, MIV-150 release peaked on day 2 also with a daily release of 44–99 µg/day for both sample sets. For the remainder of the study, release ranged from 42–71 µg/day for both sample sets.

The cumulative release graph (Figure 44 B) shows only minor differences between Device A and B for the release of NES. On day 29, MET Device A had a 10 % higher cumulative drug release than Device B and there was a higher amount of MIV-150 released with Device A than B. NES only had a 1.6% difference in release between devices. As previously mentioned, the difference between Device A and B were how they were stored during the stability study. It was concluded that the orientation of the cores during this stability study is having an effect on release.



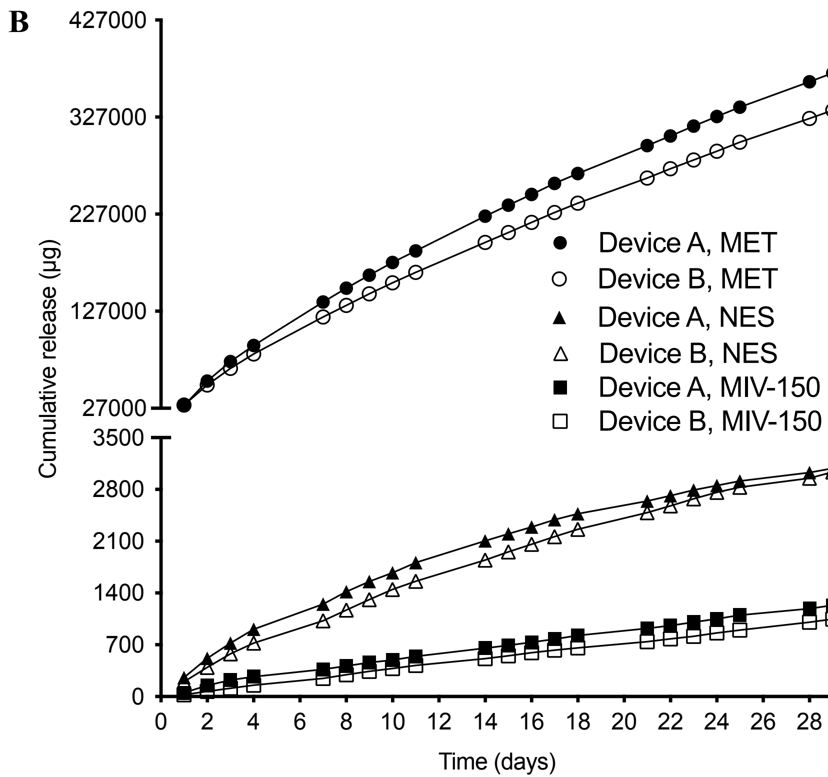


Figure 44. Daily (A) and cumulative (B) release (mean ± SD, n=4) vs time profiles for MET, NES and MIV-150 from silicone cores placed into a thermoplastic frame (T=1 month timepoint) vs time (days) for a period of 29 days.

Cumulative daily release amounts for all APIs in this ring were modelled by the zero order and Higuchi models. The graphs are presented in

Figure 45 and summary results in Table 26. As with Ring 4, MIV-150 *in vitro* release data more closely follows zero order kinetic model (R^2 = Device A = 0.99, Device B =1.00) than Higuchi (R^2 = Device A = 0.99, Device B = 0.94). As with Ring 4, MET *in vitro* release data for Device A closely follows Higuchi model than zero order (R^2 = 0.98 and 0.97 respectively) however Device B closely favoured zero order kinetics (R^2 = 0.99 and 0.96 respectively). Unlike Ring 4, NES release from both samples closely favoured the Higuchi model (R^2 = Device A = 0.98, Device B =0.99) rather than zero order kinetics (R^2 = Device A = 0.94, Device B =0.98).

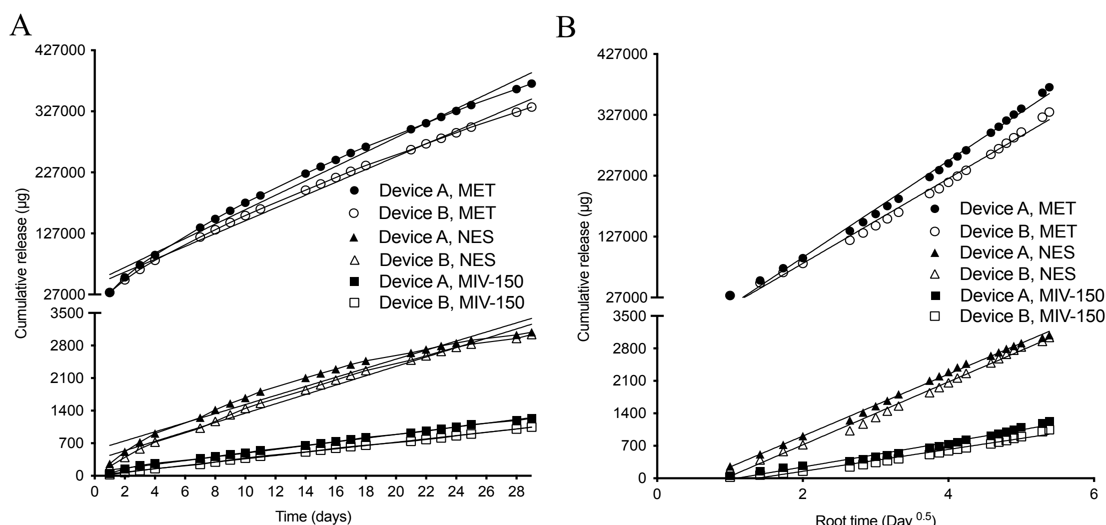


Figure 45. Graphs depicting mathematical modelling of cumulative release of MET, NES and MIV-150 from silicone cores placed into a thermoplastic frame for a period of 29 days with linear regression applied. A) Cumulative release vs time (Zero Order model), B) Cumulative release vs root time (Higuchi model)

Table 26. Summary of mathematical modelling of drug release from rings.

Ring 4 stability study sample set		Sample A			Sample B		
Model		MET	NES	MIV-150	MET	NES	MIV-150
Zero order	Release rate µg/day	11811	97.50	40.20	10505	100.60	35.69
	R^2	0.97	0.94	0.99	0.99	0.98	1.00
Higuchi	Release rate µg/day $^{0.5}$	79552	667.00	267.30	69780	10505	236.30
	R^2	0.98	0.98	0.98	0.96	0.99	0.94

Alongside this *in vitro* release study, assessment of initial drug (assay) was also conducted. This involved extracting all of the drug substances from both cores and ring frames. Separately stored ring frames from Sample set B contained no drug or degradation substances (no change from T=0 timepoint). Cores stored separately product a 100 % recovery of the theoretical drug content. Assay of Sample A found API from each of the three actives was present in the ring frame and a lower than expected content in the API-loaded cores. When these two content valves were added, they totalled the expected API content in each core. Therefore, there is migration of drug from the core into the non-medicated frame during storage, which contributes to a higher drug release and may explain changes in the drug release kinetics.

Mechanical testing of Ring 4

Mechanical testing was conducted on samples from T=0 and T=1 month timepoint. Two sets of experiments were designed, one evaluating the ring frame itself, and the other Ring 4 with cores inserted (Figure 46 A and B respectively). As expected, the deeper the compression distance, the higher the force across all samples. T=0 and T=1 Device B samples exhibit similar results for both testing regimes, T=1 Device A however, produced difference compression forces. For the compression of the ring frame only, Device A needed a higher force to compress, while when cores were inserted, it needed the least force for compression. A possible explanation for this might be that API was found in the non-medicated ring and the core content was less than expected.

During this stability study there has been differences in *in vitro* release from Devices A and B and in mechanical testing results. This discrepancy could be attributed to the configuration of the ring frame and cores during the stability study. As this MPT ring is designed to accommodate numerous different API loaded rods to treat numerous indications it potentially may never be stored with these rods inserted before consumer use. This stability study confirmed that there is potential for drug to leach into the ring frame when stored together, therefore for any additional studies, no cores will be stored inserted into the ring frame.

Using One-way ANOVA and Tukey's Multiple Comparisons test, it was calculated that there was no statistically significant difference ($p > 0.05$) between T=0 and T=1 month nor between Device A and B both with frames only and with silicone cores inserted.

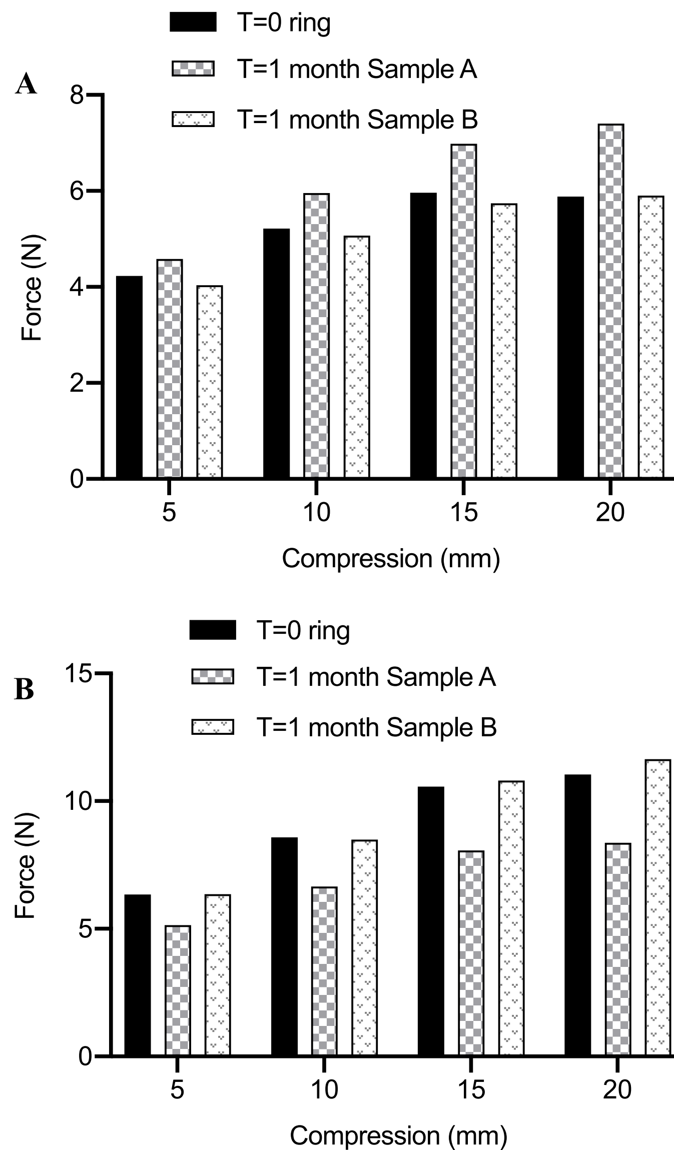


Figure 46. Force needed to compress Ring 4 a series of distances A) frames only (n=4) B) frames with silicone core inserted (n=4).

7.4 Conclusion

The *in vitro* release of various formulations shows promise to create a novel MPT ring device. MIV-150 was released alongside both MET and NES with no change in daily

and cumulative release. MIV-150 release in Ring 4 was altered with a shorter core length. For future formulations, core length will be altered rather than API loading. NES was released from a matrix core alongside MIV-150 in Ring 1 and released from a reservoir core in Ring 2 alongside MET. There was a larger cumulative NES release (30 mg) from Ring 1 (matrix core, ¼ length, 0.8% w/w loading) compared to Ring 2 (27 mg, ½ length, 2.5% w/w loading) exhibiting how release can be altered using different types of cores. MET was released alongside NES in Ring 2 and MIV-150 in Ring 3. Cumulative release showed a 68% reduction in Ring 3 compared to Ring 2 although core length was halved.

A stability evaluation of Ring 4 was conducted to assess two types of storage configurations. Device A, cores were stored in the ring frame and Device B cores were stored separately from the frame. These different conditions impacted release, content and mechanical properties. For future stability studies, its proposed that cores and ring frames are stored separately to prevent drug leaching from API loaded core to non-medicated ring frame.

These *in vitro* release results show how NES, MET and MIV-150 can be released together from a single ring device by inserting multiple cores into the ring frame. The data also illustrate how API loadings and length of cores can be manipulated to modulate release.

8

72-day *in vitro* release of MET, MIV-150 and NES from a novel ring device

8.1 Introduction

Commercial combination contraceptive rings such as NuvaRing® and Ornibel® are used for a period of 21 days followed by removal for 7 days for breakthrough bleeding, after which a new ring is inserted [87,116]. This regimen mimics that of the combination contraceptive pill and is used to provide a natural menstrual period. Other long-acting contraceptive devices, such as the Mirena IUD, are inserted and offer continuous release of a progestin for up to five years. The Mirena intrauterine device provides a dose of LNG at a rate of 20 μg/day [112]. This provides constant contraceptive use for the five years although the downside to this method is that a medical professional is needed for insertion and removal, which might not be accessible to every woman. Population Council have just gained FDA approval for a one-year contraceptive ring, named Annovera® [128]. This ring works similar to Nuvaring® and Ornibel® with an insertion period of 21 days and removal of 7 days however, with Annovera, the ring can be inserted again. This cycle can be repeated for 13 cycles.

IVRS such as Femring ®, Progering® and Estring® are used for a period of 3 months which after a new ring can be inserted [198]. Femring ® and Estring® are both used for estrogen replacement therapy with Estring® containing 2 mg of 17β-estradiol with a release rate of 7.5 μg/day and Femring® manufactured in two strengths containing 17β-estradiol-3-acetate (12.4 and 24.8 mg releasing 50 or 100 μg/day respectively). Progering® releases progesterone at a daily rate of 10 mg/day for post-partum contraception.

Boyd *et al.* has reported IVRs (both a reservoir and matrix) releasing DPV and the LNG over a period of between 60 and 180 days [89]. These IVRs are second generation following on from a DPV only IVR that is currently under regulatory review. Clark *et al.* have also reported a reservoir IVR, although this device is manufactured from polyurethane [64]. The IVR contains tenofovir (TNF) (a reverse transcriptase inhibitor) and levonorgestrel (LNG) (contraceptive) which releases at 7.5 mg and 21 μg/day respectively. Due to the difference in release rates, (7.5 mg vs. 21 μg/day), this ring with formulated with a hydrophobic and hydrophilic segment in a

dual reservoir. LNG is delivered from the hydrophobic TPU reservoir while TNF is released from the hydrophilic segment. This design has allowed a hydrophilic and hydrophobic drug to release in vitro for a period of 90 days. LNG segments alone place, in rabbits, released sustained levels which were detected in plasmas for 90 days.

Most of the rings previously discussed have one thing in common to allow sustained release, they are reservoir rings. An exception to this is Progering®. As a matrix-type ring, it aims to release high levels of progesterone (10 mg/day) over the 90 days rather than controlling a small amount, compared to Femring® [113]. In chapter 7, four formulations were reported having different core types, different loadings and different lengths of cores to modulate drug release of MIV-150, MET and NES. In this chapter, a modified IVR device is reported with increased drug loadings and different length cores to try and achieve sustained release.

8.2 Materials and methods

Materials

See chapter 2 for all materials used in this thesis.

Manufacture of silicone cores

Matrix-type silicone elastomer cores were manufactured as previously described in Chapter 6.

Manufacture of ring frames

Ring frames were manufactured from Hytrel® using the method described in Chapter 6.

In vitro release testing

In vitro release testing used up until day 39 in this chapter has been previously described in chapter 7. From day 42 to 71 twice-weekly sampling and replacement of

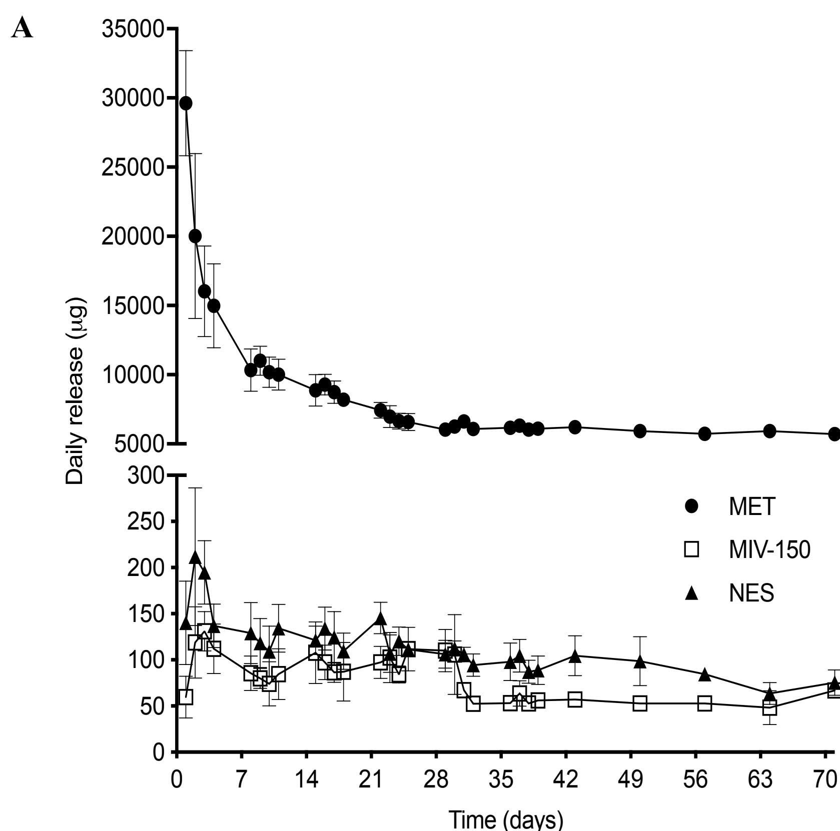
the release medium was performed on consecutive days (Days 42, 43, 49, 50, 56, 57, 63, 70 and 71), with 500 mL release medium used on the first of the two consecutive days and 1000 mL used on the second day. Samples were analysed by UPLC (refer to Chapter 2).

8.3 Results and discussion

The device comprised of a 6.4 cm length silicone matrix core containing 25 % w/w MIV-150, a 6.4 cm length silicone matrix core containing 40 % MET w/w and a 2.1 cm core containing 2.5 % w/w NES. The cores lengths and drug loaded selected here were due to in vitro release data gained from a 28-day *in vitro* release study of MET, NES and MIV-150 (Ring 4 formulation described in chapter 7). Briefly, Ring 4 contained three matrix-type silicone cores one of which was a 7.5 cm 25 % w/w MET loaded core, the second a 3.75 cm 0.8% w/w NES loaded core and a 3.75 cm core containing 25% w/w MIV-150. These cores provided continuous release for the 29-day period however failed to achieve target release. By using a modified Cranks equation (k=R ln (b/a)/L) to determine core length and a custom matrix ring loading calculator to determine loadings to achieve extended release these modified core lengths and loading values were obtained. Both of these calculation methods were used as this device has elements of both matrix and reservoir-type drug release from the drug loaded silicone core. Initially this *in vitro* release study was scheduled for 90 days; however, the study had to be concluded at Day 71 due to the Covid-19 pandemic.

The daily and cumulative release graphs are presented in Figure 47. Although all cores are matrices, only MET provided the expected initial burst release on day 1 with a release of 30 mg. MET release continued to decline steadily until day 25 where it remained constant until the end of the study (5.7–6.6 mg/day). Release of NES peaked at day 2 with a mean value of 213 µg/day. Release from day 3–24 ranged from 120–137 µg/day, after which (day 29–72) release ranged from 75–112. MIV-150 release peaked at day 3 with a release of 131 µg/day compared to day 1 release of 60 µg and a day 2 release of 120 µg/day. Release ranged from 67–112 µg/day from day 4–31 after which it remained between 53–67 µg/day until the end of the study.

MET release was targeted at 37.5 mg/day to match daily application of Metrogel®, a common vaginal gel product used for treatment of BV [223]. However, MET release only achieved close to this target on day 1. In fact, MET release for this ring closely matched MET release from a previously reported hydrophilic TPU ring, for which a segment containing 25% w/w MET released a mean of 20 mg/day over a 7-day period [159]. The hydrophilic TPU ensured complete exhaustion of MET after 7 days, making it potentially suitable for a BV treatment. With other material choices available, considerations for future formulations will determine if MET incorporated into silicone is the most suitable material for release. It may be beneficial in future work to use a thermoplastic core to provide increased release. Due to the versatility of this ring (e.g. the ease of manufacturing cores for insertion and the insertion of these cores), various polymers could be considered as release materials without effecting the release of APIs from other cores in the ring frame.



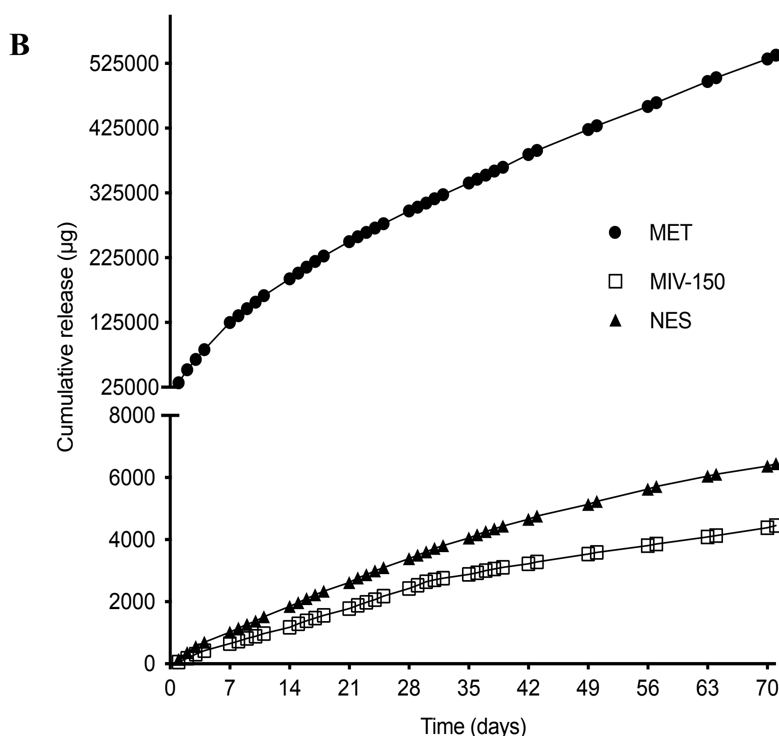


Figure 47. Daily (A) and cumulative (B) release (mean ± SD, n=4) vs time plots over 71 days for NES, MET and MIV-150 silicone cores placed into a thermoplastic frame.

NES release rate was targeted at 150 µg/day based on therapeutic levels previously reported for protection against unwanted pregnancy [96]. After 21 days, the mean release rate was 125 µg/day, and over the entire study period, mean NES release was 91 µg/day. Although NES release did not achieve the 150 µg/day target, release was greatly enhanced compared to previous formulations (Chapter 7). The large burst release observed with previous formulations was largely mitigated in this formulation by reducing core length while increasing API loading. Previous formulations had exhibited a characteristic burst release associated with matrix-type rings. However, due to the reduced surface area with this smaller core, this was not observed with this ring device. In future formulations, API loading could be increased rather than core length altered.

MIV-150 release rate was targeted at 150 µg/day, based on previous data for protection against HIV-1 acquisition [57]. Singer *et al*. reported a daily range of 33–111 µg/day from a 50 mg loaded MIV-150 silicone elastomer ring [69]. After 29 days, this ring formulation had a mean release of 87 µg/day compared to Singer *et al*. reported *in vitro* release results (54 µg/day). In *in vivo* studies, the 50 mg loaded MIV-150 silicone

elastomer ring reduced the acquisition of a chimeric human/simian virus (RT-SHIV) by 58%. This comparison suggests that even though the target daily release for this MPT ring was not achieved, that if *in vivo* studies were conducted, it would provide some protection against SHIV-RT.

Mathematical modelling

Release of all API were mathematically modelled using techniques described in chapter 7. MET, when modelled using Higuchi and zero order models, more closely follows Higuchi than zero order (R^2 = 0.93 and R^2 =0.91 respectively) (Figure 48, Table 27). As MET is very hydrophilic and therefore highly soluble in the release medium, this is typical. The Korsmeyer-Peppas model suggest anomalous transport mechanism (n=0.66), again attributable to highly soluble drugs.

When the NES *in vitro* release data is modelled using the zero order and Higuchi models, it more closely follows the Higuchi model (R^2 = 0.98 and R^2 =0.97 respectively). This high correlation with the Higuchi model, suggesting that release may well be dependent on root time kinetics however Korsmeyer-Peppas model suggests an anomalous (Non -Fickian) transport, with the *n* value being closer to zero order release than $t^{-0.5}$.

MIV-150 release more closely followed Higuchi model than zero order with a R^2=0.98 compared to R^2=0.95 for zero order. A super case II transport drug transport mechanism was suggested from the Korsmeyer-Peppas model, which is typical for poorly water-soluble drugs like MIV-150. API release of all actives follow the Higuchi model more closely than zero order. When this ring is compared to Ring 4 in chapter 7, MIV-150 and NES have changed from closely following zero order kinetics to having a high correlation with the Higuchi model. This suggests that altering core length and loading can manipulate drug release kinetics.

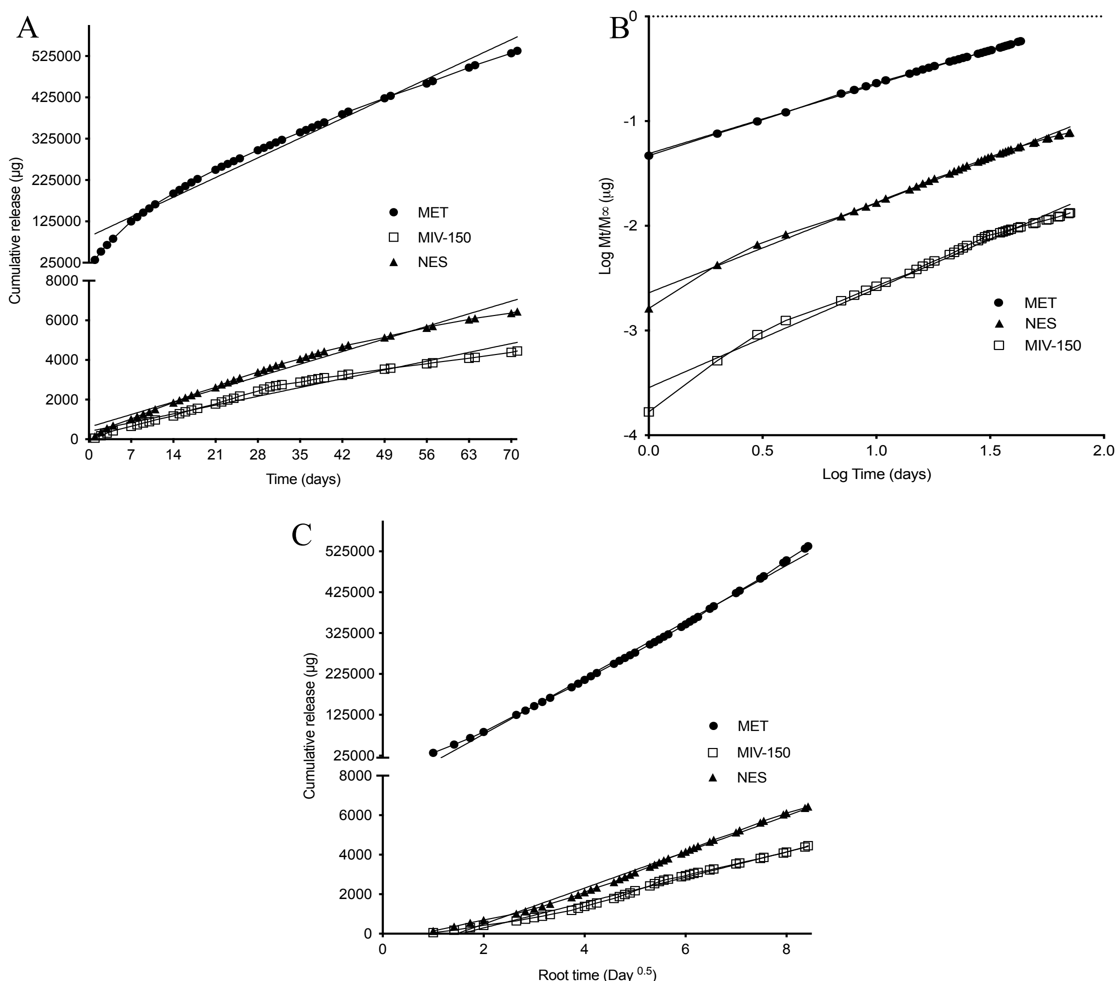


Figure 48. Graphs depicting mathematical modelling of cumulative release of NES, MET and MIV-150 from silicone cores placed into a thermoplastic frame for a period of 71 days with linear regression applied. A) Cumulative release vs time (zero order model), B) Log cumulative release vs log time (Korsmeyer–Peppas) and C) Cumulative release vs root time (Higuchi model).

Table 27. Summary of mathematical modelling results

Model		**MET**	**MIV-150**	**NES**
Zero Order	Release rate µg/day	6808	63.35	90.81
	R^2	0.91	0.95	0.97
Higuchi	Release rate µg/day $^{0.5}$	68781	640.5	913.1
	R^2	0.93	0.98	0.98
Korsmeyer–Peppas	Release exponent (n)	0.66	0.95	0.86
	Drug transport mechanism	Anomalous transport	Super Case-II transport	Anomalous transport
	Rate as a function of time	t^{n-1}	t^{n-1}	t^{n-1}

8.4 Conclusion

A new MPT ring formulation containing higher API loadings and different core lengths was tested for a period of 72 days. MET release was below target when compared to current BV treatments, although release was likened with that from a hydrophilic TPU ring [159]. Future formulations could incorporate different polymer cores like thermoplastics to increase release of MET. NES release did not achieve the target of 150 µg/day, however there was constant release over the study period out to 72 days. Depending on the desired release time for NES (either constant release or 21 day in/ 7 day out), the loading of API could be altered to achieved desired release. MIV-150 release was higher than that previously reported for a silicone elastomer ring which reduced the acquisition of SHIV-RT by 58%, suggesting a higher protection against the virus [69]. When drug release was mathematical modelled using Higuchi, zero order and Korsmeyer-Peppas models resulted differed from data obtained in chapter 7. This demonstrated that by changing core length and loaded the silicone cores with different loadings drug release kinetics can be changed.

Overall, this final MPT ring formulation in this thesis produced results that show how drug release can be manipulated by changing API loading and core length to extend release out to 72 days. Future work could focus on different drug loaded polymer core such as EVA and both hydrophobic and hydrophilic TPU to enhanced drug release further.

9

In vitro release of vaginal acidifying agents from silicone elastomer vaginal rings

9.1 Introduction

The normal vaginal pH in healthy women of child-bearing age typically ranges between 3.8 and 4.2 and is maintained by production of lactic acid resulting from the action of resident lactobacilli species upon the glycogen found in vaginal epithelial cells [242]. Lactic acid concentrations (measured *in vivo* as lactate:protein ratios) have previously been measured in the cervicovaginal lavage of healthy women with values ranging from 2–7 mol/mg [243]. The acidic vaginal environment is known to be protective against various infectious microorganisms, including HIV [4, 21,244].

Vaginal pH varies significantly during the menstrual cycle (Figure 49) due to the production of menstrual fluid and cervical mucus. Other factors are also known to influence vaginal pH, including the presence of alkaline semen after intercourse, estrogen deficiency in the vaginal tissue, and the use of antibiotics [199,245]. However, the biggest factor contributing to elevated vaginal pH is vaginal infection, and most notably bacterial vaginosis (BV).

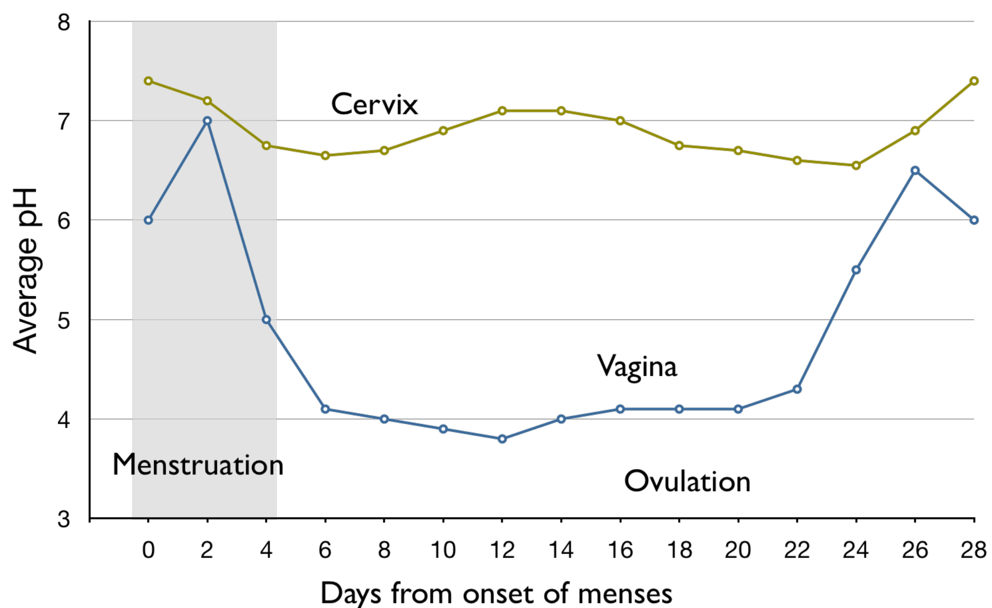


Figure 49. Average pH of the vagina and cervix during the menstrual cycle in an adult female of child-bearing age**.**

BV is a vaginal infection caused by dysbiosis of the normal vaginal microbiota. This microbial unbalance is caused by the displacement of the lactic acid-producing lactobacilli species with opportunistic bacteria – such as *Gardnerella vaginalis*,

Mycoplasma homonis, *Peptostretococci*, various *Prevotella* and *Mobiluncus* species – which leads to an increase in vaginal pH due to a reduction in lactic acid production [20,21,246]. By maintaining a healthy acidic environment, the vaginal lactobacilli can remain dominant and other unwanted bacteria are supressed.

BV has also been reported to be a leading cause of genital tract inflammation, which correlates with increased risk of HIV acquisition [17, 19,21]. McKinnon *et al.* reported the effect of genital inflammation on HIV acquisition with use of topical tenofovir gel in the CAPRISA 004 clinical trial [17]. The lowest HIV acquisition rates were observed in women who did not have genital inflammation and who reported high adherence to use of the tenofovir gel. Even for those women reporting high adherence to the gel product, high levels of genital inflammation resulted in no protection from HIV acquisition. Infection with other sexually transmitted infection (STIs) has also been shown to increase genital inflammation which increases the risk of young African women acquiring HIV [17,18, 22,247]. Some STIs present as asymptomatic, creating inflammation the individual may not be aware of and leading to transmission infection or longer exposure to higher rate of HIV acquisition [248].

A range of antibiotic dosage forms are available for the treatment of BV [249]. These include oral tablets, vaginal tablets, vaginal gels and vaginal creams, most commonly containing a 5-nitroimidazole drug (such as metronidazole (MET), tinidazole, ornidzole or secnidazole) or clindamycin (Table 28). MET is the most common prescription for the treatment of BV and is commonly administered as an oral tablet or a vaginal cream. Vaginal products are usually recommended for use at night, since this often leads to increased drug absorption as leakage is minimised while non-ambulatory. Oral dosage forms usually contain a larger dose of antibiotic compared with topical forms (Table 28).

Table 28. Overview of antibiotic treatments for bacterial vaginosis. Qd- per day.

Antibiotic	Route	Dosage form	Dosage (per day)	Frequency and Administration time	Comment	Reference
metronidazole	oral	tablet	400–500 mg	1 qd x 5–7 days		[250]
	oral	tablet	2 g	Single dose	Used if adherence is an issue	[251]
	vaginal	cream	0.75%	1 qd x 5 days	Administrated at night	[223]
clindamycin	vaginal	cream	2% (5 g per applicator)	1 qd x 7 days	Not recommended due to increased risk of pseudomembranous colitis	[252–255]
	vaginal	tablet	100 mg (2.5 g suppository)	1 qd x 3 days	Administrated at night	[256]
tinidazole	oral	tablet	2 g	Single dose	Used if adherence is an issue	[252]
	oral	tablet	500 mg	1 qd x 5 days	Administrated at night	[257]
	vaginal	tablet	500 mg	1 qd x 14 days	Administrated at night	[241]
ornidzole	oral or vaginal	tablet	500 mg	2 qd x 5 days (oral) 1 qd x 5 days (vaginal)	Administrated at night	[258]
	vaginal	tablet	500 mg	1 qd x 7 days	Administrated at night	[259]
secnidazole	oral	tablet	1 g or 2 g	Single dose	Used if adherence is an issue	[258]
	oral or vaginal	tablet	2 g 500 mg	Single dose orally 1 qd x 5 days	Used if adherence is an issue Administrated at night	[260]
rifaximin	vaginal	tablet	25 mg	1 qd x 5 days	Administrated at night	[261]

Other non-antibiotic products are also used for the treatment of BV (Table 29). For example, antiseptics such as chlorhexidine for vaginal rinsing, probiotics in the form of vaginal tablets, and acidification treatments in the form of tablets, rinses and gels are all available offering varying levels of clinical effectiveness [262]. A lactic acid (LA) gel treatment is available in which a 2.5 mg dose is recommended for daily for 7 days and thereafter twice weekly for 5 weeks. This treatment option, however, has been shown to be less effective than oral metronidazole [263]. Over-the-counter brand product such as Canestan Canesbalance® Bacterial Vaginosis Relief Vaginal Gel contains lactic acid that seeks to maintain the optimal acidic vaginal pH.

There are several key differences between antibiotic and non-antibiotic treatments for BV. Average treatment time for an antibiotic is around 5–7 days compared to up to 35 days for non-antibiotic treatment. Also, different dosage forms are used – three antibiotics are available as oral dosage forms and only two non-antibiotic oral tablets.

The semi-solid dosage forms (creams, gels, etc.) tend to be less acceptable to women, mostly on account of their tendency to leak, the need for application using an applicator, and the requirement for repeated daily doses for effective treatment [186,264].

A common side effect with using an antibiotic cream, such as metronidazole, is thrush [151]. Thrush is a common yeast infection which occurs when there is an overgrowth of fungus, mainly Candida albicans. This fungus is usually kept under control by the immune system or lactobacilli. However, antibiotic use can disrupt this balance leading to an antifungal medicine being needed.

A more user-friendly BV treatment is needed as oral and vaginal doses are dependent on daily user adherence. A device that could deliver an antibiotic to treat BV and also release an agent to maintain vaginal pH would lessen the likelihood of reoccurring BV.

Table 29. Overview of non-antibiotic treatments for bacterial vaginosis. qd –per day.

Product	Dosage form	Administration time and frequency	References
Antiseptics			
chlorhexidine	vaginal rinse	1 qd x single dose	[265]
dequalinium chloride	vaginal tablet	1 qd x 10 days	[266]
povidone iodine	vaginal rinse	1 qd x 7 days	[262]
nifuratel	vaginal or oral tablet	1 qd x 250 mg x 10 days 3 qd x 200 mg x 7 days	[262]
Acidification			
vitamin C	vaginal tablet	1 qd x 250 mg x 6 days	[267–270]
hydrogen peroxide	vaginal rinse	1 qd x 3% H_2O_2 x single dose	[271]
acetic acid	vaginal gel	2 qd x 0.92% x 7 days	[272]
lactic acid	vaginal tablet		[263]
polycarbophil-Carbopol	vaginal gel	1 qd x 35 days	[273]
Prebiotic			
lactic acid	vaginal gel	1 dd x 2.5 mg x 7 days plus 2 per week for 5 weeks	[274]
Probiotic lactobacilli			
L. reuteri and L. rhamnosus	oral or vaginal tablet	1 qd x 28 days 1 qd x 5 days	[275] [276]
L. casei rhamnosus	vaginal tablet	1 qd x 7 days	[277]
L. rhamnosus and L. gasseri	vaginal tablet	1 qd x 10 days x 3 months	[278]
L. acidophilus + 0.03 mg estriol	vaginal tablet	1 qd x 12 days	[279]

Verstraete *et al.* reported an intravaginal ring offering sustained release of LA as a prophylactic treatment for BV [159]. Sicne previous studies had indicated that ethylene vinyl acetate (EVA) matrices were not suitable for incorporation and release of lactic acid due to its plasticising effect on the polymer, this study focused on formulation of various LA loadings (0–30% w/w) using hydrophilic and hydrophobic thermoplastic polyurethanes (TPUs) with pH control assessed over 28 days [280]. For hydrophilic TPUs, lactic acid release was faster and achieved lower pH values over the first two days compared to the hydrophobic TPUs. However, the low acidity could not be maintained; hydrophilic TPUs containing ≥10% w/w lactic acid could only maintain pH under 4.5 for 17 days. A hydrophobic TPU with a loading of 30% w/w LA was able to maintain a pH of under 3.2 for the full 28 days, although pH values during days 1–11 were found to be too acidic (pH < 3). A hydrophobic TPU with a 20% w/w loading provided intermediate pH values (3.0–4.1) over 28 days. A 5-day slug mucosal irritation testing was also performed on this formulation using a contact

time of 30 mins each day. The slugs produced only a minimal amount of mucus and therefore the 20% w/w lactic acid TPU formulation was considered non-irritating.

DL-lactide is a cyclic dimer of lactic acid which can hydrolyse to form lactic acid. If DL-lactide could be successfully incorporated into and released from a silicone elastomer vaginal ring, the molecule would be expected to undergo hydrolysis to produce lactic acid. This prodrug approach to delivery lactic acid may prove advantageous over attempts to directly incorporate lactic acid into a silicone ring.

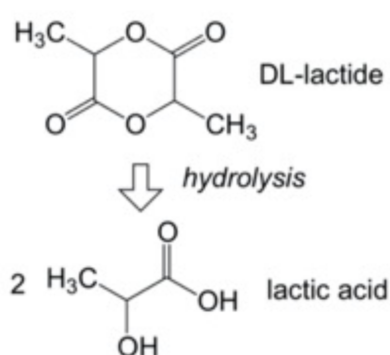


Figure 50. The hydrolysis conversion mechanism of DL-lactide to lactic acid.

Here, as part of efforts to develop a modular-type MPT vaginal ring in which one of the modules incorporates an agent to maintain an acidic vaginal pH, the feasibility of incorporating and releasing lactic acid and lactide from silicone elastomers was assessed. Initially, rheological studies were performed to assess how LT and LA affect the silicone elastomer curing characteristics. Also, the rate of hydrolysis of DL-lactide to lactic acid was studied and it's *in vitro* release from a silicone elastomer.

9.2 Materials and methods

See chapter 2 for all materials used in this chapter.

Rheological studies

DDU-4320 addition-cure silicone elastomer mixes (3.0 g) for rheological analysis were prepared by adding equal amounts of Part A and Part B (and the required amount of LT and LA) to a sealed plastic container. The active silicone elastomer mixture was

hand-mixed for 30 s and then mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixer ™ DAC 150 FVZ-K, Hauschild, Germany) (30 s, 3000 rpm).

Oscillatory rheology was performed using a TA Instrument AR2000 rotational rheometer. Silicone mixes were placed on stationary plate of the rheometer and the upper plate (40 mm crosshatch plate) lowered to give a distance of 1000 μm between the plates. Excess silicone mix was removed before the experiment was initiated. The time taken between sample placement on the lower plate and the experiment starting was typically less than 30 secs. An oscillatory frequency of 1 Hz was used to replicate the frequency a device might experience in *in vivo* conditions, as previously described by McConville *et al.* [281].

Control rheograms monitoring the isothermal cure at 40 °C, 60 °C and 80 °C of DDU-4320 without LT or LA were obtained for comparison with rheograms obtained with various loadings LT (1%, 2.5%, 5%, 11%) and LA (1%). Values for storage modulus (G'), the loss modulus (G'') and tan delta (=G''/G') were measured as a function of time.

Investigation of the rate of hydrolysis of DL-lactide to form lactic acid

200 μg/mL sample of LT in 0.2 % Tween solution was sampled everyday (except weekends) for a period of 10 days. Samples were analysed by UPLC described in chapter 2.

In vitro release study of DL-lactide from a silicone elastomer in different pH mediums

Matrix-type, silicone elastomer vaginal rings containing DL-lactide (5 or 11 % w/w) were manufactured using an injection molding machine fitted with a custom stainless-steel ring mold assembly. Separate 40 g premixes of DL-lactide in Parts A and B of the DDU-4320 addition-cure silicone elastomer system were prepared by adding weighed quantities of DL-lactide into a screw-cap polypropylene container followed by addition of the silicone part. The premixes were then hand-mixed for 30 s and then mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixer™ DAC 150 FVZ-K, Hauschild, Germany) (30 s, 3000 rpm). A and B premixes were combined in

a 1:1 ratio, which was then hand-mixed for 30 s and then DAC mixed (30 s at 3000 rpm). The mixture was manually injected into the injection molder. Rings were left to cure at 90 ˚C for 5 mins and then manually removed from the molds. Four rings were cut in half, and each half immersed in 25 mL of a 0.2% w/w Tween solution (adjusted to either pH 4.19 or pH 8.04) and placed in an orbital shaker at 37 ˚C and 60 rpm. pH was recorded over 10 days. Four of the test samples remained in the same solution for the duration of the study, while the others had their media replaced daily. Samples from the media that was changed daily were analysed by UPLC method described in chapter 2.

Comparison of *in vitro* release methods

Methods 1 & 2

Rings manufactured as per method above. Rings were cut in half (each half representing a drug-loaded module for insertion into a ring frame), and each module was immersed in 25 mL 0.2% w/w Tween solution (adjusted to pH 4.19) and placed in an orbital shaker at 37 ˚C and 60 rpm. pH was measured periodically for 10 days. Four of the test samples remained in the same solution for the duration of the study, while four had their media replaced daily. Samples from the media that were changed daily were analysed by UPLC for LA and LT content.

Method 3

A custom flow-through system for *in vitro* release testing was designed and assembled in-house. Separate 250 mL glass flasks were used to contain the donor solutions, each segment test sample and the receptor solutions. Flasks were interconnected using narrow bore PVC tubing (ID 0.25 mm) through a Gilson Minipuls Evolution peristaltic pump system (Gilson Inc, WI, US). The flow rate was set to 0.8 rev/min to give a mean flow rate of ~10 mL per day. A shaking incubator unit set at 37℃ was used to house, agitate and provide temperature control for the entire assembly.

The volume of fluid initially used in the ring flask was 25 mL. Assuming constancy of pumping, this reservoir volume should not change throughout the experiment as fresh media is fed into each flask at the same 10 mL/day rate as is sampled from the

same flask. The fluid in the terminal collection vessel was sampled and removed daily on weekdays. Samples collected were stored at 4 °C until analysis by UPLC.

9.3 Results and discussion

Rheological studies

DDU-4320 is an addition-cure medical grade silicone elastomer that has been previously reported for use in the low-temperature manufacture of intravaginal rings (IVR) [47, 89, 111, 127,218]. Addition cure silicone elastomers cure via a platinum-catalysed hydrosilylation reaction between Si–H functional groups in the poly (methyl hydrogen silane) component and the vinyl groups in the poly(methylvinylsilane) component of the silicone elastomer system. These addition cure systems are preferred over condensation type systems for fabrication of medical devices since no volatile alcohol by-products are formed during the curing reaction. Alcohol by-products have the potential to dissolve and redistribute the API within the device which impacts drug release from the elastomer and often leads to burst effects [77, 171,282].

McConville *et al.* have previously investigated cure characteristics of various medical grade silicone elastomers, including DDU-4320 [281]. A model protein, bovine serum albumin (BSA) and a model hydrophilic excipient, glycine, were incorporated into DDU-4320 to assess how the loading and cure temperature impacted the isothermal cure properties of the elastomer.

A characteristic rheogram for the isothermal cure of DDU-4320 silicone elastomer is presented in Figure 51. Three different rheological parameters are plotted as a function of time: storage modulus (G'), loss modulus (G") and tan delta (=G"/G'). G" is the measure of energy dissipated as heat and G' the measure of elastic character. As the system cures, both G" – associated with the viscous characteristic of the system – and G' – associated with the liquid nature of the silicone – increase with time with G' increasing at the faster rate due to chemical crosslinking of the elastomer. A critical point in the curing process is the transition from a predominantly viscous to a

predominantly elastic system, which occurs when G'= G" or tan ð =1. When tan ð ≥ 1, the system is dominated by elastic character.

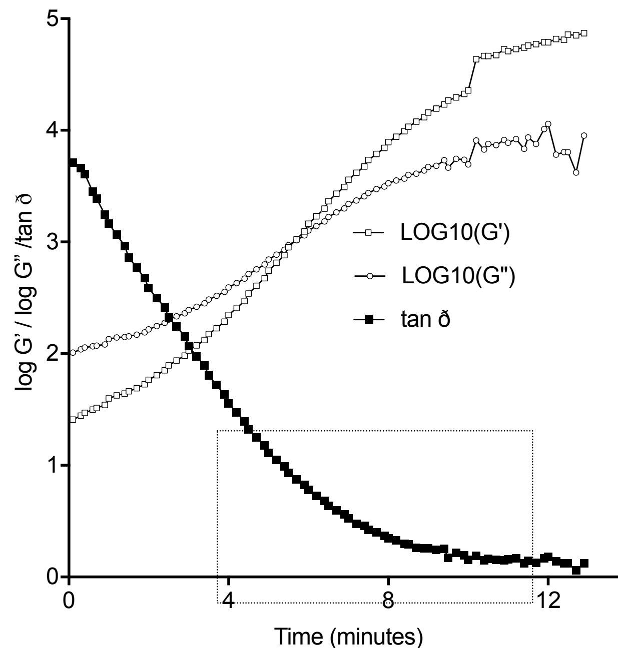


Figure 51. Oscillatory rheogram for the isothermal cure (60 °C) of silicone elastomer DDU-4320. The rheogram shows characteristic trends in G', G" and tan ð.

The portion of Figure 51 highlighted by the dashed box is presented in Figure 52 to illustrate how values for $t_{\tan ð = 1}$ and $t_{\tan ð = 0.2}$ were calculated. Three cure temperatures (40, 60 and 80 °C) were investigated for each formulation, and two critical points – time to tan ð = 1.0, 0.2 ($t_{\tan ð = 1}$ and $t_{\tan ð = 0.2}$) were measured to assess the extent of cure. These values represent the pre- and post-gel cure points and allow direct comparison between cure temperatures and % w/w LT/LA content.

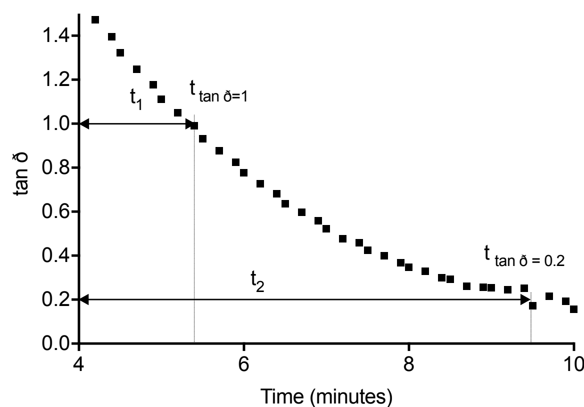


Figure 52. Zoomed region of Figure 2 showing tan ð vs. time rheogram for the isothermal cure (60 °C) of silicone elastomer DDU-4320. The rheogram also defines $t_{\tan ð = 1}$ and $t_{\tan ð = 0.2}$.

Rheological experiments were performed for 80 min to allow the formulations to cure at the designated cure temperature. However, in a pharmaceutical manufacturing setting, the long cure times used at low cure temperature cure conditions would be considered impractical. Formulations with various loadings of LT at cure temperatures of 60 °C and 80 °C followed a similar trend of increasing $t_{\tan ð = 1}$ and $t_{\tan ð = 0.2}$ values with increasing LT loadings. Formulations containing more than 1% LT did not achieve a value of tan ð =1 or tan ð = 0.2 within the 80 min timeframe at a cure temperature of 40 °C. Blank DDU-4320 $t_{\tan ð = 1}$ and $t_{\tan ð = 0.2}$ values were consistent with those reported by McConville *et al.* for all three temperatures.

Table 30. Description of formulations and their accompanying $t_{\tan ð = 1}$ and $t_{\tan ð = 0.2}$ values. Experiments were conducted for 80 mins, '–' represents replicates that did not achieve tan ð values in this time frame.

Formulation	Time (min) to tan ð = **1.0**, *0.2* [a]		
	Cure temperature (°C)		
	40	60	80
Blank	**27.7 ± 2.1**	**5.3 ± 0.1**	**2.3 ± 0.1**
	51.3 ± 0.8	*9.8 ± 0.1*	*3.7 ± 0.7*
1% LT	**51.1± 0.1**	**11.8 ± 1.9**	**2.3 ± 0.9**
	—	*34.0 ± 0.5*	*4.2 ± 1.1*
2.5% LT	—	**14.2 ± 0.4**	**2.9 ± 1.0**
	—	*42.7 ± 0.5*	*4.8 ± 0.8*
5% LT	—	**18.2 ± 2.8**	**3.5 ± 0.9**
	—	*55.5 ± 0.6*	*8.5 ± 1.2*
11% LT	—	**32.5 ± 0.8**	**3.3 ± 0.7**
	—	—	*10.3 ± 0.6*
1% LA	—	—	**18.1 ± 1.7**
	—	—	—

[a] Determined from tan ð vs t plots, according to Figure 52.

80 °C cure temperature

All systems both with and without loadings of LT achieved both tan ð =1 and tan ð = 0.2 in the 80 min time frame of the rheology experiment. tan ð =1 was achieved between 2.3–3.3 mins for all systems while and tan ð = 0.2 was achieved between 3.3–10.3 mins. LT loading did not seem to affect the pre-gel value however a wider range of 3.3–10.3 mins was needed to achieve $t_{\tan ð = 0.2}$ for the various loadings. The trend

of increasing $t_{\tan ð = 0.2}$ was evident with increasing LT % w/w content. The data demonstrate the compatibility of LT with the silicone elastomer. The system with 1% w/w loading of LA reached tan ð =1 in 18.1 min but failed to achieve tan ð =0.2 within 80 min at 80 °C, indicating that LA significantly inhibits the silicone elastomer curing reaction.

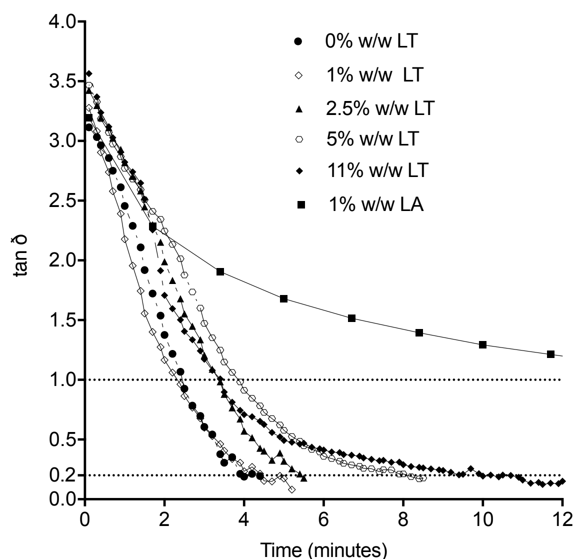


Figure 53. Tan ð versus time oscillatory rheograms for silicone elastomer systems showing the influence of LT and LA on the curing characteristics of DDU-4320 at 80°C.

60 °C cure temperature

All systems achieved tan ð =1 between 5.3–32.5 mins. tan ð = 0.2 was achieved by all systems except 11% w/w LT. As with the 80°C cure temperature condition, increasing LT content increased time to achieved specified tan ð values. Systems cured at 60°C showed increasing $t_{\tan ð = 1}$ and $t_{\tan ð = 0.2}$ values with increased LT loading. At 60°C curing of blank DDU-4320 took approximately three times longer to achieve the desired tan ð values than at 80°C. However, with increasing LT loading, $t_{\tan ð}$ values increased up to 10-fold, such as 11% w/w LT which did not reach tan ð = 0.2 within the 80 mins time frame. 1% w/w LA formulation experiment was not conducted and hence not shown in Figure 54.

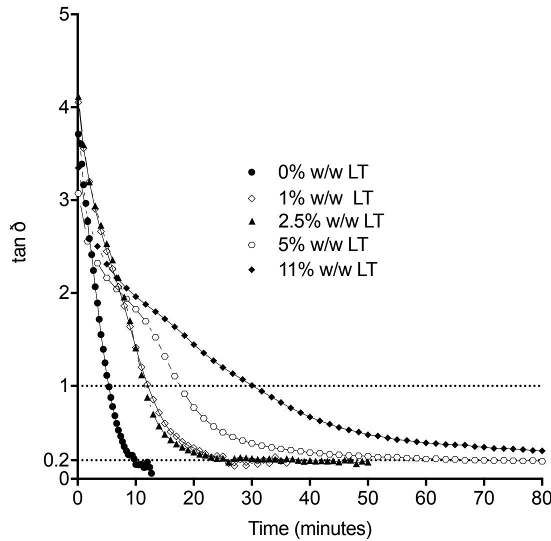


Figure 54. Tan ð versus time oscillatory rheograms for silicone elastomer systems showing the influence of LT on the curing characteristics of DDU-4320 at 60° C.

40 °C cure temperature

At this cure temperature, only blank DDU-4320 and 1% w/w LT formulations reached tan ð =1, with $t_{tan\ ð\ =1}$ value of 27.7 and 51.1 mins, respectively. tan ð = 0.2 was achieved by only the blank DDU-4320 formulations ($t_{tan\ ð\ =\ 0.2}$ = 51.3 mins). No other systems achieved tan ð = 0.2 within 80 min.

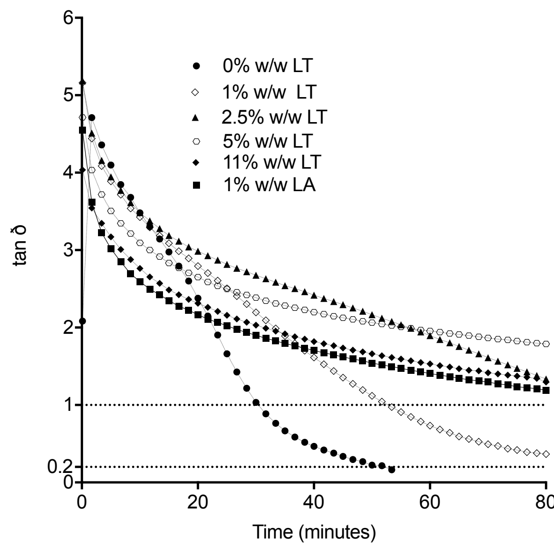


Figure 55. Tan ð versus time oscillatory rheograms for silicone elastomer systems showing the influence of LT on the curing characteristics of DDU-4320 at 40°C.

Based on trends observed at the various cure temperatures, it can be concluded that increasing the loading of w/w% LT increases the pre-gel and post-gel points. These longer values needed to achieve $t_{\tan ð =1}$ and $t_{\tan ð = 0.2}$ indicates inhibition of cure with loadings higher than 1% w/w LT at lower temperatures.

Cure characteristics vs LT loadings

Here, the t_1/t_2 ratios were used to compare the effect of LT upon the cure characteristics of the formulations at various temperatures. t_1/t_2 ratios were calculated for all LT loaded formulations cured at 80°C. For formulations cured at 60°C and 40°C, t_1/t_2 ratios could not be calculated since $t_{\tan ð = 0.2}$ values could not be determined due to slow or limited cure. By measuring the time required to achieve specific tan ð values the ratio t_1/t_2 can be used to compare formulations of various loading and over various temperatures.

The t_1/t_2 ratios of various loadings across the three cure temperatures was plotted as a function of cure temperature (Figure 56). This ratio was not calculated for lower temperature systems as no *tan ð* =1 or *tan ð*=0.2 was achieved. For those formulations for which t_1/t_2 ratio could be calculated, a trend of decreasing t_1/t_2 ratio is evident with decreasing cure temperature. As t_1 and t_2 increase with decreasing cure temperature, it is the disparity between t_1 and t_2 that gives this ratio the characteristic of being temperature dependent and that the rate of cure pre-gel point is slower than post gel point with increasing temperature. This decrease in t_1/t_2 ratio with decreasing temperature for DDU-4320 has previously been reported by McConville *et al.* [281].

A comparison of t_1/t_2 ratio of different loadings of LT for one temperature was plotted to assess LT effected cure time (Figure 57). Due to faster curing, t_1/t_2 ratios are larger at 80°C compared with 60°C cure temperature. The largest t_1/t_2 value was measured for blank DDU-4320 and the lowest for the 11% LT formulation. This decrease of value with increasing % w/w loadings suggests a faster pre-gel point cure than post gel with increased temperature. The general trend of decreasing t_1/t_2 ratio with increased LT loading is exhibited at 80° C, however a loading of 2.5% w/w LT does not follow this trend. At 60° C, the t_1/t_2 ratio initially decreases with increasing drug loading, remains almost constant between 1 and 5% w/w LT loading, and then

increases for the 11% loading. McConville *et al.* reported that the increasing the loading of model protein and excipient provided a significant decrease in t_1/t_2 which is also shown here.

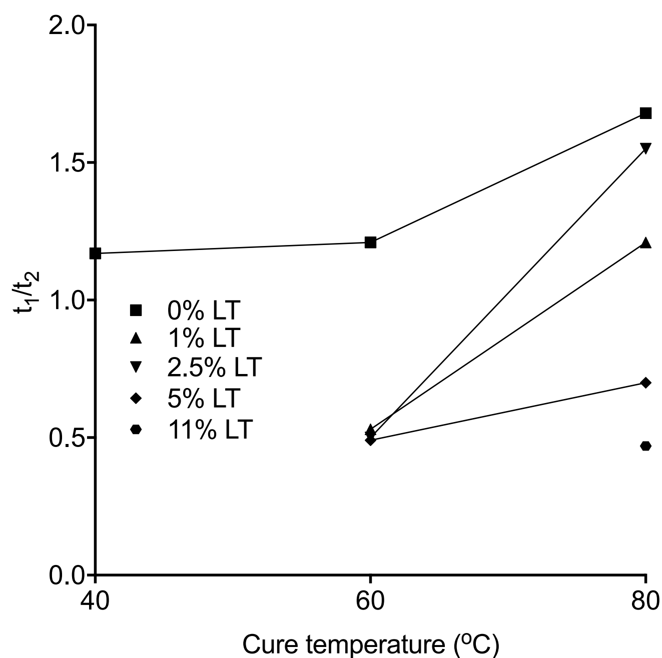


Figure 56. Influence of cure temperature and loading of LT in DDU-4320 on t_1/t_2 ratio.

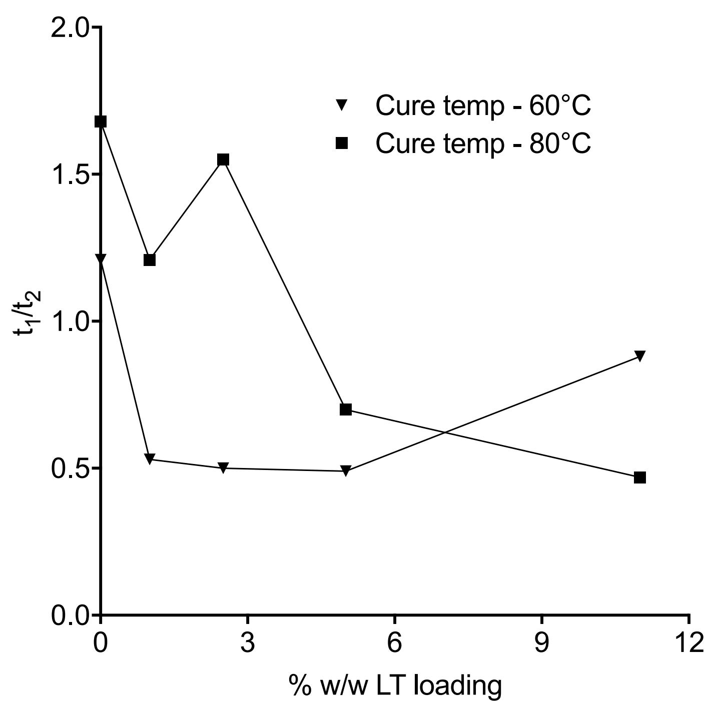


Figure 57. Influence of cure temperature and loading of LT in DDU-4320 on t_1/t_2 ratio.

Investigation of the rate of hydrolysis of DL-lactide to form lactic acid

Preliminary experiments (not reported here) demonstrated that DL-lactide in water can be readily hydrolysed to lactic acid by addition of 1N NaOH. Confirmation of the hydrolysis was obtained by analysing the solution by UPLC following neutralisation of solution. The aim of this experiment was to monitor the kinetics of DL-lactide hydrolysis in a 0.2 % v/v Tween solution at both room temperature (20 °C) and body temperature (37 °C).

DL-lactide concentrations in the solution decreased with time with concomitant increase in the lactic acid concentrations, as measured by the UPLC peak areas associated with the two species (Figure 58). The hydrolysis rate constants were determined by plotting a graph of $\ln(C_0/C_t)$ vs time, where the initial concentration of DL-lactide = C_0, and concentration at time = C_t. Linear regression was performed, and the slope of the linear fit provides the rate constants reported in Table 31. Not surprisingly, the rate of hydrolysis of DL-lactide to lactic acid is faster at body temperature compared to room temperature. *In vivo*, the presence of hydrolytic and/or esterase enzymes would likely further modulate the rate of conversion.

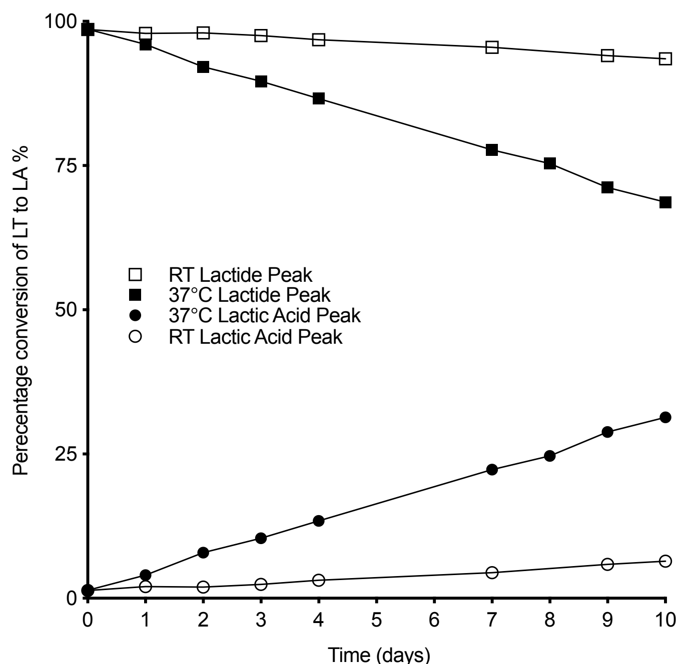


Figure 58. Percentage conversion of lactide to lactic acid in 0.2% v/v solution of Tween over 10 days at room temperature and 37ºC.

Table 31. Rate constants for the hydrolysis of DL-lactide to lactic acid

Temperature	Calculated rate constant (days^{-1})
20 °C	0.019
37 °C	0.057

In vitro release study of DL-lactide from a silicone elastomer in different pH mediums

A study was performed to assess extent to which LT reduced the pH of the *in vitro* release medium. 0.2 % w/w Tween 80 solutions having two different pH values were selected for testing: pH 4.2, representing normal vaginal pH, and pH 8 representing a worst-case scenario for BV infection [199,283].

In all experiments, pH was significantly reduced over the study period, dropping to an average pH of 2.77 on day 1 for both ring segments in pH 4.19. Where the media was not changed, pH steadily reduced, reaching a constant low pH value (pH 2.5 for pH 4.19 and pH 2.3 in pH 8.04 media. Where the media was changed daily, the overall extent of pH reduction was reduced compared to no replacement of medium (Figure 59, Figure 60).

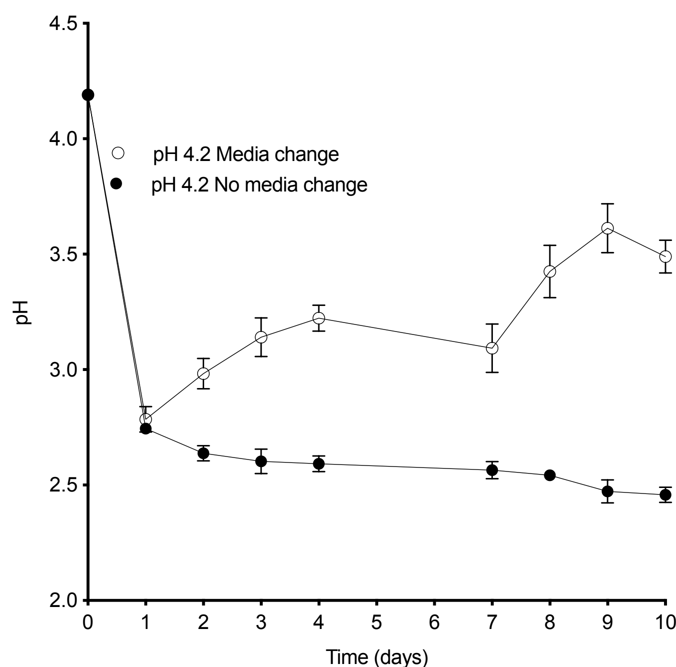


Figure 59. pH analysis of 11% w/w DL-lactide samples in 0.2% w/w Tween solution, pH 4.19. Error bars in graph represent ± standard deviation of four replicates; some error bars were smaller than the plot symbols.

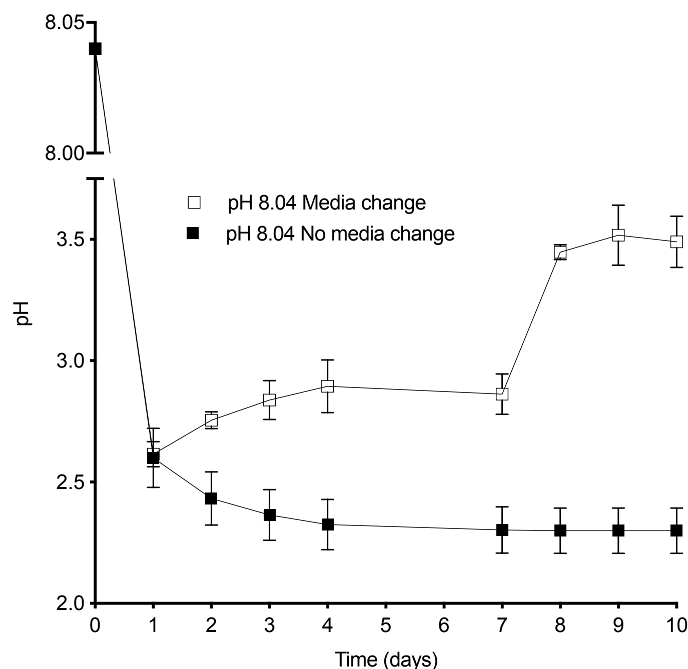


Figure 60. pH analysis of 11% w/w DL-lactide samples in 0.2% w/w Tween solution, pH 8.04. Error bars in graph represent ± standard deviation of four replicates; some error bars were smaller than the plot symbols

LT release from both media show a burst over the first 4 days followed by decreased release rates for the remainder of the study (Figure 61). This behaviour is typical of matrix-type drug delivery devices [164, 237,284,285]. LT release in pH 4.19 was 20 mg and in pH 8.04, 29 mg on day 1. Measurement of LA in the media increased with time, reflecting the time taken to hydrolyse LT to LA; average Day 1 release values for LA were 1 and 5 mg in pH 4.19 and pH 8.04, respectively. In the second half of the study period there is a substantial increase in the LA detected in the release samples with an average release value of 3.45 mg in pH 4.19 and 2.90 mg in pH 8.04 from day 8–10. This is thought to be due to the penetration of the media through pores created by the releasing LT. As the media penetrates further into the centre of the rod, more LT is available to hydrolyse to LA providing a reservoir of LA in the rod. As both LT and LA are detected in release samples, it is clear that it is the LT hydrolysing to produce the LA detected here.

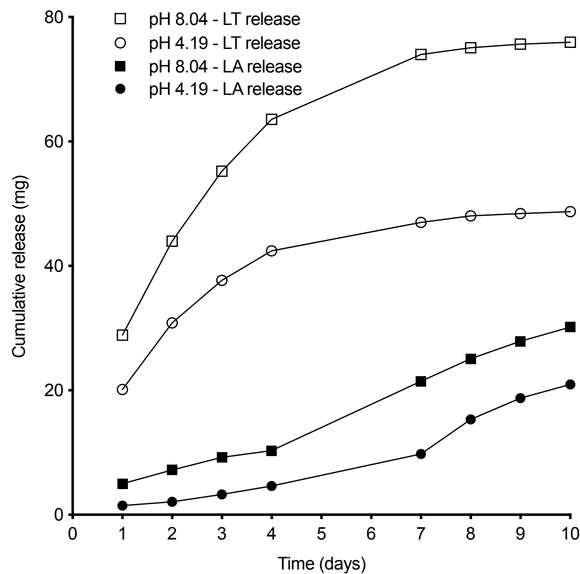


Figure 61. Cumulative release of DL-lactide and lactic acid into mediums of various pH.

Comparison of *in vitro* release methods

Three *in vitro* release test methods were assessed: 25 mL 0.2% Tween solution adjusted to pH 4.2 and replenished daily; 25 mL 0.2% Tween solution (pH 4.2) with no daily replacement; and 25 mL 0.2% Tween solution (pH 4.2) in a 10 mL per day flow-through system. The first method is similar to those currently used for marketed rings and other vaginal rings in development. The second method is designed to overcome the requirement for daily replacement of release medium. The third method is intended to better represent the dynamics of fluid flow and loss in the vagina.

Daily pH comparison of Method 1, 2 and 3

In all experiments, pH was significantly reduced over the study period, dropping to an average pH of 2.77 on day 1 for Method 1, 2.75 for Method 2 and 2.81 for Method 3 (Figure 62). With Method 1, the overall extent of pH reduction was less than that for Method 2, attributed to the daily replenishment of the release medium which depleted LT in the ring therefore increasing pH. For Method 2, where the media was not changed, pH steadily reduced, reaching a constant low pH value (pH 2.5). With Method 3, pH remained constantly low (pH 2.66–2.63) between day 2–8 followed a slight increase in pH on days 9 and 10.

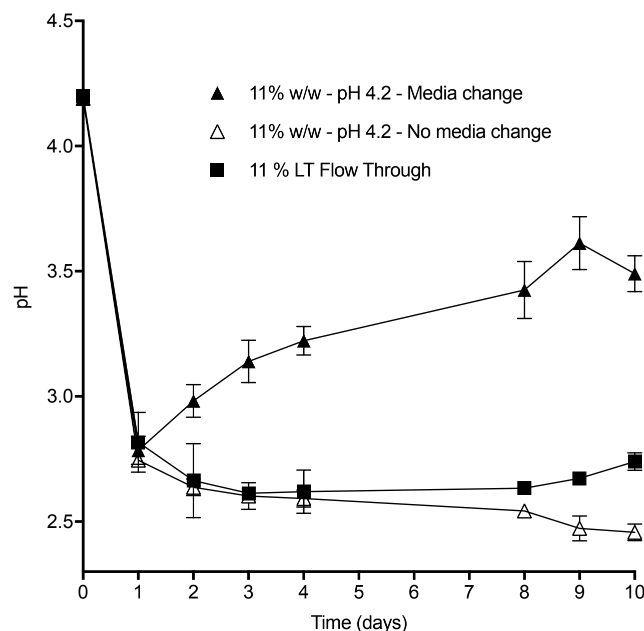


Figure 62. pH as a function of time from samples in Methods 1, 2 and 3 over the 10-day study. Method 1, 25 mL 0.2% Tween solution adjusted to pH 4.2 and replenished daily; Method 2, 25 mL 0.2% Tween solution, pH adjusted with no daily replacement; Method 3, 25 mL 0.2% Tween solution, pH adjusted in a 10 mL per day flow-through system.

Verstraete *et al.* reported that 30% w/w LA incorporated into a TPU vaginal ring maintained a low acidity for 30 days compared to 10% w/w LA ring which only lasted 17 days [159]. In that study, vaginal ring segments of length 4.5 cm were placed in 3 mL of deionised water and stored for 28 days at room temperature without stirring. pH measurements and media replenishment occurred daily. Segments having 30% w/w loading produced pH values that were considered too acidic. In fact, similarly low values were obtained here using Methods 2 and 3, in which pH remained below pH 2.81 for the duration of the study. By comparison, Method 1 provided similar desirable results as a 10% w/w LA/TPU delivering of a solution of medium acidity (pH 3.0 –4.1) for the 10-day study except for day 1 where pH dropped to 2.79.

Over-the-counter vaginal products containing lactic acid, such as Canesten Canesbalance® vaginal gel, are available, for the treatment and prevention of BV. Canesbalance® provides a 5 mL daily dose of a pH 3.8 gel for use over 7 days. This pH is more consistent with those obtained for the ring segments using Method 1.

Using One-way ANOVA and Tukey's Multiple Comparisons test, it was calculated that there was no statistically significant difference ($p > 0.05$) in the initial pH (day 1)

however by day 10, there was a statistical difference ($p < 0.05$) between the three methods.

DL-lactide and lactic acid release

Release of LT and LA release was measured from samples taken using Methods 1 and 3; since Method 2 did not provide daily release data, release was not measured. LT release using both methods showed a day 1 burst (20.1 mg and 19.2 mg for Methods 1 and 3, respectively). This burst behaviour is common with matrix-type drug delivery devices [164, 237,284,285]. LT release using Method 1 showed a steady decline over the 10-day period with 3.0 mg LT released on Day 10. For Method 3, mean LT release was 14.3 mg between day 2–4 and 4.4 mg on Days 8–10. Since Method 1 used daily replacement daily of the 25 mL release medium, the depletion of LT is greater than that of Method 3 were 10 mL is constantly moving through the sample.

As LA is formed slowly in the release medium by hydrolysis of LT, samples were analysed daily to provide an accurate measurement of both. LA concentrations on Day 1 were 1.7 mg and 5.3 mg for Methods 1 and 3, respectively (Figure 63). As with previous studies described above, there was considerable increase in the LA detected in release samples with an average value of 3.7 mg in Method 1 and 6.6 mg in Method 3 from day 8–10. As releasing LT crystals dissolve, it is thought that media fills these orifices, allowing access to LT contained in the centre of the module, allowing hydrolysation to LA to occur. The simultaneous detection of both LT and LA in release samples indicates that hydrolysis of LT is not completed in the samples. The higher release of LA in Method 3 is due to the constant flow and longer time in the sample flask, allowing more time for LT to hydrolyse to LA.

Canesten Canesbalance® vaginal gel provides daily dosing of 225 mg LA for 7 days, although a portion of the dose is likely lost due to leakage [286]. The 11% w/w LT module provided around only 10% of this dose on day 1 with daily release declining further over the 10-day study. A method to further enhance daily release such as increasing LT loading or using a different polymer such as a TPU could be used. Other lactic acid gel are available in a 2.5 mg dose for a treatment time of 7 days [263]. This dosage is similar to that measured using Methods 1 and 3 with an average of 4.4 mg

of LA being released daily. Although this dosage was found to be less effective than oral metronidazole for the treatment of BV, it could be used as a prophylactic treatment in cases of reoccurring BV and to promote a healthy vaginal pH.

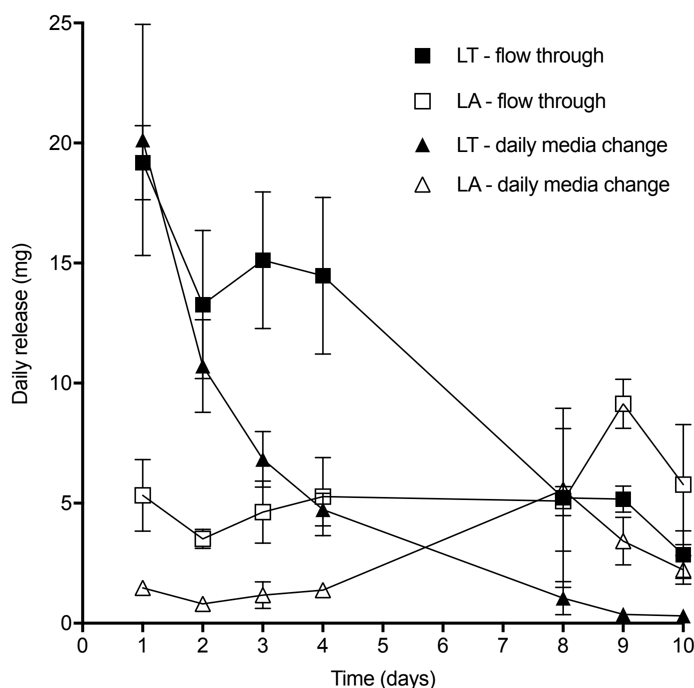


Figure 63. Daily release of LT and LA from Methods 1 and 3 into a 0.2 % Tween 80 solution adjusted to pH 4.2.

9.4 Conclusion

Oscillatory rheology was used to assess the cure characteristics of blank, LT and LA-loaded DDU-4320 silicone elastomer formulations at different cure temperatures. $t_{\tan ð =1}$ and $t_{\tan ð = 0.2}$ values were recorded and compared across different temperatures with various loadings of LT and LA. The time required to achieve these values increased with decreasing temperature and increasing LT loading. From $t_{\tan ð =1}$ and $t_{\tan ð = 0.2}$, a ratio t_1/t_2 was calculated. This ratio showed that by decreasing temperature and increasing LT loading t_1/t_2 decreased. The results indicate that optimum curing temperature for any LT loading (up to 11% w/w) is 80 °C as all loadings achieved a tan ∂ = 0.2 within 10.3 mins. The highest loaded LT system (11% w/w) did not attain this post gel point at 60 °C and 40 °C. From the perspective of establishing a viable manufacturing method, it was deemed unrealistic to cure these DDU-4320 formulations at temperatures less than 80°C due to impractically long cure times. The

addition of LA, even at a low loading of 1% w/w, impacted curing properties and a post gel value is not seen before 80 mins at all temperatures. It is clear from these results that LA significantly inhibits the DDU-4320 curing reaction, even at low concentrations.

From this series of studies, it was concluded that LT can be effectively incorporated into a silicone elastomer module and is released under *in vitro* conditions from the module, as demonstrated by the detection of LT in the release medium. LT is hydrolysed to LA, either within the module by the penetration of the release medium or in the release medium, following the release of LT. A reduction in pH of the release medium is observed due to the production of LA.

Although the three *in vitro* test methods investigated provide different release vs. time profiles; we do not know which of the three methods best mimics release *in vivo*. It is concluded that LT incorporated into a silicone elastomer has the potential to be useful in a modular MPT, delivering LA to decrease pH and promote a healthy vaginal environment.

10

General Discussion

The aim of this thesis was to examine a new type of MPT device, a vaginal ring frame that cores that can be loaded with different APIs could be inserted. The story within this thesis shows of the different considerations that were taken into account during pre-formulation studies to prototype testing that investigated surface area, solubility, different materials and a combination of APIs.

Firstly, UPLC methods were developed and validated to the industry standard recognition ICH guidelines. These validated methods were used throughout this thesis to detect and determine amounts of NES, MET, DPV and MIV-150 released and contained from silicone and in media. In chapter 3, DSC was also utilized to determine the amount of NES, MIV-150 and MET soluble in DDU-4320 (an addition cure silicone elastomer system) at the respective API melting point. This high temperature solubility value reflected the maximum solubility of each API at their melting temperatures. A room temperature drug uptake study was also conducted that evaluated drug ingress into different polymers like DDU-4320 and a range of thermoplastics. This study evaluated some potential ring frame candidates which showed that more crystalline polymers are most resistant to drug ingress, this being one of the traits that would be ideal in a ring frame polymer.

After exploring drug ingress and solubility, ring frames were manufactured from various thermoplastics, EVA-17.5%, Pebax®, a thermoplastic elastomer made of polyether and polyamide, Estane®, a thermoplastic polyurethane, Hytrel®, a polyester elastomer, Vistamaxx™, an isotactic propylene repeated unit with random ethylene distribution and Desmopan®, a thermoplastic polyurethane (TPU). The thermoplastic ring frames were tested using mechanical test methods designed to replicate not only *in vivo* use but to demonstrate reliability to regulatory authorities. Tests such as a 5 mm compression was used to compare numerous rings while a 28-day deformation test was designed to mimic compression during clinical use. Alongside these two tests another compression test was designed, a compression to 50 % OD, a deeper compression which allowed additional comparison to be made across the range of thermoplastics. For the compression testing, a single non-drug loading silicone core was inserted to generate additional data on the full device rather than the ring frame alone. These mechanical tests determined a trend in the range of polymers with Desmopan, Vistamaxx and Hydtrel providing the most favourable characteristics

looked for this novel ring frame. For example, they were exhibited enough strength to hold a core in place however were more flexible of the selection. This selection also provided best recovery values in the 28-day deformation deeming them the polymers to carry on to the next stage of testing.

Chapter Five explored problems and issues that had been envisioned to be associated with this novel ring device. Firstly, a medium to be used throughout this thesis was chosen, a 0.2% Tween solution with was determined to be the most efficient medium to make and also provided comparable drug release. Secondly issues such involving available surface area were explored. The first surface area related issue analysed was that of a matrix-core placed inside a prototype 'skeleton' section. This allowed a short 10 –day study to be conducted on the release of NES, MET, DPV and MIV-150 from separate matrix-type drug loaded elastomers placed inside. This data set showed that drug release decreased when the surface area of the matrix section was reduced compared to a control sample. This data also provided the first *in vitro* release results of the APIs that would be used throughout this thesis. Secondly, reservoir cores were explored. This time surface area was increased by cutting the same size of rod into different sizes but overall, the rod length stayed the same. The increased surface area arose from the ends of the cut reservoir rods which was demonstrated to increase the rate of *in vitro* release giving name to 'end effects'. This useful data envisioned how that the rate of release could be increased from set lengths of rods placed in the ring frame without changing the composition of the drug loading.

After preliminary mechanical and *in vitro* release testing was conducted, the three chosen polymers VX, HY and DS were manufacturing in to prototype ring frames to be used in *in vitro* release testing. Into these frames separately matrix-type drug loaded silicone cores of NES, MIV-150 and MET were inserted. Singularly these API were sampled over a 10-day period in four different sample sets. The first set of samples explored the drug release from the free cores, a sample set to act as a control. These control sets were split into two different lengths, a half-length core and two quarter length cores. This again explored the idea of increasing surface area by exposing the ends, this time in a matrix-type core rather than a reservoir type core which was explored in chapter 5. These two configurations where then inserted into the selection of polymer ring frames, a set containing the half cores, the other containing two quarter

cores across the three APIs. Cumulative release across the three APIs concluded a number of trends across the board. Firstly, drug release from cores only provided more release than those inserted into the frame, and secondly that drug release from two quarters provided slightly more release than release from one single half-length core. There was also a different in release of API between the different polymer frames. VX provided the highest release across of APIs and provided very little drug uptake was observed in the content studies however its physical properties were deemed that it did not provide a secure enough fit hold the silicone rods therefore increasing the surface area that came into contact with the medium. In content studies DS proved to be the polymer that drug ingress was most prominent, leaving HY which provided a well round set of results. HY polymer was then taken into the next step of formulation.

In chapter 7, for the first-time release of two or more APIs were released in unison from this novel ring device. Different combinations involving NES, MET and MIV-150 contained in matrix or reservoir silicone cores were placed in a ring frame manufactured from the previously selected HY polymer. Four ring devices were created using different lengths of drug-loaded silicone core. Ring 1 contained two matrix-type silicone cores one loaded with MIV-150 and the other NES. Ring 2 comprised of one matrix-type and one reservoir type core containing MET and NES respectively. Ring 3 contained two matrix-type silicone cores loaded with MET and MIV-150 respectively. Ring 4 comprised of three silicone matrix-type cores containing all three APIs separately. Ring 1 –4 devices were placed on *in vitro* release for 28 days with Ring 4 undergoing additional testing in the form of mechanical and accelerated stability testing. The initial *in vitro* release testing concluded that MIV-150, NES and MET, when co-released from the device in different combinations exhibited no change in daily and cumulative release. NES release was altered using different types of cores where a shorten, lower drug loaded matrix –type core provided slightly more cumulative release over the time period than a longer higher loaded reservoir-type core. A stability study assessed two types of possible storage configurations that this novel ring device could be stored in. One, the medicated cores would be stored in the device and the other the cores would be stored separately. After the accelerated stability was finished and *in vitro* release study was conducted on the all the device configurations with the conclusion that storage conditions impacted released, content and mechanical properties. Overall these studies demonstrated the

release of all three APIs from this novel ring device and provided initial data for future storage conditions.

After the release of NES, MET and MIV-150 for 28 days from the novel ring device, this data was exploited and manipulated using custom equations to create a long acting prototype. This device contained the same API although core length and drug loading were altered compared to previous devices to allow constant release for 72-days. MIV-150 % w/w loading remained the same compared to previous formulations with NES and MET loading increasing (0.8 to 2.5 % and 25 to 40% respectively). At the end of the study all API were still detectable in the release medium with steady state being achieved from around day 35 for all APIs. The release of MIV-150, (although not achieving a target release rate of 150 ug/mL) was more than that of a previously reported study with achieved 58% protection against the acquisition of SHIV-RT. This chapter concluded the journey of the novel ring device in this thesis. It demonstrated how that by altered core length and drug loading that API release can altered to last a period of 72- days. This study was aimed to run until 90 days however due to the Covid-19 situation the study had to be stopped at 72 days. By looking at the daily release graphs, an extra 12 days of daily release would have been achieved.

Chapter 9, an extra project that could tie in with future studies involving this novel ring explored APIs to maintain vaginal pH. Firstly, lactic acid and lactide was examined incorporated into the silicone elastomer DDU-4320 using oscillatory rheology. It was found that when lactic acid was added to the silicone elastomer, even at a 1 % w/w loading, it inhibited the cure of the system. It was found that when lactide was added to the silicone system, the optimum curing temperature was 80 °C at a 11 % w/w loading which was carried on to further studies. LT loaded silicone elastomer where then assessed in *in vitro* release studies which examined the reduction of pH during the study and also the release of LT. It was found that LT hydrolyses in water to produce LA, which a reduction in pH was due to. Another study examined three potential type of sampling during a short 10 day *in vitro* release study. Method 1 consisted of solutions being sampling as per usual while Method 2 did not change the media. Method 3 consisted of a peristaltic pump device that provided a constant replenishment of release medium throughout the study. All methods provided different

release vs time profiles however it was not known when method would best mimic release in an *in vivo* situation. Overall this chapter explored a new concept, a device that would reduce vaginal pH rather than the gels or tablets that are currently on the market. This type of core could be easily inserted into the novel ring device to work alongside MET to treat BV which is associated with a high pH environment.

Overall this PhD project has taken a concept of a novel ring device and taken it through preformulation studies, polymer selection studies to various prototype formulations, finishing with one that could theoretically last for up to 90 days. The data accumulated in this thesis provided a basis for various future prototype ring devices. It has been an exciting and interesting project throughout these three years.

11

Placement at Population Council, New York

My PhD project was partly sponsored and funded by Centre of Biomedical Research (CBR) of Population Council. Population Council (PC) is a non-profit, non-governmental organization (NGO) based in New York City, USA who conduct biomedical, social science and public health research [287]. A major focus in recent years has been around HIV/AIDS prevention and reproductive health, including how poverty, youth and gender relates to reproductive health. PC operates a 'bench to bedside' approach, affording formulation scientists to work alongside social scientists to create and develop unique and much-needed products for low-income countries.

PC was first established by John D. Rockefeller III in 1952 with the Centre for Biomedical Research, located in Rockefeller University on the Upper East Side in New York City. Rockefeller had a vision to create an organisation that would allow woman to have a choice in their reproductive options, in both large economic climates and in low income countries. Although PC headquarters are still located in New York to this day, PC has a global network of offices across Africa, Asia, Latin America and the Middle East with research and implementation programs in more than 50 countries [288].

PC's 'bench to beside' approach has been hugely successful, with a raft of female contraceptive products having reached market, including the Copper T intrauterine device, Progering®, Norplant®, Mirena® and – most recently in 2018 – Annovera™, a one-year contraceptive vaginal ring system.

For three months during 2019 (September to November), I had the opportunity to visit and work at PC's CBR as part of my PhD project. During that time, I gained invaluable insights into the organisational structure and operations at PC and contributed to a number of research projects.

OPERM

Oregon Permanent Contraception Research Centre (OPERM) is an organisation created to research and test new approaches to nonsurgical female contraception. One of their products, a foam-based product containing the active agent polidocanol (a sclerosant and irritant used to treat varicose veins), is currently being trialled as a

permanent contraceptive foam. When injected into the uterus via a catheter, the foam creates tubal occlusion of the fallopian tubes [289]. PC were tasked with developing, testing and manufacturing a polidocanol foam (PDF) formulation. *In vitro* testing included assessment of foam viscosity and potential interactions between the formulation components. As part of my placement, I was tasked with designing a conceptual replica uterine model to assess the pressure that the foam exerts on the uterus. This model was intended to reduce the reliance on animal testing and to generate biorelevant data. Using a uterus teaching model supplied by an online medical supply firm, I created an anatomical correct human uterus model from a silicone elastomer. Due to time constraints, this project was not completed by the end of my placement. I also attended a scientific advisory board meeting for this project. This allowed me to see how high-level decisions are made, their impact on the progress of the project, and how some aspects of the project were more important for donors than others.

PrEP and contraception formulation projects

Although various single indication products are available for prevention of unwanted pregnancy or HIV prevention, there are no combination products that simultaneously address both. Further, there is considerable stigma in sub-Saharan Africa in taking PrEP medicines such as Truvada, since the tablets are prescribed in bottles which look very similar to those in which antiretrovirals (ART) are supplied. This similarity between bottles leads to non-adherence to PrEP as users are concerned their peers will think they are taking ART. There is also a large population, and especially among young woman, who do not think they are at risk of contracting HIV. The possibility of combining contraceptive and antiretroviral actives in a single product would likely increase the PrEP uptake in communities where the combined oral contraceptive pill (COC) is already widely used. PC are working on two projects offering different approaches to solve this issue.

Co-encapsulation

One project, funded by National Institute of Health, will conduct a two-arm 12-month clinical trial in Zimbabwe. In one arm, participants will use a single capsule containing

contraceptive and antiretroviral tablets, allowing both tablets to be taken at the same time. In the second control arm, women will be assigned to tablets taken separately. The study aims to recruit participants already familiar with taking oral contraceptives and PrEP and to assess if there is a preference for taking one large tablet or separate tablets. I was involved with the formulation aspects of the project during my placement, Specifically, I helped assess the moisture sensitivity of the PrEP component of the combined pill. PrEP is usually prescribed in a plastic pill bottle containing a desiccant which helps maintain a low humidity environment and prevents degradation of the APIs. However, combined oral contraceptive pills are normally supplied in 21 or 28-day blister packs with the day clearly indicated for each pill, allowing the woman to clearly see whether or not they have taken their daily dose. This presented a problem, as most manufacturing sites have dedicated hormone only packaging areas in which no other product other that COCs are allowed to enter. I participated in vendor phone calls, enquiring about the types of packaging available and if the vendor would have the capacity to enable a COC and PrEP to be blistered together. Another interesting aspect of the product related to stability and material testing. A vendor has recommended a company called FreeThink, specialising in enhanced stability testing and computer modelling to generate two years' stability data in 6 weeks. The company required only minimum number samples and could test different packaging combinations, which would allow accelerated testing of several vendor materials. A company working in partnership with FreeThink had just submitted a New Drug Application (NDA) to the FDA which included such accelerated stability data, and the data were well received by the FDA. This project gave me lots of insights into the type of stability data and information required to support a clinical study.

Co-formulation

This co-formation project consisted of a new drug product combining a COC and PrEP. Even though the APIs were already included in separate marketed products, co-formulation was to prove challenging. The daily dosing requirements for the PrEP drug was >100-fold greater than for the contraceptive drugs, making blending, moisture sensitivity and content uniformity difficult to control. Part of the project involved a compatibility study with API and excipients from both brands of the chosen

COC and PrEP products. I was involved in weekly updates from a third-party laboratory that was conducting the work. I was able to apply my knowledge of analytical chemistry (e.g. HPLC, PXRD and Karl Fisher analysis) to contribute to the conversation around the compatibility of APIs and excipients.

Another aspect of the project involved receiving quotations from different potential pharmaceutical manufacturing companies who had submitted bids for this scope of work. I was tasked with in comparing the quotations and compiling a report to help in deciding which company was awarded the contract. I also visited one of the GMP hormone manufacturing facilities in Baltimore. Although I have previously worked in a manufacturing facility, every company is different and it was interesting to see the approach this company used, especially in their analytical department.

These two projects were especially interesting to me given the topic my PhD project, and have the potential to address the unmet need a combined COC and PrEP product for women.

Social science project

PC are not only are leaders in the field of biomedical science, but they also employ a team of social scientists working providing and commissioning projects that help the lives of young woman. The GIRL (Girl Innovation, Research and Leadership) Centre is based in NY but works with young women world-wide generating research and evidence to put recommendations and policies in place. I was able to meet with some of these researchers and understand first-hand the important work they were pursuing.

In the final weeks of my placement, I attend an event held by the Tri-State Area Africa Funders, titled 'Healthcare for At-Risk Populations: Progress on HIV care and outcomes-Case study from South-Africa'. At panel at this event included: The Honourable Edwin Cameron, a gay-rights activist and a recently retired justice of the Constitutional Court of South Africa; Dr Bruce D. Walker, founding director of the Ragon Institute and Director of the Harvard University Centre for AIDS Research; and Dr Jean Bassett, executive director at Witkoppen Clinic. The question-and-answer session helped me appreciate the importance of lab-based researchers working in

conjunction with activists and social scientists to successfully address global health issues. Although the members of the panel had not met previously met, they held each other in deep respect and highlighted the common issues around funding, opposition from government and social misconceptions with had led to this panel discussion. Their in-depth knowledge and personal first-hand experiences were moving. I realised that the issues they face are not only hugely challenging, but that that those like me living in developed countries often take high quality healthcare systems for granted.

Reflecting as a laboratory-based scientist, I sometimes focus too much on the small issues that arise in the laboratory – an instrument not operating correctly or a standard dilution that is outside of specification. My time at Population Council opened my eyes to the bigger picture and gave me invaluable insights into how a large organisation operates collectively to get difficult tasks completed. The supportive community at PC was like nothing I have experienced before. I appreciate that the non-profit status of the organisation and the passion and work ethic of its many employees likely make a very significant contributed to the overall outlook of the organisation and the purposeful work environment. At the end, I was sad to leave but I knew the time spent there allowed me to gain new insights into my PhD and what the research I was conducting could contribute in a small but meaningful way to the health and well-being of young woman worldwide.

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