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In vitro drug release, mechanical performance and stability testing of a custom silicone elastomer vaginal ring releasing dapivirine and levonorgestrel

Diarmaid J. Murphy [a], Yahya H. Dallal Bashi [a], Clare F. McCoy [a], Peter Boyd [a], Leeanne Brown [b], François Martin [b], Nicole McMullen [b], Kyle Kleinbeck [c], Bindi Dangi [c], Patrick Spence [c], Bashir Hansraj [c], Bríd Devlin [c], R. Karl Malcolm [a,*]

[a] *School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK*
[b] *Elkem Silicones, Two Tower Centre Boulevard, Suite 1802, East Brunswick, NJ 08816, USA*
[c] *International Partnership for Microbicides, Silver Spring, MD 2910, USA*

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ABSTRACT

We have previously reported a multipurpose silicone elastomer vaginal ring providing sustained release of dapivirine (an antiretroviral) and levonorgestrel (a progestin) for HIV prevention and hormonal contraception. During initial development, issues arose due to reaction between the ethynyl group in the levonorgestrel molecule and the hydride-functionalised polydimethylsiloxane components in the silicone elastomer formulation. This unwanted reaction occurred both during and to a lesser extent after ring manufacture, impacting the curing process, the mechanical properties of the ring, and the *in vitro* release of levonorgestrel. Recently, we reported custom silicone elastomer grades that minimise this reaction. In this follow-on study, we describe the manufacture, *in vitro* drug release, mechanical, and pharmaceutical stability testing of ring formulations prepared from a custom silicone elastomer and containing 200 mg dapivirine and 80, 160, 240 or 320 mg levonorgestrel. The rings showed mechanical properties similar to marketed ring products, sustained *in vitro* release of both drugs over 30 days in quantities deemed clinically relevant, offered acceptable assay values, and provided good product stability over 15 weeks at 40 °C and 75% relative humidity.

1. Introduction

A silicone elastomer vaginal ring containing 25 mg of the antiretroviral drug dapivirine (DPV) has been shown to be effective in reducing women's risk of HIV acquisition in two Phase III clinical trials (Baeten et al., 2016; Nel et al., 2016). Two subsequent open-label trials with the DPV ring reported higher levels of ring adherence as measured by lower levels of residual DPV in rings returned after use and increased estimated levels of HIV risk reduction, albeit tempered by the lack of a placebo group (Baeten et al., 2021; Nel et al., 2021). In July 2020, the DPV ring received a positive opinion from the European Medicines Agency under the Article 58 procedure, and in January 2021 a recommendation from the World Health Organization, paving the way for country approvals and introduction (EMA, 2020; World Health Organization, 2021).

The successful development and testing of a microbicide-releasing vaginal ring product for HIV prevention is the result of over 20 years effort (Malcolm et al., 2016). For the past ten years, the field has also been considering and developing next-generation products – referred to as multipurpose prevention technologies (MPTs) – which seek to address multiple sexual and reproductive health indications within a single product (Boyd et al., 2016; Malcolm et al., 2014). Given the historical use of vaginal rings for hormonal contraception (Barriga Pooley et al., 2020; Dezarnaulds and Fraser, 2002; Monteiro et al., 2018), an obvious option is to develop a MPT vaginal ring containing both an antiretroviral and a contraceptive progestogen (Boyd et al., 2016; Thurman et al., 2018). Levonorgestrel (LNG), a progestogen with a long history of clinical use in both long acting intrauterine devices as well as an emergency contraceptive (Buhling et al., 2014; Mansour, 2012; Shen et al., 2019), has long been considered a lead candidate for inclusion in such a combination product (Malcolm et al., 2014; Romano et al., 2013; Young Holt et al., 2018).

* Corresponding author at: School of Pharmacy, Medical Biology Centre, Queen's University Belfast, Belfast BT9 7BL, UK.
E-mail address: k.malcolm@qub.ac.uk (R.K. Malcolm).

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Table 1
Summary of the tests conducted on the DPV-LNG rings at each stability time point.

Test	Stability time point		
	0	*4 weeks*	*15 weeks*
Ring content	x	x	x
Shore M hardness	x	x	x
Twist during compression	x	x	x
5–20 mm compression	x	x	x
1000 cycle compression	x	–	x
28-day static compression	x	–	x
IVRT* acetate buffer + Kolliphor® HS 15	x	–	x
IVRT* IPA + water	x	–	x

* IVRT – *In vitro* release testing.

As part of efforts to develop a combination DPV-LNG vaginal ring, we reported an unwanted hydrosilylation reaction between LNG (and other steroids with non-aromatic unsaturated chemical functional groups) and platinum catalysed silicone elastomers which leads to irreversible binding of the steroid with the cured silicone elastomer (McCoy et al., 2018; Murphy et al., 2016). In addition to investigating the nature of this reaction, we also explored various strategies aimed at minimising its impact on development of a fixed-dose combination ring (Dallal Bashi et al., 2019; McCoy et al., 2018; Murphy et al., 2016). Importantly, LNG binding to the silicone elastomer not only reduced the LNG content available within the ring device for release, but also negatively impacted the mechanical properties of the cured elastomer by interfering with the usual curing reaction (McCoy et al., 2018; Murphy et al., 2016). This is an important issue as the mechanical properties of rings can impact clinical performance; poor mechanical performance of rings may impact user comfort, rates of unintended ring expulsion, and user adherence (Boyd et al., 2020; McCoy et al., 2019).

We have reported previously on efforts to minimise the extent of LNG binding and its impact on the ring product (Dallal Bashi et al., 2019). However, there has been only limited effort to explore use of different or modified silicone elastomers system to minimise the drug binding reaction. Condensation cured silicone systems – an obvious alternative to platinum-catalysed addition cured systems – make use of a organometallic tin-based catalyst which produces an alcohol by-product during the curing reaction (Malcolm et al., 2016). The alcohol produced can dissolve the solid drug dispersed within the ring, resulting in drug migration and deposition of the drug at the ring surface following evaporation, with substantial impact on the distribution of drug within the ring matrix (Nel et al., 2009).

The above considerations led to the development and investigation of a range of custom silicone elastomer formulations designed to offer improved mechanical properties and reduced LNG binding. We recently published details of the initial screening and ranking of these custom elastomers (Dallal Bashi et al., 2021). Several of the silicone formulations tested produced rings with mechanical properties similar to currently marketed rings while minimising LNG binding. In particular, one formulation (DEV SILB BIO LSR D1XX-TB Lot 07117–14) – having increased amounts of crosslinker and filler and a reduced polymer molecular weight – was considered most suitable for further investigation. Details of this custom silicone elastomer material are provided in Table 1 of that published article.

Here we examine the pharmaceutical stability, mechanical performance, and *in vitro* release profiles of a range of fixed-dose combination DPV-LNG rings produced using the lead silicone elastomer candidate identified previously (Dallal Bashi et al., 2021).

2. Materials and methods

2.1. Materials

Micronised DPV was supplied by Ajinomoto OmniChem n.v. (Wetteren, Belgium). Non-micronised LNG (nmLNG) was supplied by CHEMO Group (Industriale Chimica s.r.l., Saronno, Italy). A custom silicone addition-cure elastomer formulation – formally DEV SILB BIO LSR D1XX-TB Lot 07117–14, a modification of the commercially available Silbione™ LSR D135-QB – was supplied by Elkem (Elkem Silicones, NJ, USA). Potassium dihydrogen orthophosphate, sodium acetate and sodium hydroxide were purchased from VWR International Ltd. (Dublin, Ireland). HPLC-grade acetonitrile and acetone, phosphoric acid (85% *w/w* in water) and Kolliphor® HS 15 were 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 manufacture

Matrix-type vaginal rings containing 200 mg DPV and 80, 160, 240 or 320 mg nmLNG (hereafter referred to *via* the coding system X-Y, where X and Y are numbers designating the initial theoretical milligram loadings for DPV and LNG, respectively) were manufactured from the custom grade silicone elastomer on a Babyplast™ 6/10P injection molding machine (Chronoplast, Spain). The supplied silicone Parts A and B were stored at −20 °C for at least 30 min prior to use. The two active part premixes (Part A + DPV + LNG, and Part B + DPV + LNG) were prepared by mixing the required quantities of Part A or Part B, DPV and nmLNG in a 100 g plastic Speedmixer™ container. Briefly, the silicone part (A or B) was added to the container first and then the DPV, followed by mixing with a spatula for ≥30 s until the powder was fully wetted. The contents of the container were then mixed in a Speedmixer™ DAC 150 FVZ-K (Hauschild, Germany) at 3000 rpm for 30 s before being stored at −20 °C for at least 10 min. The nmLNG was then added and the same two-stage mixing protocol applied, except for a reduced time of 15 s in the Speedmixer™. The active premixes were stored at −20 °C for 10 min before further use.

Immediately prior to injection molding, ~50 g portions of Part A premix and Part B premix were sequentially added to a large plastic Speedmixer™ container (Max 300 long) until ~200 g in total had been transferred. The contents were then mixed by hand for approximately 10 s before mixing in a DAC 600 Speedmixer™ (Hauschild, Germany) at 1500 rpm for 30 s. This active mix was stored at −20 °C for 10 min, mixed with a spatula for 10–20 s to ensure no agglomerates, and then transferred to a Babyplast™ injection cartridge. The cartridge was transferred to a pre-chilled (−20 °C) cartridge holder and rings were manufactured at 100 °C for 95 s using predefined injection molding parameters.

2.3. Stability study protocol

A stability study investigating *in vitro* release and mechanical properties of rings stored at 40 °C / 75% relative humidity (RH) was conducted according to the schedule outlined in Table 1. Three rings were tested for content and *in vitro* release shortly after manufacture (T0) and after 15 weeks (T15). Six rings were used for mechanical testing at T0 and T15 with three rings used at 4 weeks (T4). Rings were initially weighed and examined for surface defects before either being used for testing or placed on storage – unpackaged, in individually labelled plastic weigh boats – in a Binder KBF115 stability chamber at 40 °C and 75% relative humidity (RH).

2.4. Mechanical testing

2.4.1. Shore M hardness test

Shore M hardness of rings was measured using a Checkline Europe® RX-DD-M Shore M durometer held in an OS-3 stand to conform with ASTM D-2240 (Type M scale) requirements. Pen markings on the ring surface were used to define test sites. Six measurements were performed per ring and mean values were calculated and reported.

2.4.2. Ring compression test to 5, 10, 15 and 20 mm

An aluminium plate with multiple rectangular grooves for ring placement was mounted on the lower fixed platform of a Shimadzu EZ Test Universal Tester. An upper Perspex® plate with identical grooves and mounted on the upper moveable arm of the tester (such that lower and upper grooves were aligned) was lowered until it touched the top of the rings; in this manner, the rings were held vertically in the test jig with slight pre-compression. Rings were cyclically compressed through 5, 10, 15 and 20 mm at a speed of 5 mm/s; each ring was compressed seven times at 5 mm and then six times at each of the other compression distance in a sequential manner. The forces required to compress the rings were recorded; the first 5 mm compression value was omitted from calculation of the mean values.

2.4.3. Twist during compression test

A custom twist-test jig was fitted to the EZ Test Universal Tester (Shimadzu, UK). Rings were mounted and compressed by lowering the tester cross-arm. The distance between the cross-arm and the ring holder was determined by extrapolation or interpolation using the mean external diameter for each ring formulation (in accordance with test specifications for diaphragm devices defined in ISO8009:2014). The degree of twist (angular rotation) was measured for rings during compression as indicated by movement of the pointer on the angular scale away from the starting (zero) position.

2.4.4. Static 28-day compression test

Each ring was placed in an individual chamber within a custom designed aluminium compression jig and secured with a Perspex cover. A threaded bolt was inserted into each chamber and the ring compressed to 25 ± 5% of its original outer diameter; if this was not possible due to the ring cross-sectional diameter, the ring was compressed until the ring sides touched. After 28 days, rings were removed from the compression jigs, allowed to recover for a period of 15–20 s, and the percentage recovery relative to the original ring diameter measured using a ring recovery gauge.

2.4.5. 1000-cycle compression test

This test was set up per the compression test described previously. Rings were cyclically compressed 1000 times from 100% to 25 ± 5% of their original outer diameter at a test speed of 15 mm/s. Where compression to 25% of the outer diameter could not be achieved, rings were compressed until the ring sides touched. Ring diameters were assessed using a ring gauge and expressed as a percentage recovery relative to the original outer ring diameter.

2.5. Drug content assay

Rings were weighed, sliced into sections (~ 2 mm thickness), and transferred into individually labelled 250 mL glass flasks. Acetone (200 mL) was added to each flask, the flasks sealed, and then placed in an Incu-Shake FL16–2 orbital shaking incubator (SciQuip, UK) at 37 °C and 60 rpm for 72 h. On removal from the incubator, flasks were allowed to cool to room temperature for ~60 min before sampling. A 1.0 mL aliquot of the acetone extraction solution was transferred to a 100 mL volumetric flask and diluted to near volume using a 1:1 mixture of acetonitrile + water. Samples were allowed to equilibrate at ambient temperature for at least 10 min before final dilution to volume with acetonitrile. Samples (1–2 mL) were transferred to labelled HPLC vials and analysed against standard solutions of known DPV and LNG concentrations.

2.6. In vitro release testing

Rings ($n = 3$ per formulation) were tested for *in vitro* release at T0 and T15 in two different release media: (i) 25 mM sodium acetate buffer pH 4.2 with 2% *w/v* Kolliphor® HS15 and (ii) 1:1 *v*/v isopropanol (IPA) + water medium. Both media have been used previously for release testing of rings (Boyd et al., 2016, 2019; Murphy et al., 2021; Murphy et al., 2016). On Day 0, each ring was placed into a 250 mL Duran bottle containing 100 mL of release media before being placed in a SciQuip Incu-Shake FL16–2 orbital shaking incubator (37 °C, 60 rpm, 25 mm orbital throw). The release medium was sampled (1–2 mL) and completely replaced (100 mL) after 3 h initially and then daily (except for weekends when 200 mL was added to maintain release rates). The amounts of DPV and LNG released were quantified by reverse-phase HPLC with UV detection.

Table 2
DPV and LNG content values for rings used in the stability study assessed at T0, T4 and T15 weeks.

Formulation (mg DPV-mg LNG)	Stability timepoint (weeks)	Mean DPV recovery (mg) ± SD	Mean DPV recovery (%)	Mean LNG recovery (mg) ± SD	Mean LNG recovery (%)
200–80	0	196.9 ± 1.3	98.4	75.4 ± 2.0	94.2
	4	204.0 ± 0.6	102.0	76.5 ± 0.7	95.7
	15	204.7 ± 0.7	102.3	75.4 ± 0.6	94.3
200–160	0	199.7 ± 1.5	99.8	154.7 ± 2.4	96.7
	4	202.7 ± 0.3	101.3	157.1 ± 1.7	98.2
	15	203.5 ± 1.5	101.7	159.6 ± 1.6	98.1
200–240	0	203.3 ± 2.1	101.7	240.2 ± 3.3	100.1
	4	204.8 ± 2.4	102.4	239.6 ± 4.0	99.9
	15	204.4 ± 2.6	102.2	237.9 ± 1.8	99.1
200–320	0	202.6 ± 0.8	101.3	318.4 ± 5.2	99.5
	4	200.5 ± 0.6	100.3	317.7 ± 1.1	99.3
	15	204.0 ± 1.2	102.0	320.8 ± 1.4	100.3

2.7. HPLC analysis

All *in vitro* release and content samples were analysed on a Waters HPLC system (Waters Limited, Elstree, UK) consisting of a 1525 Binary HPLC pump, a 717 Plus Autosampler and a 2487 Absorbance Detector. Samples (25 µL) were injected onto a Thermo Scientific BDS Hypersil™ C18 HPLC column (150 mm ⨯ 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 comprising 58% HPLC-grade acetonitrile and 42% pH 2.4 phosphate buffer (prepared using 7.7 mM pH 3.0 phosphate buffer modified by addition of 2.2 mL of 85% H_3PO_4 and 1 mL of 10 M NaOH), a flow rate of 1.2 mL/min and a run time of 5 min. DPV was detected at 210 nm after approximately 2.5 min and LNG at 240 nm after approximately 3.5 min (Dallal Bashi et al., 2019).

2.8. Statistical analyses

Where appropriate, data were statistically analysed using either a student *t*-test or a one-way analysis of variance (ANOVA) followed by *post hoc* analysis using the Tukey–Kramer multiple comparisons test. In all cases, a p value of ≤ 0.05 was deemed significant. Statistical analyses were performed using GraphPad Prism (Version 9.1.2).

Table 3
Mean Shore M hardness values for ring formulations at different stability time points ($T = 0$, 4 and 15 weeks).

Formulation (mg DPV-mg LNG)	Mean Shore M hardness ± SD ($n = 6$) at different stability time points	
	T0	*T15 weeks*
200–80	60.5 ± 0.1	62.6 ± 0.7
200–160	60.5 ± 0.4	62.3 ± 0.8
200–240	59.9 ± 0.7	62.9 ± 0.4
200–320	62.5 ± 0.5	63.1 ± 0.3

3. Results and discussion

3.1. Ring manufacture

Matrix-type rings containing 200 mg DPV and various nmLNG loadings (80, 160, 240 and 320 mg) were successfully manufactured on a Babyplast 6/10P. Upon inspection, rings appeared homogeneous and off-white in colour. The mean ring weight was 8.1 g and ring weight was not dependent upon total drug loading.

3.2. Drug content assay

Mean drug content assay values are presented in Table 2 for the various ring formulations; values are presented for the total amount recovered and the percentage amount recovered. Mean DPV content varied between 98.4 and 102.4% of the nominal ring loading, with no loss upon storage. Mean LNG content ranged between 94.2 and 100.3% of the nominal ring loading. Rings with lower LNG loadings (80 and 160 mg) showed significantly lower LNG recovery percentages (94–95% for the 80 mg rings and 96–98% for the 160 mg rings), reflecting the relatively greater impact of LNG binding on low drug loadings (Dallal Bashi et al., 2019; Murphy et al., 2016). At 240 and 320 mg LNG loadings, drug loss through binding is more difficult to detect, with recovery values measured between 99 and 100% for both 240 and 320 mg rings. Overall, no significant changes in LNG assay values were observed over fifteen weeks storage for any of the ring formulations.

3.3. Mechanical testing

Several mechanical tests were performed on ring formulations at each stability time point to monitor any changes in physical properties with time. A summary of the mean Shore M hardness values is presented in Table 3. As anticipated, initial post-manufacture hardness values for these silicone systems were ~ 60, exceeding the minimum hardness value target of 50 and exceeding the Estring® value of 54. The small increase in hardness values measured at 15 weeks is in line with expectation of some residual curing of the silicone occurring after initial manufacture. Significant differences in Shore hardness were noted for 200–320 T0 rings compared to rings having lower LNG loadings, while no significant differences were noted between any ring formulations at T15 weeks.

The mean forces required to compress the various ring formulations through distances of 5, 10, 15 and 20 mm at stability timepoints T0, T4 and T15 weeks are presented in Fig. 1 A–D. Significant increases in the measured mean force were observed with increasing compression distances across all formulations. In general, increases in LNG loading of 160 mg or more resulted in significant increases in mean compression

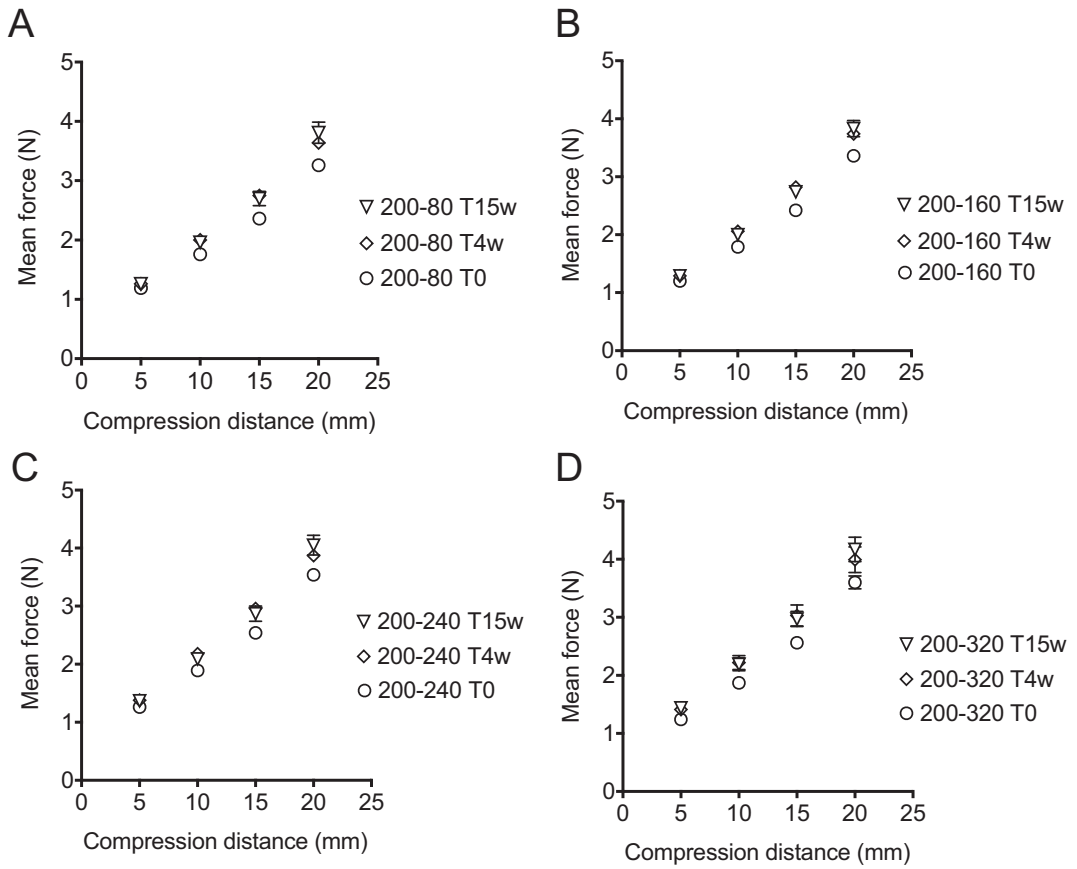

Fig. 1. Mean force required to compress the various DPV-LNG rings (A – 200-80; B – 200-160; C – 200-240; D – 200-320; the numbers refer to DPV-LNG loadings in milligrams) by 5, 10, 15 and 20 mm at each stability timepoint (T0, T4 and T15 weeks). Error bars, which are often smaller that the plot symbols, represent standard deviations.

Table 4
Mean angular rotation under compression values for rings at different stability timepoints ($T = 0$, 4 and 15 weeks).

Ring formulation (mg DPV-mg LNG)	Mean angular rotation (°) ± SD		
	T0	T4 weeks	T15 weeks
200–80	69.3 ± 0.8	68.0 ± 1.0	67.5 ± 1.0
200–160	69.8 ± 1.2	67.0 ± 1.0	67.3 ± 1.2
200–240	69.3 ± 1.0	68.7 ± 0.6	66.5 ± 2.3
200–320	68.8 ± 1.2	67.0 ± 1.0	66.8 ± 2.8

force, while smaller 80 mg increments did not. A small but significant increase – typically 5–16% – in the force required to compress the rings was measured between T0 and T4. At the 20 mm compression distance (but not the other distances) a further significant increase in the force required was measured between T4 and T15, suggesting that larger compression distances may provide a more sensitive measure of post-manufacturing curing. Post-manufacture increases in compression force are common with silicone elastomer drug delivery systems, since products are usually demolded as soon as possible (to minimise the potential for drug degradation or reaction) and certainly before complete cure is achieved. For addition-cure silicone elastomers, the characteristic hydrosilylation reaction continues to occur up to several weeks after removal from the mold.

The measured compression force at each distance also correlates with the total DPV + LNG loading in the ring devices (280, 360, 440 and 520 mg) (Supplementary Fig. S1). Most solid substances added to silicone elastomers – including both dedicated fillers and solid crystalline drug substances – will exert this reinforcing effect (Barman et al., 2020; Kopylov et al., 2011).

Data for the mean twist during compression test are presented in Table 4. Angular rotation values across all formulations at T0 were close to 69°. No significant differences in angular rotation were measured between formulations within a stability time point, nor across stability time points for any one formulation. All values fall within the range previously reported for marketed ring products (McCoy et al., 2019). All rings recovered to 90–100% of the original outer diameter after 28-day static compression and 1000 cycle compression (Supplementary Information, Tables S1 and S2), as reported previously for marketed rings (McCoy et al., 2019).

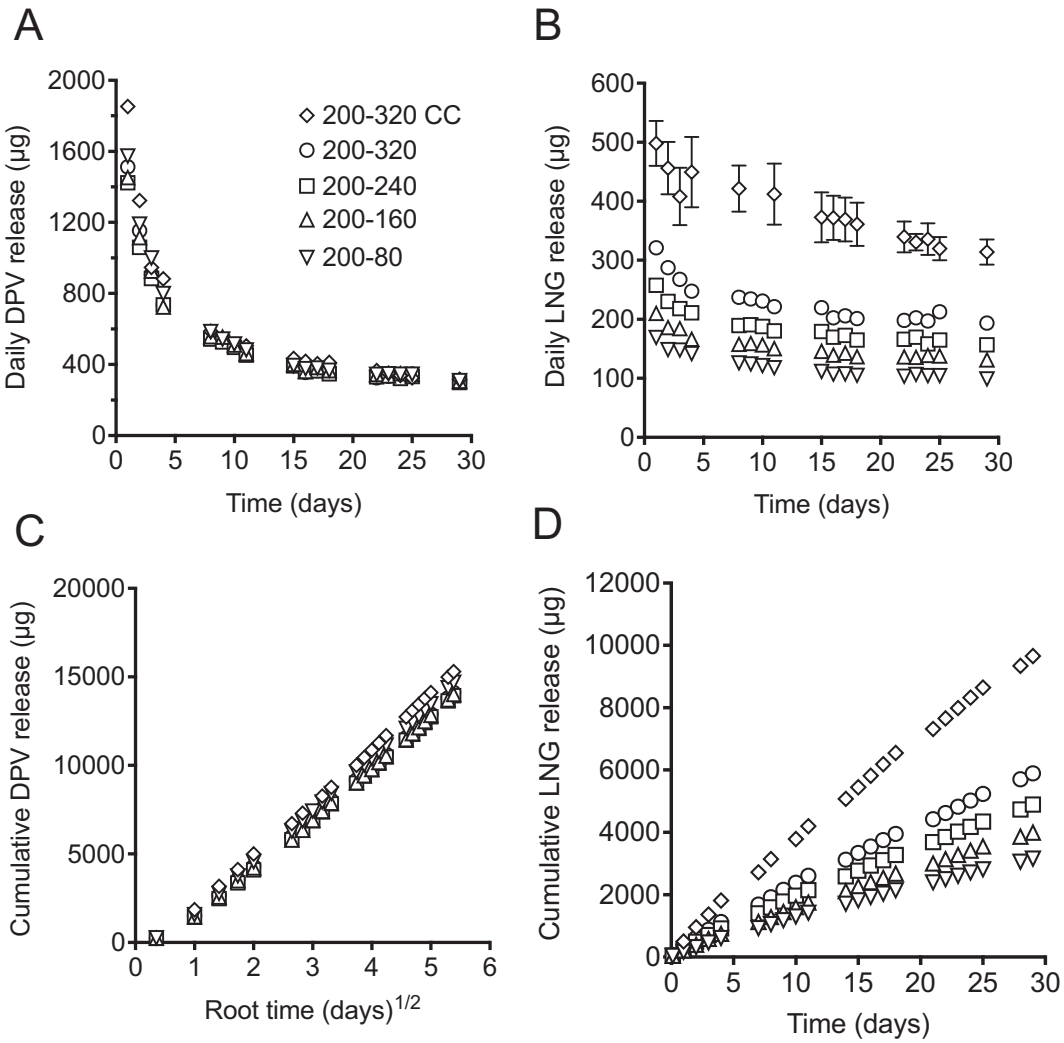

Fig. 2. *In vitro* release data over 29 days for DPV-LNG rings into 25 mM sodium acetate buffer pH 4.2 with 2% *w/v* Kolliphor® HS15 at the T0 stability timepoint. A – daily DPV release *vs* time; B – daily LNG release *vs* time; C – cumulative DPV release *vs* root time; D – cumulative LNG release *vs* time. The legend refers to DPV-LNG loadings in milligrams. Data showing release from a comparator 200–320 ring (CC) that uses a different silicone elastomer is also presented in each graph (open diamonds). The figure legend for A also applies to B, C and D. Error bars have been omitted to aid clarity – representative errors bars are included for one profile in panel B, and errors bars in panel A are always <5% of plotted values.

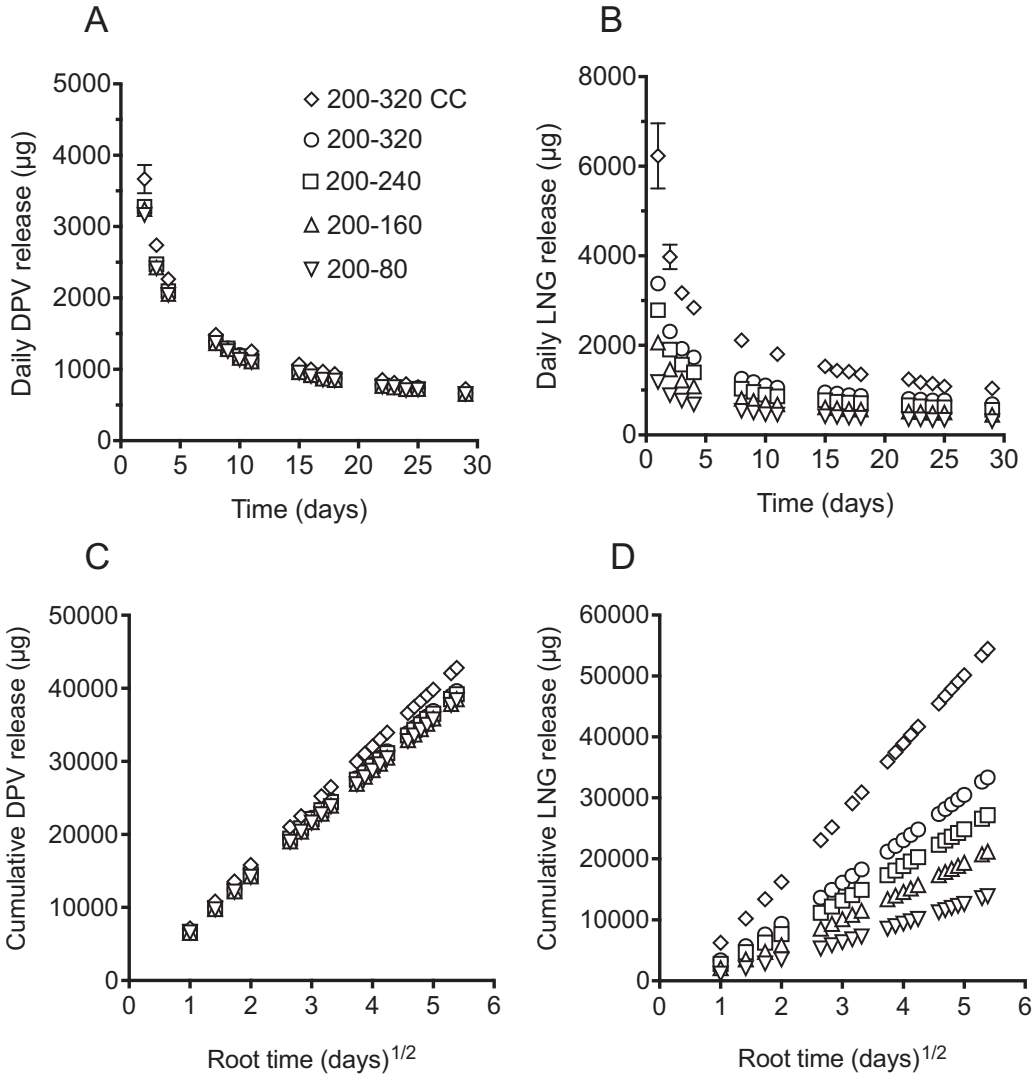

Fig. 3. Daily (A and B) and cumulative (C and D) release profiles for DPV (A and C) and LNG (B and D) release into 1:1 *v*/v IPA + water medium over 29 days at T0 with equivalent release of a 200–320 ring made using a comparator commercial silicone (CC). The figure legend for A also applies to B, C and D; the numbers in the legend refer to DPV-LNG loadings in milligrams. Error bars have been mostly omitted to aid clarity, although representative errors bars are included for one profile in each of A and B. All other error bars are <5% of plotted values and commonly smaller that the plot symbol.

3.4. In vitro release testing

In vitro release testing was conducted using two different release media – acetate buffer + Kolliphor® HS 15 and IPA + water – at stability timepoints T0 and T15. The acetate buffer + Kolliphor® HS 15 medium more closely reflects the total quantity of drugs released *in vivo* (where drug solubility is a limiting factor). However, for DPV-only rings, the IPA + water medium has been shown to be more discriminatory in some instances, although it overestimates the total quantity of DPV released *in vivo* (Boyd et al., 2019; Malcolm et al., 2016; Tietz and Klein, 2018; Tietz, 2019). Representative plots of daily and cumulative release *versus* time for DPV and LNG at T0 into acetate buffer + Kolliphor® HS 15 and IPA + water medium are presented in Figs. 2 and 3, respectively. In both figures, release from a 200–320 mg ring made with an alternative commercially available silicone system (DDU-4320; NuSil) is included for reference. Plots for T15 are presented in the Supplementary Information (Figs. S2 and S3). Summary data obtained from linear regression analysis of the cumulative release *vs* root time plots – including release rates (gradients), 95% confidence intervals and coefficients of determination – are presented in Tables 5 and 6 for release into acetate buffer + Kolliphor® HS 15 and IPA + water medium, respectively.

The release profiles for DPV into acetate buffer + Kolliphor® HS 15 (Fig. 2A and Supplementary Figs. S2A and S2C) are consistent with permeation-controlled drug release from a matrix-type ring (Boyd et al., 2016; Fetherston et al., 2013; Malcolm et al., 2003; Wang et al., 2018). The DPV cumulative release profiles for each formulation at T0 and T15 were similar with mean release rates in the range 2700–2900 µg/day$^{1/2}$. All confidence intervals overlapped (Table 5) independent of LNG loading indicating that increasing LNG loading had no impact on the release of DPV into this release medium. DPV release from the comparator silicone formulation was similar to that measured for the new silicones at the equivalent loading of both DPV and LNG. However, DPV release was higher from the 200320 comparator ring than the other elastomer formulations with lower LNG loadings.

The LNG daily release profiles (Fig. 2B and Supplementary Fig. S2B for T0 and T15, respectively) were relatively flat, resulting in linear cumulative release *versus* time profiles (Fig. 2D and S2D). This is often indicative of a solubility-controlled (pseudo zero order kinetics) drug release mechanism (Boyd et al., 2016; Malcolm et al., 2005, 2016; Matlin et al., 1992; Wang et al., 2018). However, cumulative release rates for LNG at both T0 and T15 also increased with initial LNG loading (no overlap of the 95% confidence intervals, Table 5). Some deviations

Table 5
Summary release parameters (mean release rate, 95% confidence intervals, and r^2 correlation coefficient) derived from the cumulative release profiles following DPV and LNG release from various DPV-LNG ring formulations (200–80, 200–160, 200–240, 200–320 for the custom silicone elastomer, and 200–320 for the commercial silicone elastomer) into pH 4.2 acetate buffer + Kolliphor® HS 15. Summary release parameters for DPV are derived from cumulative release *vs* root time plots (μg/day½), while summary release parameters for LNG are derived from cumulative release *vs* time plots (μg/day). Data for stability timepoints T0 and T15 weeks are presented.

T0 stability timepoint						T15 week stability timepoint				
	DPV-LNG loadings (mg)						DPV-LNG loadings (mg)			
	200–80	*200–160*	*200–240*	*200–320*	*200–320 CC*		*200–80*	*200–160*	*200–240*	*200–320*
DPV release										
Release rate	2929	2801	2800	2809	3053	*Release rate*	2873	2889	2834	2763
95% CI	2829 to 3029	2752 to 2849	2739 to 2861	2745 to 2872	3021 to 3085	*95% CI*	2834 to 2912	2838 to 2939	2781 to 2888	2688 to 2838
R^2	0.9818	0.9952	0.9924	0.9920	0.9967	R^2	0.9971	0.9952	0.9943	0.9883
LNG release										
Release rate	106.7	135.2	165.6	198.5	326.5	*Release rate*	110.2	153.0	170.6	189.1
95% CI	101 to 112	131 to 139	160 to 172	192 to 205	319 to 334	*95% CI*	108 to 112	149 to 157	165 to 176	185 to 193
R^2	0.9562	0.9850	0.9791	0.9836	0.9837	R^2	0.9932	0.9889	0.9853	0.9922

Table 6
Summary release parameters (mean release rate, 95% confidence intervals, and r^2 correlation coefficient) derived from the cumulative release *versus* root time profiles following DPV and LNG release from various DPV-LNG ring formulations (200–80, 200–160, 200–240, 200–320 for the custom silicone elastomer, and 200–320 for the commercial silicone elastomer) into 1:1 v/v IPA + water medium. Data for stability timepoints T0 and T15 weeks are presented.

T0 stability timepoint						T15 week stability timepoint				
	DPV-LNG loadings (mg)						DPV-LNG loadings (mg)			
	200–80	*200–160*	*200–240*	*200–320*	*200–320 CC*		*200–80*	*200–160*	*200–240*	*200–320*
DPV release										
Release rate	7242	7273	7410	7481	8087	*Release rate*	7451	7554	7594	7686
95% CI	7185 to 7298	7182 to 7364	7358 to 7461	7446 to 7516	8005 to 8168	*95% CI*	7367 to 7535	7451 to 7657	7511 to 7678	7666 to 7707
R^2	0.9990	0.9975	0.9992	0.9996	0.9969	R^2	0.9980	0.9970	0.9981	0.9999
LNG release										
Release rate	2852	4301	5489	6743	11,187	*Release rate*	3345	4820	5890	7131
95% CI	2790 to 2913	4185 to 4418	5399 to 5579	6638 to 6849	11,012 to 11,361	*95% CI*	3259 to 3431	4718 to 4922	5731 to 6049	6985 to 7277
R^2	0.9926	0.9883	0.9957	0.9961	0.9927	R^2	0.9895	0.9929	0.9885	0.9933

from linearity, particularly at T0, were noted ($R^2 < 0.99$). Also, for equivalent formulations, LNG release from rings manufactured from the comparator commercial silicone was approximately 65% greater over the 29-day study compared to the custom silicones. This difference for LNG is surprising given the similarity of the DPV release. Without specific knowledge of the proprietary components/ratios for each silicone, we are not able to propose a hypothesis to explain this difference.

There were no substantial changes in the cumulative release rates for DPV or LNG comparing between the same formulations at T0 and T15 (Table 5). One exception was the release of LNG from 200-160 rings which showed a significantly lower mean release rate at T0 compared to T15 (135 μg/day compared to 153 μg/day, respectively). The veracity and practical significance of this increase is unclear as similar effects were not seen at any other loading and there is no obvious rationale to explain this effect. Most likely, it is due to the small sample size in the experiment.

The daily release *vs.* time and linear cumulative release *vs.* root time profiles for DPV and LNG from matrix-type rings into IPA + water medium (Figs. 3 and S3) show classic permeation-controlled release behaviour under sink conditions (Boyd et al., 2019; Malcolm et al., 2016). There are substantial increases in the DPV and LNG release rates observed with this medium (~2.5-fold for DPV and > 10-fold for LNG) compared to the acetate buffer + Kolliphor® medium, reflecting its greater solvating power for these poorly water soluble actives. Also, unlike the acetate buffer + Kolliphor® release medium, DPV release into IPA + water medium increased with LNG loading at both T0 and T15, although these differences were not always significant (Table 6). The lack of consistency in results and the lack of significant differences at T4 are likely due to the small sample size ($n = 3$). A contributing factor may also be the increase in ring surface area generated as a result of the dissolution and release of the relatively large particles of LNG. However, given the very limited solubility of LNG in aqueous fluids, such an increase in available surface area is likely to be less relevant *in vivo*. LNG release into IPA + water medium was also dependent on the LNG loading. In line with expectations, the square of the release rates were approximately linear ($r^2 > 0.99$) with the drug loading per unit volume of ring (Supplementary Fig. S4) (Chien et al., 1974).

For any given formulation, significant increases in the release rates for both DPV and LNG into the IPA + water medium were noted between T0 and T15 (Table 6, no overlap of 95% confidence intervals). Most of these differences seem due to increased release quantities during first couple of days of release. The percentage increase in drug release rates in

IPA + water medium between T0 and T15 weeks were similar for DPV across all formulations but tended to decrease with increasing LNG loading. Changes in release rate between T0 and T15 observed in the IPA + water medium were not observed in the acetate buffer + Kolliphor® HS 15 system, probably due to the higher solubility afforded by the IPA + water medium making the test more sensitive to small changes occurring over time. Given that this change was not observed in the acetate buffer + Kolliphor® HS 15 system, its practical relevance *in vivo* is uncertain.

Small deviations from anticipated daily release values into IPA + water medium were observed in samples from day 24 on, in the T15 release profiles (SI Fig. 3A and B). These deviations are thought to be due to poorly fitting HPLC vial closures allowing evaporation of some release media before analysis. These unusual values did not strongly impact the cumulative release plots for either DPV or LNG.

Comparing DPV and LNG release into IPA + water medium between the new elastomer formulations and the commercial comparator IPA + water medium shows an increase in DPV release over the new elastomer formulations (not seen in acetate buffer + Kolliphor® HS 15). A significant increase in LNG release was also measured with rates again approximately 65% higher than the equivalent LNG loaded ring prepared with the new elastomer. The reductions in LNG release relative to a commercial comparator could be due to either the formulation changes made to improve the mechanical or LNG binding properties of the new elastomers or size variation in the non-micronised LNG particles used in each manufacture. The former could be mediated through increased cross-linker concentration and reduction in the polymer molecular weight leading to increased cross-link density, or increased filler concentrations leading to a reduction in drug release. Further work would be required to verify the cause of these differences between silicone excipients.

4. Conclusions

A custom silicone elastomer formulation with improved mechanical properties and relatively little LNG binding showed no untoward changes in mechanical properties, LNG binding, or drug release when stored at 40 °C, 75% RH for up to 15 weeks. Measured ring mechanical properties were similar to currently marketed vaginal ring products at all time points. Cumulative release profiles for LNG into acetate buffer + Kolliphor® HS 15 suggested an increase in the amount released with increasing drug loading, despite release being largely solubility-controlled. Whether this effect would be seen *in vivo*, where drug release is also likely to be largely solubility-controlled, is unknown. A reduction in total release of DPV and LNG was measured compared to a commercial comparator silicone. This may be due to formulation changes to the elastomer slowing release or other factors in ring manufacture.

Author contribution to manuscript

All authors contributed to the design of experiments and analysis of data. DJM, YDB and CFM conducted the experimental work. The manuscript was drafted by DJM and RKM, with input from all other authors. All authors approved submission of the manuscript.

Credit author statement

Diarmaid Murphy: Investigation; Methodology; Data analysis; Writing – Original draft, review and editing.

Yahya Dallal Bashi: Investigation; Methodology; Data analysis; Writing – Review and editing.

Clare McCoy: Investigation; Methodology; Data analysis; Writing – Review and editing.

Peter Boyd: Conceptualisation; Supervision; Methodology; Data analysis; Writing – Review and editing.

Leeanne Brown: Investigation; Writing – Review and editing.

François Martin: Investigation; Writing – Review and editing.

Nicole McMullen: Investigation; Writing – Review and editing.

Kyle Kleinbeck: Conceptualisation; Project administration; Methodology; Supervision Writing – Review and editing.

Bindi Dangi: Project administration; Supervision.

Patrick Spence: Conceptualisation; Project administration; Supervision Writing – Review and editing.

Bashir Hansraj: Conceptualisation; Project administration; Supervision Writing – Review and editing.

Brid Devlin: Conceptualisation; Project administration; Supervision Writing – Review and editing.

Karl Malcolm: Conceptualisation; Methodology; Supervision; Writing – Original draft, review and editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Prof. Karl Malcolm reports financial support was provided by Queen's University Belfast. Prof. Karl Malcolm has patent #US20190105192A1 pending to International Partnership for Microbicides. Prof. Karl Malcolm has patent #EP3209251A1 pending to International Partnership for Microbicides.

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Appendix A. Supplementary data

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