Matrix and reservoir-type multipurpose vaginal rings for controlled release of dapivirine and levonorgestrel


Published in:
International Journal of Pharmaceutics

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2016 Elsevier. This manuscript is distributed under a Creative Commons Attribution-NonCommercial-NoDerivs License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Open Access
This research has been made openly available by Queen's academics and its Open Research team. We would love to hear how access to this research benefits you. – Share your feedback with us: http://go.qub.ac.uk/oa-feedback
Matrix and reservoir-type multipurpose vaginal rings for controlled release of dapivirine and levonorgestrel

Peter Boyd¹, Susan M. Fetherston², Clare F. McCoy¹, Ian Major³, Diarmaid J. Murphy¹, Sandeep Kumar¹, Jonathon Holt⁴, Andrew Brimer⁴, Wendy Blanda⁴, Brid Devlin⁴, R. Karl Malcolm¹*

¹School of Pharmacy, Queen’s University Belfast, Belfast BT9 7BL, UK; ²QPharma, Malmo, Sweden, ³Athlone Institute of Technology, Athlone, Ireland, ⁴International Partnership for Microbicides, Silver Spring, MD 20910, USA

*Corresponding author. Tel: +44 (0)28 9097 2319; Fax: +44 (0)28 9024 7794;
E-mail: k.malcolm@qub.ac.uk

Short title: Dapivirine and levonorgestrel vaginal rings

Keywords: HIV microbicide; Hormonal contraception; Silicone elastomer vaginal ring; Multipurpose prevention technology; MPT; Formulation development.
Abstract

A matrix-type silicone elastomer vaginal ring providing 28-day continuous release of dapivirine (DPV) – a lead candidate human immunodeficiency virus type 1 (HIV-1) microbicide compound – has recently demonstrated moderate levels of protection in two Phase III clinical studies. Here, next-generation matrix and reservoir-type silicone elastomer vaginal rings are reported for the first time offering simultaneous and continuous in vitro release of DPV and the contraceptive progestin levonorgestrel (LNG) over a period of between 60 and 180 days. For matrix-type vaginal rings comprising initial drug loadings of 100, 150 or 200 mg DPV and 0, 16 or 32 mg LNG, Day 1 daily DPV release values were between 4132 and 6113 μg while Day 60 values ranged from 284 to 454 μg. Daily LNG release ranged from 129 to 684 μg on Day 1 and 2–91 μg on Day 60. Core-type rings comprising one or two drug-loaded cores provided extended duration of in vitro release out to 180 days, and maintained daily drug release rates within much narrower windows (either 75–131 μg/day or 37–66 μg/day for DPV, and either 96–150 μg/day or 37–57 μg/day for LNG, depending on core ring configuration and ignoring initial lag release effect for LNG) compared with matrix-type rings. The data support the continued development of these devices as multi-purpose prevention technologies (MPTs) for HIV prevention and long-acting contraception.
Abbreviations

DAC, dual asymmetric centrifuge; DPV, dapivirine; DSC, differential scanning calorimetry; HIV-1, human immunodeficiency virus type 1; HPLC, high performance liquid chromatography; LNG, levonorgestrel; IPM, International Partnership for Microbicides; MPT, multipurpose prevention technology; NNRTI, non-nucleoside reverse transcriptase inhibitor; STI, sexually transmitted infection; SVF, simulated vaginal fluid
1. Introduction

Vaginal rings offering sustained or controlled release of antiretroviral drugs have been at the forefront of efforts over recent years to develop microbicide products for prevention of sexual transmission of human immunodeficiency virus type 1 (HIV-1) (Malcolm et al., 2016). A matrix-type silicone elastomer vaginal ring containing dapivirine (DPV; Figure 1A) – an experimental non-nucleoside reverse transcriptase inhibitor (NNRTI) – and intended for 28-day continuous use is being developed by the International Partnership for Microbicides (IPM) (R Karl Malcolm et al., 2012; Nel et al., 2011, 2009). This DPV ring recently completed two Phase III clinical studies (the Aspire Study and The Ring Study) designed to support licensure of the ring for preventing infection with HIV in women (Baeten et al., 2016; Nel et al., 2016b). Results from these studies showed that the ring reduced HIV infection by 27% and 31%, respectively, compared with a placebo ring (Baeten et al., 2016; Nel et al., 2016b). Post hoc sub-group analyses in the Aspire Study revealed a 37% reduced risk after excluding two sites with the lowest rates of retention and adherence, a 56% reduced risk when only women older than 21 years were considered, and a 61% reduction in women aged 25 and older (Baeten et al., 2016). In The Ring Study, sub-analysis by age revealed no significant benefit for women younger that 21 years, and a 37.5% reduced risk in women aged >25 years (Nel et al., 2016b).

Despite the fact that a safe and effective vaginal microbicide product to protect against HIV infection has yet to reach market, there is already considerable interest and early-stage development activity around next-generation multipurpose prevention technology (MPT) products that seek to combine HIV prevention with contraception and/or prevention/treatment of other sexually transmitted infections (STIs) (Fernández-Romero et al., 2015; Malcolm and
Fetherston, 2013; Malcolm et al., 2016, 2014; Romano et al., 2013; Woodsong et al., 2015).

With 86 million unintended pregnancies (Sedgh et al., 2014) and 2.1 million new HIV cases
around the world every year (Joint United Nations and HIV/AIDS, 2016), reformulation of the
DPV ring to additionally include a continuous-use progestin-only contraceptive is an obvious
next step, especially since most existing hormonal birth control methods offer no protection
against HIV or other STIs. Furthermore, a vaginal ring with a use indication for both prevention
of pregnancy and HIV infection may result in increased user adherence compared with a
product preventing only HIV, since women’s perceived risk of pregnancy is usually higher than
that for HIV infection (Woodsong and Holt, 2015).

Many of the MPT products currently undergoing development, including a number of vaginal
ring devices, have prioritised use of levonorgestrel (LNG; Figure 1B) as the contraceptive
hormone component based on its historical record of safety and effectiveness and its suitability
for continuous use without need for a monthly withdrawal period (Mansour, 2012; Romano et
al., 2013; Ugaonkar et al., 2015; Woodsong et al., 2015). In addition to its current use as a long-
acting contraceptive in intrauterine devices and subdermal implants (Eisenberg et al., 2015;
Gonzalo et al., 2002; S Koetsawang et al., 1990; Rose et al., 2009), LNG has also previously
been investigated extensively for delivery from silicone elastomer vaginal rings (Bounds et al.,
1993; S Koetsawang et al., 1990; S. Koetsawang et al., 1990a, 1990b; Mishell et al., 1975;
Murphy et al., 2016b). Recently, as part of continued efforts to develop a MPT vaginal ring
offering simultaneous release of DPV and LNG, we reported on various formulation strategies to
reduce the extent of LNG binding to addition cure silicone elastomer materials (Murphy et al.,
2016b). Here, we report for the first time assessment of the preclinical feasibility of matrix-type
and reservoir-type silicone elastomer vaginal rings offering continuous release of both DPV and LNG for at least 60 days and preferably at least 90 days in quantities anticipated to offer clinical effectiveness.

2. Materials and methods

2.1. Materials

Micronised DPV was supplied by S.A. Ajinomoto OmniChem N.V. (Wetteren, Belgium). Non-micronised LNG (Batch No: 120101) was supplied by Haorui Pharma-Chem Inc. (Irvine, CA, US). MED-4870 and DDU-4320 silicone elastomer kits were purchased from NuSil Technology LLC (Carpinteria, CA, US). HPLC-grade acetonitrile, HPLC-grade isopropanol and potassium dihydrogen orthophosphate (AnalR analytical reagent) were purchased from VWR International Ltd. (Dublin, Ireland). Phosphoric acid (85% w/w in water) was purchased from Sigma-Aldrich (Gillingham, UK). A Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK) was used to obtain HPLC-grade water.

2.2 Ring release rate targets

The aim of this study was to develop a MPT vaginal ring offering at least 60-day in vitro release, and preferably 90-day release, of DPV and LNG at levels likely to be effective for HIV prevention and contraception. In comparison, the existing Dapivirine Vaginal Ring-004 contains only 25 mg DPV and is intended for 28 days of use (Nel et al., 2009). For the DPV component of the MPT ring, the in vitro release rate on Day 60 or Day 90 was targeted to be equal to or greater than the Day 28 in vitro release value from the Dapivirine Vaginal Ring-004 (i.e. 200 μg). This value was determined from historical data across multiple batches of Ring-004 and measured
experimentally under the same \textit{in vitro} release conditions as those used to test the MPT rings described in this study. Two target (lowest acceptable) \textit{in vitro} release rates – 35 µg/day and 70 µg/day – were defined for LNG based on our analysis of previously reported data in the scientific literature (Clark et al., 2014; Eisenberg et al., 2015; Jackanicz, 1981; S Koetsawang et al., 1990; S. Koetsawang et al., 1990a; Landgren et al., 1994a, 1994b; Xiao et al., 1985). Vaginal rings with \textit{in vitro} LNG release rates ranging from 20–30 µg/day have been investigated previously (Clark et al., 2014; Jackanicz, 1981; S. Koetsawang et al., 1990a; Landgren et al., 1994a, 1994b; Xiao et al., 1985). Systemic LNG levels peaked at between 300 to 800 pmol/L shortly after ring insertion and remained relatively stable with an average decline of 23–26% during the 3 months of use (S Koetsawang et al., 1990; Landgren et al., 1994b; Xiao et al., 1985). However, new ring designs targeting higher LNG \textit{in vitro} release rates (e.g. 35 µg/day) have been advocated due to concern with the higher pregnancy rates observed among heavier women in clinical trials (Brache et al., 2000).

\textbf{2.3. Differential scanning calorimetry}

Samples of micronised DPV, non-micronised LNG and physical mixtures of the two drugs at 10% w/w intervals were prepared for DSC analysis. Each mixture was mixed thoroughly, first by hand using a spatula and then in a Speedmixer™ at 3000 rpm. Samples were analyzed by DSC (TA Instruments 2920 modulated DSC) in standard heating ramp mode. Approximately 5–10 mg of each sample was accurately weighed into an aluminum pan and heated from 20 to 250°C at a rate of 10°C per min alongside an empty reference pan. For each sample, the following parameters were noted for any melting transitions that were observed: onset temperature (°C), peak temperature (°C) and enthalpy (ΔH, J/g). A minimum of four replicates was used to
calculate mean values for each sample mixture. DSC analysis was similarly performed on silicone elastomer samples loaded with various concentrations and ratios of DPV only, LNG only and DPV+LNG in order to characterize the nature of the drugs in the rings.

**2.4. Matrix-type vaginal ring manufacture**

The DPV-only matrix-type vaginal ring (Ring-004) that recently completed being tested in two Phase III clinical trials in Africa contains 25 mg DPV and is intended for 28-day use (Baeten et al., 2016). In order to extend DPV release from a matrix-type device out to at least 60 days, it was necessary to increase the DPV loading in the matrix-type ring, in accordance with the relevant theory of drug release kinetics (Malcolm et al., 2003; Siepmann and Peppas, 2011). Three higher DPV loadings were selected for further investigation in this study: 100 mg, 150 mg and 200 mg. Two LNG loadings – 16 mg and 32 mg – were also selected, based on previous data generated as part of the project (data not published). In total, 11 different matrix-type vaginal ring formulations were manufactured based on various combination loadings of DPV and LNG (Table 1). Matrix-type, silicone elastomer vaginal rings (cross-sectional diameter 7.8 mm, outer diameter 56.7 mm) were manufactured using a Babyplast 6/10P horizontal injection molding machine fitted with a custom stainless steel ring mold assembly and a silicone dosing system. Separate 50 g premixes of DPV and/or LNG in Parts A and B of the MED-4870 addition-cure silicone elastomer system were prepared by adding weighed quantities of DPV and LNG into a screw-cap polypropylene container followed by addition of the silicone part. The premixes were then mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixer™ DAC 150 FVZ-K, Hauschild, Germany) (180 s, 3000 rpm) before storing in the fridge. On the day of ring manufacture, the premixes were removed from the fridge, hand-mixed (30 s) and then DAC
mixed (120 s, 3000 rpm). A and B premixes were combined in an overall 1:1 ratio, according to the following procedure: (i) 25 g weights of each premix were alternately added to a large screw-cap polypropylene container to a final weight of 100 g; (ii) this active silicone elastomer mixture was hand-mixed for 30 s and then DAC mixed (30 s at 3000 rpm); (iii) this process was repeated four times for each formulation to produce 400 g total of the active mix. The 400 g active mix was transferred to a 500 g polypropylene SEMCO® injection cartridge designed for use with the dosing system on the Babyplast injection molder. The ring mold assembly on the Babyplast machine was heated via 2 x 200 W heater cartridges fitted to both the fixed and mobile plates. Rings were manufactured by injecting the active mix into the heated ring mold assembly, under the following conditions: 100 bar clamping pressure, 50 bar injection pressure, 160 °C mold temperature, 60 s cure time. Rings were subsequently demolded, deflashed (where necessary) and stored at ambient temperature until further testing.

2.5. Core-type vaginal ring manufacture

Two different configurations of human-sized, reservoir-type, silicone elastomer rings containing DPV and LNG (Formulations L and M, Table 2) were manufactured using a three-step injection molding process (Figure 2). Each step was similar to that described previously for the manufacture of the matrix-type rings (Section 2.3). However, given their greater complexity, the reservoir-type rings were manufactured on a laboratory-scale injection-molding machine using the DDU-4320 grade of addition-cure silicone elastomer, which offers lower cure temperature, lower viscosity and improved flow characteristics compared to the MED-4870 silicone elastomer. Formulation L reservoir-type rings comprised a full-length DDU-4320 silicone elastomer core containing both solid crystalline micronised DPV and solid crystalline non-
micronised LNG, each at a loading of 2% w/w. The drug-loaded core was subsequently overmolded in two steps using custom molds with a drug-free DDU-4320 silicone elastomer sheath (rate-controlling membrane). All mixing procedures were conducted as described for the matrix-type rings. However, cure of the drug-loaded cores was performed at 90 °C for 30 s, producing cores with the following dimensions: 54.9 mm outer diameter, 4.5 mm cross-sectional diameter. The overmolded, non-medicated, rate-controlling membrane was cured at 90 °C for 90 s. The fully manufactured core rings had the following dimensions: 58.0 mm outer diameter, 7.6 mm cross-sectional diameter. The thickness of the non-medicated membrane was therefore \((7.6 - 4.5) / 2 = 1.55\) mm. Formulation M reservoir-type rings were manufactured in the same manner, except with two separate half-length cores – one containing only 2% w/w DPV and the other containing only 2% LNG (Table 2).

2.6. In vitro release testing

Matrix-type rings

On Day 0, matrix-type rings were placed individually into 250 mL glass bottles containing 200 mL 1:1 mixture of isopropanol and water 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 (2 mL) for subsequent HPLC analysis and the entire remaining volume replaced with a fresh 100 mL of isopropanol/water mixture. This sampling and 100 mL replacement of the release medium was performed daily out to Day 30, except on Fridays when, after sampling, the flask was replenished with a 200 mL volume of release medium and no further replacement or sampling performed until the following Monday. From Day 30 through to Day 60, twice-weekly sampling and replacement of the release medium was performed on consecutive days (Days 38,
39, 45, 46, 52, 53, 59 and 60), with 100 mL release medium used on the first of the two consecutive days and 200 mL used on the second day. Release testing was extended out to Day 92 for matrix-type vaginal ring formulations C and K with twice-weekly sampling (Days 66, 67, 73, 74, 80, 81, 87, 88, 91, 92) following the protocol described earlier. The amount of drug in each sample was quantified by reverse-phase HPLC with UV detection (Section 2.6).

Core-type rings

In vitro release testing of reservoir-type rings over 180 days was performed in a similar manner to that for matrix-type vaginal rings. Daily sampling and replacement was performed (50 mL; 100 mL at weekends) out to Day 30, twice-weekly sampling and replacement on consecutive days (50 mL first day, 200 mL second day) out to Day 95, and twice-fortnightly sampling and replacement on consecutive days (50 mL first day, 200 mL second day) out to Day 180. The smaller 50 mL volume used here compared with the 100 mL volume used when testing matrix-type rings is acceptable given the significantly lower drug release rates from reservoir-type rings.

2.7. HPLC method

A Waters HPLC system (Waters Corporation, Dublin, Ireland) consisting of the following components was used for all HPLC analysis: 1525 Binary HPLC pump, 717 Plus Autosampler, In-line Degasser AF Unit, 2487 Dual λ Absorbance Detector, 1500 Column Heater. Samples were injected (25 µL) onto a Thermo Scientific BDS Hypersil C18 column (150 mm x 4.6 mm, 3 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 phosphate buffer (pH 3.0) and 45% HPLC-
grade acetonitrile (1.2 mL/min) with a run time of 9 min. DPV was detected using a wavelength of 210 nm after 6.2 min, while LNG was detected after 7.7 min using a wavelength of 240 nm.

2.8. Statistical analyses

DPV and LNG in vitro release was compared for each ring set using a one-way ANOVA, followed by post-hoc analysis using the Tukey-Kramer multiple comparisons test. The following results were compared for both drugs: Day 1 release, Day 30 release, Day 60 release, total release over 60 days. Analysis was conducted using GraphPad Prism software and significance was noted for a P value of less than 0.05: * = significant (0.01 < P < 0.05), ** = very significant (0.001 < P < 0.01), *** = extremely significant (P < 0.001), ns = not significant (P > 0.05).

3. Results and Discussion

DSC thermal analysis

DSC analysis of the pure DPV and LNG substances showed sharp endothermic transitions at 219 and 238 °C, respectively, indicative of crystalline melting (Figure 3A). The additional endothermic transition observed at ~100 °C in the DPV trace is due to a known polymorphic transition (crystalline form I to II) (Murphy et al., 2014). For all the ring formulations tested in this study, the concentrations of DPV and LNG incorporated into the silicone elastomer material were so low (0.2–2.5% w/w, Tables 1 and 2) that no discernible crystalline melting endotherms were observed by DSC; at the high temperature of DSC analysis, the drug loading fully dissolves in the silicone elastomer (Gramaglia et al., 2005). However, evidence that DPV and LNG exist in the solid crystalline state within the rings was provided using silicone elastomer samples containing much higher (10% w/w) drug loadings for which the endotherms associated with
melting of the pure drug substances were observed at 219 and 238 °C (Figure S1, Supplementary Material). Coupled with the white opaque appearance of the matrix rings (particularly those containing DPV; Figure 4) and the drug-loaded cores of the reservoir rings (Table 2), the DSC data strongly indicate that both drug substances are at least partially present in the solid crystalline state within the silicone elastomer materials.

DSC analysis of physical mixtures of crystalline DPV and LNG revealed reduced melting behaviour for both drugs (Figure 3A and 3B), a eutectic composition at 40% LNG concentration (Figure 3C), and a eutectic melt temperature of ~192°C (Figure 3A and 3B). Once again, the rings of this study did not contain sufficiently high concentrations of DPV and LNG to show discernible DSC peaks. However, it is assumed that the same reduced melting behaviour also applies to the drugs within the rings.

In vitro release from matrix-type vaginal rings

Dapivirine is an exceptionally poorly water-soluble (< 1 mcg/mL) antiretroviral drug (Murphy et al., 2014). Various release media have been used for in vitro release testing of dapivirine-releasing rings during the past twelve years of development, including simulated vaginal fluid (SVF; a substantially aqueous, non-buffered medium), various buffer systems, aqueous media incorporating surfactant(s), and various organic solvent/water mixtures (Fetherston et al., 2013a, 2013b; Malcolm et al., 2005; R. Karl Malcolm et al., 2012; Murphy et al., 2016a, 2016b, 2014; Woolfson et al., 2006). SVF is unquestionably the most physiologic medium here, but it affords very low in vitro release of dapivirine (in the order of low micrograms per day) even when relatively large volumes (> 100 mL) are used, due to the poor aqueous solubility of dapivirine.
Moreover, *in vitro* dapivirine ring release using SVF does not correlate with release *in vivo*, based on post-use residual dapivirine content data (unpublished data). (It is worth noting that the daily production of human vaginal fluid is around 6 g/day, with approximately 0.5–0.75 g present in the vagina at any one time (Owen and Katz, 1999).) Use of buffered aqueous release media for *in vitro* release testing is not preferred since vaginal fluid has only limited buffering capacity (Tevi-Bénissan et al., 1997; Wagner and Levin, 1984). Therefore, protocols for *in vitro* release testing of vaginal rings containing poorly water-soluble drugs have inevitably had to make use of solvent enhancement strategies to come close to measured *in vivo* release rates. Both organic solvent/water mixtures and surfactant-containing aqueous media have been used and are widely reported in the literature. For most of its development program, an isopropanol/water mixture (1:1 volume ratio) has been used for the *in vitro* testing of the dapivirine ring, primarily for the purpose of screening and comparing different formulations during preclinical development. We have extensive unpublished data to confirm that this solvent mixture does not cause the rings to swell and that solvent extraction is not responsible for the release of dapivirine. We also have extensive data to confirm that a conventional permeation-controlled release mechanism operates in this medium. Use of isopropanol/water also permits use of much lower (and more practical) volumes of release media; 100 mL per day is typically used for a human-sized ring, which, although still relatively large compared to vaginal fluid volumes, is significantly less than the litres required when using purely aqueous media. Finally, measurement of residual dapivirine content following clinical use and testing in sheep of the 25 mg dapivirine ring for 28 days indicates that the total amount of dapivirine released (~4 mg) is broadly similar to that measured following *in vitro* release testing using 1:1 isopropanol/water over the same time period (Fetherston et al., 2013a; Holt et al., 2015; Nel et al., 2016a; Spence et al., 2016). For
these reasons, a 1:1 isopropanol/water mixture was selected as the in vitro release medium in this study. The solubility of DPV in different isopropanol/water mixtures has been reported previously (Woolfson et al., 2010).

All of the matrix-type rings containing DPV (Formulations A–C and F–K; Table 1) were white and opaque in appearance (Figure 4), consistent with uniform distribution of the white micronised DPV particles throughout the silicone elastomer matrix. By comparison, the 16 and 32 mg LNG rings (Rings D and E, Table 1) were partially transparent (Figure 4), with the non-micronised LNG particles clearly visible within the matrix as discrete particles upon close inspection. Ring weights for all matrix-type ring formulations were close to 8 g (Table 1).

A validated HPLC-UV method was developed for quantification of in vitro release of DPV and LNG from the vaginal ring formulation. Full details – including representative chromatogram, baseline quality, precision, recovery, resolution and linearity – are provided in the Supplementary Material (Figures S3 and S4, Tables S4, S5, S6, S7 and S8). Graphs depicting DPV and LNG release from the matrix-type rings over the 60-day test period are presented in Figures 5 and 6, respectively, while summary release data are presented in Supplementary Material (Tables S1 and S2). For all ring formulations containing DPV, DPV release showed a burst release on Day 1 (ranging between 4132 and 6038 µg, depending upon initial DPV loading within the ring) followed by steadily declining daily release quantities with time (Figure 5A). By Day 30, daily DPV release was within the range 407–634 µg, and by day 60 284–454 µg (Table 2). In accordance with theory (Malcolm et al., 2003; Siepmann and Peppas, 2011) and based on previously reported in vitro release data for 25 mg DPV-only rings under similar experimental
conditions (Fetherston et al., 2013a), cumulative DPV release on Day 30 showed an approximate
two-fold increase for every four-fold increase in DPV loading.

Day 60 DPV release values for these matrix-type rings were significantly higher than both the
predetermined minimum acceptable value of 200 µg and the 136 µg/day mean release rate
reported previously for DPV release from a reservoir-type silicone elastomer ring (Malcolm et
al., 2005). The cumulative release versus root time graph (Figure 5B) more clearly illustrates the
impact of initial DPV loading upon release. Increasing the DPV loading produced a significant
increase in the DPV release rate (P < 0.001 for all relevant comparisons). However, the
additional presence of LNG in rings having a fixed DPV loading did not significantly influence
DPV release. For rings containing 100 mg DPV and 0, 16 or 32 mg LNG (formulations A, F and
G), there was no significant difference in DPV release for any of the comparisons made (P >
0.05), with the exception of 60-day cumulative release for formulations A and F (P < 0.01). The
total release of DPV from formulations A and F was 36.7 and 36.1 mg, respectively (Table S1 in
the Supplementary Material), a difference unlikely to manifest itself in vivo. The same is true for
rings containing 150 mg DPV (B, G and H) and 200 mg DPV (C, J and K). Very low percentage
RSD values for the daily release data were observed, indicating that ring manufacture and in
vitro release are highly reproducible. All cumulative DPV release versus root time profiles were
linear (Figure 5B), with coefficient of variation (R²) values very close to unity (Table S1 in the
Supplementary Material), indicating a permeation-controlled release mechanism for DPV from
these rings (Malcolm et al., 2003). Based on the DPV in vitro release data generated, each
formulation tested has potential as a 60-day product.
In vitro LNG release from the matrix-type rings is rather more nuanced than that for DPV. In general, the daily LNG release versus time profiles are also indicative of matrix-type kinetics with highest release occurring on Day 1, followed by declining daily release over time (Figure 6A). In general LNG release from the rings fall into four distinct groups in order of increasing LNG release: 16 mg LNG ring < 32 mg LNG ring < 16 mg LNG + DPV ring < 32 mg LNG + DPV ring. Release from the LNG-only rings D and E was clearly much lower than that for combination rings having the same initial LNG loading (Figure 6A) and shows significant deviation from root time kinetics based on linear regression modelling (Table S2, Supplementary Material). This suggests either a release-enhancing effect in the presence of DPV or a lack of LNG availability / inhibition of LNG release in the absence of DPV. The non-linear cumulative release versus square root time profiles for the LNG-only rings (Rings D and E, Figure 6B) further suggest that only a fraction of the initial LNG loading is capable of being released from the rings; at Day 60, only 2.0 and 5.2 µg LNG were released from Rings D and E, respectively (Table S1 in the Supplementary Material).

We have recently reported that a hydrosilylation reaction occurs between LNG and the hydride-functionalised polydimethylsiloxane component of addition-cure silicone elastomer system leading to irreversible covalent binding of LNG with the silicone and ultimately reduced LNG release (Murphy et al., 2016b). This binding phenomenon is almost certainly occurring in both the LNG-only and the LNG+DPV rings of this study. However, it is clearly not the only mechanism affecting LNG release, since LNG release is very significantly increased by the presence of DPV when LNG-only rings are compared to DPV+LNG rings with equivalent LNG loading (Figure 6, Table S2 in the Supplementary Material) (P < 0.001 for all comparisons).
Each of the combination rings released significant quantities of LNG on Day 60 (23–91 µg), culminating in total release of 31–36% of the nominal LNG loading over the course of the release experiment (Table S2 in the Supplementary Material). There are several possible explanations for the enhanced release of LNG in the presence of DPV. The presence of DPV in the silicone elastomer may modify the silicone elastomer environment so as to enhance the solubility of LNG in the elastomer, resulting in a corresponding increase in release. This phenomenon has been reported previously for *in vitro* release of DPV from a silicone elastomer ring when maraviroc (MVC) is incorporated as a second microbicide agent (Fetherston et al., 2013a), and is attributed to 'pore-forming' theory first postulated for drug/excipient loaded silicone elastomers back in the 1980s (Carelli et al., 1989; Di Colo, 1992; Golomb et al., 1990). Additionally, and supported by the DSC experiments previously discussed in this study for powder mixtures of DPV and LNG, DPV and LNG might form a solid state eutectic-type mixture within the silicone elastomer matrix, as reported previously in other combination drug delivery systems, including vaginal rings (Liu et al., 2006; Stott et al., 1998; van Laarhoven et al., 2002). The reduced melting temperature for each drug component in the eutectic would result in its increased solubility in the silicone elastomer and increased drug release. Finally, incorporation of DPV in the rings will lead to competition for the solubility sites in the silicone elastomer which will reduce LNG solubility in the elastomer leading to reduced exposure to and reaction with the hydrosilane groups in the silicone elastomer formulation (Murphy et al., 2016b). Given the complexity of the system, it is very difficult to determine the relative contribution of these various mechanisms to the enhanced LNG release in the presence of DPV.
Based on previous unpublished data from preliminary studies on matrix-type vaginal rings containing both DPV and LNG, the LNG loadings for rings in this study (16 and 32 mg) were selected to target Day 60 LNG release values of 35 and 70 µg. For rings F, H and J, each containing 16 mg LNG, LNG release on Day 60 was in the range 23–29 µg (Table S2 in the Supplementary Material), slightly below the target value (Section 2.2). For Rings G, I and K, each containing 32 mg LNG, Day 60 release ranged between 84 and 91 µg (Table S2 in the Supplementary Material), significantly above the 70 µg target (Section 2.2).

Two matrix-type ring formulations – Ring C containing 200 mg DPV and Ring K containing 200 mg DPV and 32 mg LNG – were selected for extended *in vitro* release testing in order to determine the feasibility of a matrix-type ring as a 3-month product. Both ring formulations provided similar DPV release on Day 92 (301 and 299 µg; formulations C and K, respectively), significantly in excess of the 200 µg minimum daily release rate (Section 2.2). For formulation K, LNG release was 46 µg on Day 92, above the lower target of 35 µg (Section 2.2).

Based on these data, the matrix-type DPV and LNG ring may be suitable for extended use over 3 months. By adjusting the initial loadings of DPV and LNG within the matrix ring the *in vitro* release behaviour of both drugs could be further modified. One of the difficulties with this approach, and a consequence of the kinetic model used to describe drug release from matrix-type rings, is that any changes in loading to affect drug release near the end of the intended use period have a disproportionate effect on the initial burst release of the drug, which may have implications for drug product safety. This issue is considered more pertinent to the LNG component within the matrix ring. Future clinical development of this matrix-type MPT ring
should seek to evaluate the relationships between drug loading, pharmacokinetic / pharmacodynamic behaviour, and product safety.

In vitro release from reservoir-type vaginal rings

Daily and cumulative in vitro release versus time graphs for reservoir-type vaginal ring formulations L and M over 180 days are presented in Figure 7. Daily DPV release from ring formulation L (Table 2; comprising a full length core loaded with 51.2 mg each of DPV and LNG) ranged from 131 μg on Day 1 through to 75 μg on Day 180, representing a 42% decline (Figure 7A; Table S3 in the Supplementary Material). By comparison, the dual half-core ring configuration (ring formulation M; Table 2) provided Day 1 release of 61 μg and Day 180 release of 37 μg, exactly half the values for the full-length reservoir-type ring formulation L (Figure 7A; Table S3 in the Supplementary Material). This linear relationship between daily release and length of drug-loaded core is in accordance with Crank’s equation (Woolfson et al., 2003, 1999). After 180 days, total cumulative DPV release was 17.0 and 9.1 mg for Rings L and M (Figure 7C, Table S3 in the Supplementary Material), respectively, equivalent to 33.2% and 35.7% of initial DPV loading, respectively (Table S3 in the Supplementary Material).

Ring formulations L and M (Table 2; comprising a full length core loaded with 51.2 mg each of DPV and LNG) showed distinct lag effects in the graphs of daily LNG release versus time (Figure 7C). Both rings show negligible release on Day 1 (Table S3 in the Supplementary Material), and maximum daily release is only achieved on Day 15 for Ring L (149.9 μg) and Day 25 for Ring M (57.2 μg). This behaviour is clearly very different from that of DPV (Figure 7A). Lag effects are commonly observed in reservoir-type rings when insufficient time has passed
between ring manufacture and release testing or clinical use to permit equilibration of dissolved drug between core and sheath components; this effect is exacerbated at low curing temperatures.

However, this explanation does not account for the very substantial lag effects observed for LNG in the rings of this study. Rather, as postulated previously for the unusual release characteristics observed for the LNG-only matrix-type rings (Rings D and E; Figure 6), the lag effect here is most likely attributed to a hydrosilylation reaction between the ethinyl functional group in dissolved LNG molecules and excess silane groups in the silicone elastomer system leading to irreversible chemical binding (Murphy et al., 2016b). LNG release rates steadily increased during the initial release period (Figure 7B), suggesting that LNG binding within the non-medicated silicone elastomer rate-controlling sheath predominates until all of the excess silane groups have reacted. Thereafter, solubilised LNG molecules diffused through the sheath layer uninhibited resulting in the expected zero-order kinetic profile (Figure 7B). After 180 days, total cumulative LNG release was 21.5 and 8.6 mg for Rings L and M (Figure 7D, Table S3 in the Supplementary Material), respectively, equivalent to 42.0% and 33.4% of initial LNG loading, respectively (Table S3 in the Supplementary Material). That Ring M comprising the half-length LNG core provides LNG release characteristics that are slightly lower than expected compared to Ring L comprising the full-length core DPV+LNG core (Table S3 in the Supplementary Material) is attributed to LNG binding in the non-medicated silicone elastomer sheath layer. This represents a confounding factor to accurate modelling of LNG release and underlines the need for experimental determination of drug release.

Comment on stability of DPV and LNG
Although pharmaceutical stability data are not presented in this manuscript, both DPV and LNG generally show good long-term stability in silicone elastomer rings. DPV Ring-004, containing 25 mg DPV in an addition-cure silicone elastomer, has recently completed Phase III clinical testing and shows long-term stability performance over its 36-month shelf life (Devlin et al., 2013). Stability performance for a combination microbicide ring device containing DPV and MRV has been published previously (Fetherston et al., 2013a). Stability data for LNG-only and DPV+LNG rings are currently unpublished, but are planned for inclusion in a future publication.

4. Conclusions

Extending the duration of DPV release over the current 28-day 25 mg DPV-only vaginal ring and developing a MPT ring combining DPV with a contraceptive agent are important next steps in the development of practical and effective HIV microbicide products. The data presented here highlights the feasibility of pursuing either a 60-day matrix-type ring or a 90-day reservoir-type ring for simultaneous release of DPV and LNG as a viable MPT strategy.
Acknowledgements

The work was supported by a grant to Queen’s University Belfast from The International Partnership for Microbicides, through generous support from the Ministry of Foreign Affairs of the Netherlands and the American people through the United States Agency for International Development (USAID) through the President’s Emergency Plan for AIDS Relief (PEPFAR).

Transparency declarations

The authors declare no conflicts of interest.
References

Govender, V., Mgozi, N.M., Matovu Kiweewa, F., Nair, G., Mhlanga, F., Siva, S.,
Bekker, L.-G., Jeenanain, N., Gaffoor, Z., Martinson, F., Makanani, B., Pather, A.,
Naidoo, L., Husnik, M., Richardson, B.A., Parikh, U.M., Mellors, J.W., Marzinke,
M.A., Hendrix, C.W., van der Straten, A., Ramjee, G., Chirenje, Z.M., Nakabiito, C.,
Taha, T.E., Jones, J., Mayo, A., Scheckter, R., Berthiaume, J., Livant, E.,
Jacobson, C., Ndase, P., White, R., Patterson, K., Germuga, D., Galaska, B.,
Bunge, K., Singh, D., Szydlo, D.W., Montgomery, E.T., Geschke, B.S., Torjesen, K.,
Grossman, C.I., Chakhtoura, N., Nel, A., Rosenberg, Z., McGowan, I., Hillier, S.,

Bounds, W., Szarewski, A., Lowe, D., Guillebaud, J., 1993. Preliminary report of
unexpected local reactions to a progestogen-releasing contraceptive vaginal ring.

Brache, V., Alvarez-Sanchez, F., Faundes, a, Jackanicz, T., Mishell, D.R.,

elastomer through controlled polymer cracking: an extension to macromolecular
drugs. Int. J. Pharm. 50, 181–188. doi:10.1016/0378-5173(89)90120-8

Segmented Dual-Reservoir Polyurethane Intravaginal Ring for Simultaneous
doi:10.1371/journal.pone.0088509

Devlin, B., Nuttall, J., Wilder, S., Woodsong, C., Rosenberg, Z., 2013. Development of
dapivirine vaginal ring for HIV prevention. Antiviral Res. 100, S3–S8.

Di Colo, G., 1992. Controlled drug release from implantable matrices based on
hydrophobic polymers. Biomaterials 13, 850–6. doi:1457678

2015. Three-year efficacy and safety of a new 52-mg levonorgestrel-releasing
intrauterine system. Contraception 92, 10–16.
doi:10.1016/j.contraception.2015.04.006

Fernández-Romo, J.A., Deal, C., Herold, B.C., Schiller, J., Patton, D., Zydowsky, T.,
Romano, J., Petro, C.D., Narasimhan, M., 2015. Multipurpose prevention
doi:10.1016/j.tim.2015.02.006

Fetherston, S.M., Boyd, P., McCoy, C.F., McBride, M.C., Edwards, K., Ampofo, S.,
containing two microbicides with different mechanisms of action. Eur. J. Pharm.

Fetherston, S.M., Geer, L., Veazey, R.S., Goldman, L., Murphy, D.J., Ketas, T.J.,
protection against multiple RT-SHIV162P3 vaginal challenge of rhesus macaques
by a silicone elastomer vaginal ring releasing the NNRTI MC1220. J. Antimicrob.
Chemother. 68, 394–403. doi:10.1093/jac/dks415
doi:10.1016/0168-3659(90)90088-B
Gonzalo, I.T.G., Swerdlow, R.S., Nelson, A.L., Clevenger, B., Garcia, R., Berman, N.,
Wang, C., 2002. Levonorgestrel implants (Norplant II) for male contraception
Endocrinol. Metab. 87, 3562–72.
speed DSC (hyper-DSC) as a tool to measure the solubility of a drug within a solid
Holt, J.D.S., Cameron, D., Dias, N., Holding, J., Muntendam, A., Oostebring, F., Dreier,
P., 2015. The Sheep as a Model of Preclinical Safety and Pharmacokinetic
Evaluations of Candidate Microbicides 3761–3770. doi:10.1128/AAC.04954-14
Jackanicz, T.M., 1981. Levonorgestrel and estradiol release from an improved
Koetsawang, S., Gao, J., Krishna, U., Cuadros, A., Dhali, G.I., Wyss, R., la Puenta,
J.R., Andrade, A.T.L., Khan, T., Kononova, E.S., Lawson, J.P., Parekh, U., Elstein,
M., Hingorani, V., Wang, N., Yao, Z., Landgren, B.-M., Boukhris, R., Lo, L.,
Boccard, S., Machin, D., Pinol, A., Rowe, P.J., 1990a. Microdose intravaginal
levonorgestrel contraception: A multicentre clinical trial: I. Contraceptive efficacy
Koetsawang, S., Gao, J., Krishna, U., Cuadros, A., Dhali, G.I., Wyss, R., la Puenta,
J.R., Andrade, A.T.L., Khan, T., Kononova, E.S., Lawson, J.P., Parekh, U., Elstein,
M., Hingorani, V., Wang, N., Yao, Z., Landgren, B.-M., Boukhris, R., Lo, L.,
D'Arcangues, C., Boccard, S., Machin, D., Pinol, A., Rowe, P.J., 1990b. Microdose
intravaginal levonorgestrel contraception: A multicentre clinical trial: III. The
doi:10.1016/0010-7824(90)90143-J
Koetsawang, S., Ji, G., Krishna, U., Cuadros, A., Dhali, G.I., Wyss, R., Rodriguez la
levonorgestrel contraception: a multicentre clinical trial. IV. Bleeding patterns.
World Health Organization. Task Force on Long-Acting Systemic Agents for Fertility
ring releasing levonorgestrel at an initial rate of 27 micrograms/24 h when used
alone or in combination with transdermal systems releasing estradiol.
Contraception 50, 87–100.
pharmacodynamic effects of vaginal rings releasing levonorgestrel at a rate of 27
rate of drugs via eutectic mixtures: itraconazole – poloxamer188 system. Asian J.
Pharm. Sci. 1, 213–221.
Influence of silicone elastomer solubility and diffusivity on the in vitro release of
doi:10.1111/1471-0528.12852
Malcolm, R.K., Boyd, P.J., McCoy, C.F., Murphy, D.J., 2016. Microbicide vaginal rings:
doi:10.1016/j.addr.2016.01.015
Malcolm, R.K., Fetherston, S.M., 2013. Delivering on MPTs: addressing the needs,
rising to the challenges and making the opportunities. Contraception 88, 321–325.
doi:10.1016/j.contraception.2013.06.009
doi:10.2147/IJWH.S36282
Malcolm, R.K., Veazey, R.S., Geer, L., Lowry, D., Fetherston, S.M., Murphy, D.J., Boyd,
P., Major, I., Shattool, R.J., Klasse, P.J., Doyle, L.A., Rasmussen, K.K., Goldman,
CMPD167 and maraviroc from vaginal rings in rhesus macaques. Antimicrob.
term, controlled release of the HIV microbicide TMC120 from silicone elastomer
Mansour, D., 2012. The benefits and risks of using a levonorgestrel-releasing
intrauterine system for contraception. Contraception 85, 224–234.
doi:10.1016/j.contraception.2011.08.003
Mishell, D.R., Lumkin, M., Jackanicz, T., 1975. Initial clinical studies of intravaginal rings
containing norethindrone and norgestrel. Contraception 12, 253–260.
doi:10.1016/0010-7824(75)90086-4
Murphy, D.J., Amsssoms, K., Pille, G., Clarke, A., Harra, M.O., Roey, J. Van, Malcolm,
R.K., 2016a. Sustained release of the candidate antiretroviral peptides T-1249 and
JNJ54310516-AFP from a rod insert vaginal ring. doi:10.1007/s13346-015-0273-8
Murphy, D.J., Boyd, P., McCoy, C.F., Kumar, S., Holt, J.D.S., Blanda, W., Brimer, A.N.,
Malcolm, R.K., 2016b. Controlling levonorgestrel binding and release in a multi-
doi:10.1016/j.jconrel.2016.02.020
Murphy, D.J., Desjardins, D., Dereuddre-Bosquet, N., Brochard, P., Perrot, L., Pruvost,
A., Le Grand, R., Lagatie, O., Vanhooren, L., Feyaerts, M., van Roey, J., Malcolm,
R.K., 2014. Pre-clinical development of a combination microbicide vaginal ring
doi:10.1093/jac/dku160
Nel, A., Bekker, L., Bukusi, E., Hellstr, E., Kotze, P., Louw, C., Martinson, F., Masenga,
G., Montgomery, E., 2016a. Safety, Acceptability and Adherence of Dapivirine
Vaginal Ring in a Microbicide Clinical Trial Conducted in Multiple Countries in Sub-


doi:10.1007/s13669-014-0107-6
Woodsong, C., Holt, J.D.S., 2015. Acceptability and preferences for vaginal dosage
Rev. doi:10.1016/j.addr.2015.02.004
intravaginal ring for the controlled delivery of 17b-estradiol as its 3-acetate ester. J.
doi:10.1016/S0168-3659(03)00277-3
mucoadhesive system for vaginal delivery of the HIV microbicide, dapivirine:
optimisation by an artificial neural network. Int. J. Pharm. 388, 136–43.
studies of vaginal rings releasing low-dose levonorgestrel. Contraception 32, 455–
71.
FIGURE CAPTIONS

Figure 1. Chemical structures for dapivirine (A) and levonorgestrel (B).

Figure 2. Three stages of manufacture of a reservoir-type vaginal ring: (A) core; (B) half-overmolded core; (C) final fully overmolded ring device; (D) cross sectional view of ring. In these representative photos, both the sheath layer and core consist of blank silicone elastomer. However, a red dye has been incorporated into the silicone elastomer of the core for illustration purposes only. Note that the core (A) was cut prior to overmolding to compensate for shrinkage upon cooling.

Figure 3. A – Representative DSC traces showing thermal behaviour of DPV, LNG and their mixtures. The traces are presented in concentration order, with 100% LNG at the top of the figure and then each subsequent trace representing a 10% interval. In addition to a crystalline melt, DPV also shows a polymorphic transition ~100°C. B – Eutectic phase diagram for DPV and LNG constructed from crystalline melt data from A. C – Estimation of eutectic composition (dashed line) from heat of fusion vs LNG concentration plot.

Figure 4. Representative photographs of each ring formulation, presented according to DPV and LNG loading (images not to scale). Letters in the centre of each photograph denote the formulation code (Table 1). Rings D and E appear are semi-transparent due to their low drug loading.
Figure 5. Mean daily release versus time (A) and cumulative release versus root time (B) profiles for release of DPV from MED-4870 matrix-type vaginal rings containing DPV (100, 150 and 200 mg per ring), with or without LNG (0, 16 and 32 mg per ring), over 60 days. Error bars in graph A represent ± standard deviation of six replicates; error bars were often smaller than the plot symbols.

Figure 6. Mean daily release versus time (A) and cumulative release versus root time (B) profiles for release of LNG from MED-4870 matrix rings containing LNG (16 and 32 mg per ring), with or without DPV (0, 100, 150 and 200 mg per ring), over 60 days. Error bars in graph A represent ± standard deviation of six replicates; error bars were often smaller than the plot symbols.

Figure 7. Mean daily and cumulative release versus time profiles reservoir-type vaginal rings L and M containing DPV and LNG. Each data point in the daily release graphs represents the mean ± standard deviation of 6 replicates.
Table 1. Description of the various matrix-type vaginal ring formulations containing DPV and LNG.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Target DPV loading</th>
<th>Target LNG loading</th>
<th>Mean ring mass (g) (± SD; n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/ring</td>
<td>% w/w</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>100</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>150</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>200</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>–</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>–</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>100</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>100</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>150</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>150</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>200</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>200</td>
<td>2.50</td>
<td></td>
</tr>
</tbody>
</table>
**Table 2.** Description of core-type vaginal rings containing DPV and LNG. Values in brackets represent standard deviations (n=6).

<table>
<thead>
<tr>
<th>Ring Formulation L</th>
<th>Ring Formulation M</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ring type</strong></td>
<td>core-type (reservoir)</td>
</tr>
<tr>
<td><strong>Core</strong></td>
<td>single full-length core loaded with both DPV (2% w/w) and LNG (2% w/w)</td>
</tr>
<tr>
<td><strong>Sheath</strong></td>
<td>non-medicated DDU-4320 1.55 mm thick</td>
</tr>
</tbody>
</table>

**Representative image***

<table>
<thead>
<tr>
<th>Mean ring mass (g)</th>
<th>7.45 (± 0.02)</th>
<th>7.46 (± 0.02)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean core mass (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core 1</td>
<td>2.56 (± 0.01)</td>
<td>1.27 (± 0.02) (DAP)</td>
</tr>
<tr>
<td>Core 2</td>
<td>–</td>
<td>1.28 (± 0.01) (LNG)</td>
</tr>
<tr>
<td>Mean sheath mass (g)</td>
<td>4.89 (± 0.02)</td>
<td>4.91 (± 0.02)</td>
</tr>
<tr>
<td>Mean theoretical drug loading (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPV</td>
<td>51.2 (± 0.3)</td>
<td>25.5 (± 0.3)</td>
</tr>
<tr>
<td>LNG</td>
<td>51.2 (± 0.3)</td>
<td>25.6 (± 0.3)</td>
</tr>
</tbody>
</table>

* Note the visible gap between the two ends of the core in Ring Formulation L due to the cut made in the core prior to overmolding. For Ring Formulation M, the two separate half-length cores are clearly visible in this image; the white core is the DPV-loaded segment (white appearance due to the use of micronized DPV), while the more transparent core is the LNG-loaded segment (LNG was not micronized; small particles of LNG were clearly visible in the silicone elastomer, although these may not be evident from the image in the table.)
<table>
<thead>
<tr>
<th>DAP loading (mg)</th>
<th>LNG loading (mg)</th>
<th>0</th>
<th>16</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td></td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure(s)