Controlling Levonorgestrel Binding and Release in a Multi-Purpose Prevention Technology Vaginal Ring Device

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ABSTRACT

Despite a long history of incorporating steroids into silicone elastomers for drug delivery applications, little is presently known about the propensity for irreversible drug binding in these systems. In this study, the ability of the contraceptive progestin levonorgestrel to bind chemically with hydrosilane groups in addition-cure silicone elastomers has been thoroughly investigated. Cure time, cure temperature, levonorgestrel particle size, initial levonorgestrel loading and silicone elastomer type were demonstrated to be key parameters impacting the extent of levonorgestrel binding, each through their influence on the solubility of levonorgestrel in the silicone elastomer. Understanding and overcoming this levonorgestrel binding phenomenon is critical for the ongoing development of a number of drug delivery products, including a multi-purpose technology vaginal ring device offering simultaneous release of levonorgestrel and dapivirine – a lead candidate antiretroviral microbicide – for combination HIV prevention and hormonal contraception.

1. Introduction

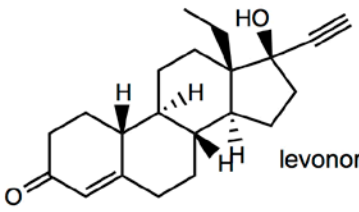
Silicone elastomers have been widely used in controlled release drug delivery applications since Dzuik and Cook first demonstrated in 1966 that various steroid molecules were capable of effectively permeating and releasing from silicone rubber capsules subcutaneously implanted in ewes [1]. Numerous steroid-releasing silicone elastomer devices, including subdermal implants, vaginal rings and intrauterine systems, have since reached market (Table 1). The past ten years have seen considerable interest in silicone elastomer vaginal ring technology for controlled release of antiretroviral (ARV) drug molecules for prevention of sexual transmission of human immunodeficiency virus (HIV) (Table 1) [2–9]. The International Partnership for Microbicides (IPM) and the Microbicide Trial Network (MTN) are currently in Phase III clinical trials in Africa with a matrix-type silicone elastomer vaginal ring developed by IPM. This ring device provides controlled release of dapivirine (Figure 1A), a non-nucleoside reverse transcriptase inhibitor, over 28 days and has already been shown to be safe and well tolerated *in vivo*. If successful, the dapivirine ring will likely provide both further impetus and a viable technology platform for development of multi-purpose prevention technologies (MPTs) aimed at combining HIV prevention with prevention of unintended pregnancy and prevention/treatment of other sexually transmitted infections (STIs) through use of a single formulation or drug-device combination product [10–13]. Many of the MPT products currently undergoing development have prioritised use of levonorgestrel (Figure 1A) as the contraceptive hormone component, based on its historical record of safety and efficacy [12–14].

Table 1. Controlled release drug delivery devices for humans that use silicone elastomer as the rate controlling material. Marketed products (current or previous), discontinued development products and products presently undergoing clinical testing are included. VR – vaginal rings

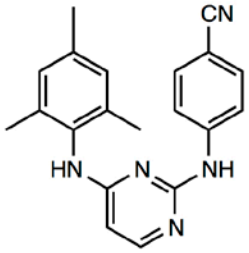
Product name	Product type	Drug(s)	Indication / duration of action	Developer	Stage
Norplant®	reservoir-type subdermal implant	levonorgestrel	female contraception / 5 years	Population Council	Discontinued
Jadelle® (Norplant II)	reservoir-type subdermal implant	levonorgestrel	female contraception / 5 years	Population Council	Marketed
Mirena®	reservoir-type intrauterine system	levonorgestrel	female contraception / 5 years	Bayer	Marketed
Skyla®	reservoir-type intrauterine system	levonorgestrel	female contraception / 5 years	Bayer	Marketed
Femring®	reservoir-type VR	17β-estradiol-3-acetate	estrogen replacement therapy / 3 months	Warner Chilcott	Marketed
Estring®	reservoir-type VR	17β-estradiol	estrogen replacement therapy / 90 days	Pfizer	Marketed
Progering®	matrix-type VR	progesterone	female contraception / 1 year	Population Council	Marketed (South America only)
Fertiring®	matrix-type VR	progesterone	in vitro fertilization / hormone replacement therapy / 3 months	Population Council	Marketed
–	matrix-type VR	progesterone	luteal phase support	Italfarmaco	Phase I/II
–	matrix-type VR	progesterone	luteal phase support	TEVA	Discontinued
–	reservoir-type VR	oxybutynin	overactive bladder	TEVA	Discontinued
–	reservoir-type VR	nestorone and ethinyl estradiol	female contraception	Population Council	Phase III
–	matrix-type VR	dapivirine	HIV prevention / 30 days	IPM	Phase III
–	matrix-type VR	dapivirine and maraviroc	HIV prevention / 30 days	IPM	Phase I
–	matrix-type VR	dapivirine and levonorgestrel	HIV prevention / 90 days	IPM	Phase I

Silicone elastomers for use in medical and pharmaceutical applications are prepared through the chemical crosslinking of functionalised, linear, polydimethylsiloxane molecules. The most important chemical crosslinking mechanisms involve either condensation-cure or addition-cure chemistries. Condensation-cure systems involve the tin-catalysed reaction between hydroxy-terminated polydimethylsiloxane molecules and a tetraalkoxysilane, resulting in the formation of the cured elastomer and an alcohol by-product [15,16]. Although the chemistry of this silicone elastomer crosslinking reaction is generally compatible with a very wide range of chemical functional groups, the alcohol produced can be problematic when the incorporated drug(s) is highly soluble in the alcohol [17,18]. Crosslinking of addition-cure silicone elastomer systems relies on the platinum-catalysed hydrosilylation reaction between hydride- and vinyl-functionalised polydimethylsiloxane molecules (Figure 1B). No by-product is formed with this reaction. However, the platinum catalyst is particularly sensitive to poisoning by certain chemical functional groups, most notably organotin, organosulfur and certain amine containing compounds.

A

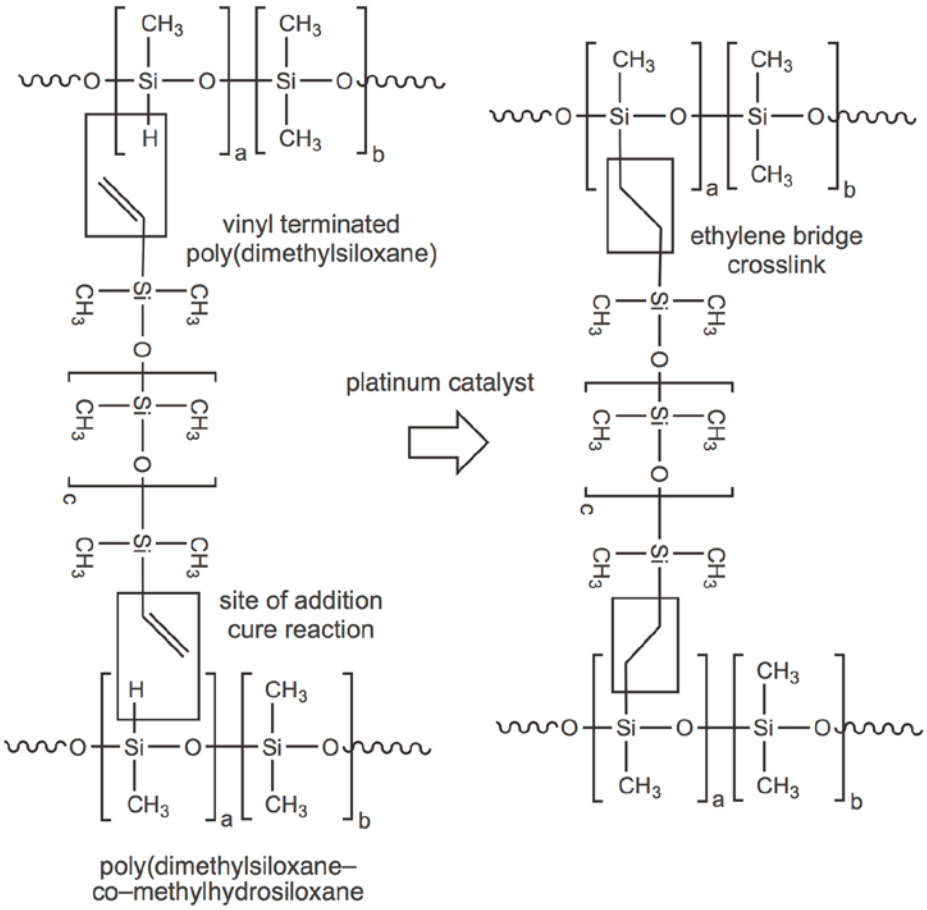


levonorgestrel

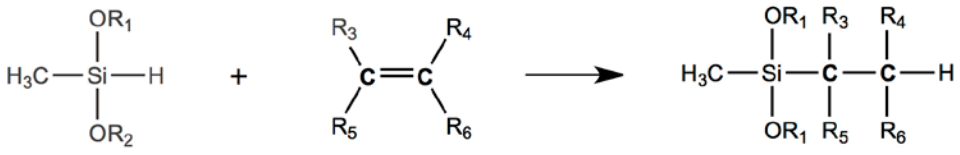


dapivirine

B



C



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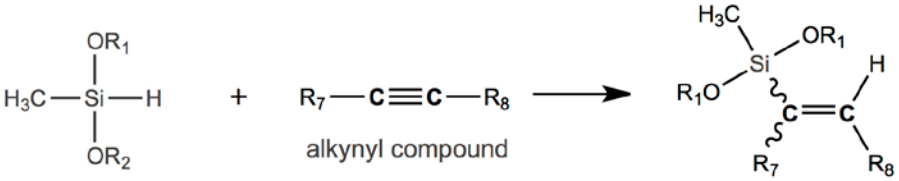

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Figure 1. A – Chemical structures of levonorgestrel and dapivirine. B – Addition-cure mechanism for silicone elastomers showing the platinum-catalysed hydrosilylation reaction between Si–H and vinyl groups. C – General hydrosilylation reaction between the Si–H groups in poly(methyl hydrogen silane) and alkenyl compounds. D – General hydrosilylation reaction between the Si–H groups in poly(methyl hydrogen silane) and alkynyl compounds.

It is well established that small molecules containing ethylenic (C=C) and acetylenic (C≡C) functional groups can undergo hydrosilylation reaction with molecules containing hydrosilane (Si–H) groups (Figures 1C and 1D, respectively) [19–23]. In general, the alkyne hydrosilylation reaction catalysed by platinum proceeds at a faster rate compared to alkenes, and is less susceptible to many electronic and structural factors that may impede alkene hydrosilylation [19]. Given the large number of steroid molecules containing ethylenic or acetylenic functional groups that have been previously formulated in silicone elastomers, it is rather surprising that only a single article (a 1980 US patent) has reported the potential for covalent binding of such steroids to the silicone elastomer [24]. Furthermore, the patent states that the quantity of drug that reacts with the silicone elastomer is “negligible for the sustained drug release rate”. On the contrary, here we report that levonorgestrel, a common contraceptive progestin, reacts with addition-cure silicone elastomer systems such that a very significant fraction of the incorporated levonorgestrel can be irreversibly bonded to the silicone elastomer impacting levonorgestrel release rates. The extent of binding is dependent on the silicone elastomer cure conditions and the particle size of the levonorgestrel material used. Aside from recent U.S. patent applications by IPM [25,26], this issue has not been reported previously for

90 levonorgestrel, despite its long history of incorporation into addition-cure silicone elastomer
91 drug delivery systems (Table 1).

2. Methods and Materials

2.1. Materials

Medical grade, addition-cure silicone elastomers DDU-4320 and MED-4870, condensation-cure silicone elastomer MED-6382, and MED-360 silicone oil were supplied by NuSil Silicone Technology Inc. (Carpinteria, CA, USA). Micronized dapivirine was supplied by S.A. Ajinomoto OmniChem N.V. (Wetteren, Belgium). Micronised levonorgestrel was supplied by CHEMO Group (Saronno, Italy). Non-micronized and sieved fractions of non-micronized levonorgestrel (non-micronised levonorgestrel) were supplied by Tecoland (Irvine, CA, US) and CHEMO Group (Saronno, Italy); except where explicitly stated, non-micronised levonorgestrel in the text refers to material sourced from Tecoland. Particle size data (d10, d50 and d90; measured via laser diffraction) for each of the levonorgestrel materials was provided by the suppliers (Table 2). HPLC-grade acetonitrile, isopropanol and dichloromethane, and phosphoric acid (85% w/w in water) were purchased from Sigma Aldrich (Gillingham, UK). HPLC-grade water was obtained using a Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK). 19-norethindrone was supplied by LGM Pharma (Boca Raton, Florida, USA) and used as an internal standard for HPLC. Analytical grade potassium dihydrogen orthophosphate was obtained from VWR (Dublin, Ireland).

Table 2. Influence of levonorgestrel particle size on its recovery from 0.4% w/w levonorgestrel-loaded DDU-4320 silicone elastomer slabs cured at 100 °C for 90 s. Each levonorgestrel recovery value is the mean of four replicates and reported errors denote standard deviations.

levonorgestrel batch	Particle size d90, d50, d10 (μm)	% levonorgestrel recovery
1 (micronized)	6.11, 2.18, 0.72	41.3 ± 6.5
2 (sieved fraction)	294, 81, 5	56.7 ± 5.2
3 (sieved fraction)	384, 170, 6	55.6 ± 2.8
4 (non-micronized) *	542, 348, 156	98.4 ± 11.4

* This non-micronized material was supplied by CHEMO.

2.2. Manufacture of silicone rings and slabs

Matrix-type, silicone elastomer vaginal rings containing 200 mg micronized dapivirine and 32 mg levonorgestrel (either micronized or non-micronized) and measuring 57 mm overall diameter x 7.8 mm cross-sectional diameter were manufactured by reaction injection molding of active silicone elastomer mixes using a Babyplast 6/10P injection-molding machine in semi-automatic mode fitted with custom, stainless steel, single-cavity injection molds. These rings were cured at 160˚C for 90 s. Briefly, the appropriate quantities of dapivirine and levonorgestrel powders were added to both Parts A and B of the addition-cure silicone elastomer system MED-4870 and mixed at 3000 rpm for 3 min using a SpeedMixer DAC 150 FVZ-K (Synergy Devices, UK). These active premixes were stored at 4˚C until use. Prior to combining the premixes, they were first hand-mixed with a spatula for 30 s and then speedmixed for 120 s at 3000 rpm. Equal weights of Part A and Part B active premixes were then combined, handmixed for 30 s, speedmixed for 30 s at 3000 rpm, and then transferred to a 65 cc low-density polyethlyene Semco 220316 cartridge system (Polymer Systems Technology Ltd., Buckinghamshire, UK) that

operates with the dosing meter fitted to the Babyplast injection molder.

Silicone elastomer slabs (20.0 × 30.0 × 2.0 mm) or vaginal rings containing levonorgestrel or dapivirine were also manufactured in a similar fashion using a custom, aluminium, multi-cavity mold fitted to a electrically-heated, laboratory-scale injection molding machine. For rings and slabs prepared using the DDU-4320 silicone elastomer (a low temperature cure system), cure time and temperature were varied between 1.5–120 min and 60–160˚C, respectively. For slabs prepared with the MED-4870 silicone elastomer (a high temperature cure system), cure time and temperature were varied between 1.5–120 min and 120–200˚C, respectively. Levonorgestrel-loaded silicone elastomer slabs were also prepared using condensation-cure MED-6382 silicone elastomer using cure conditions of 80°C / 5 min. For some silicone elastomer slabs, the drug powder was first dispersed in MED-360 silicone oil prior to preparing the silicone elastomer premixes.

2.3. Quantification of levonorgestrel by HPLC

Levonorgestrel concentrations were quantified by HPLC using a BDS Hypersil C18, 3 µm column (150 x 4.6 mm; Thermo Scientific, UK), fitted with an Analytical Guard Cartridge System (Phenomenex, UK), at 25˚C on a system comprising a Waters 1525 binary HPLC pump, 717 Plus autosampler, in-line degasser AF unit, and 2487 dual absorbance detector. Isocratic HPLC was performed with a mobile phase of 7.7 mM phosphate buffer, pH 3.0 and acetonitrile (55:45) at flow rate of 1.2 mL/min with detection at 240 nm. The retention times of norethindrone (internal standard) and levonorgestrel were 5.2 and 8.2 min, respectively. All chromatograms

were processed using the supplied Waters Breeze software.

2.4. In vitro release testing of rings

In vitro levonorgestrel release from vaginal rings (n=6) was assessed over a 15-day period. Each vaginal ring was placed into a glass flask containing 200 mL of isopropanol (IPA)/water mixture (1:1 volume ratio). This IPA/H_20 mixture has been widely used for *in vitro* release testing of silicone elastomer vaginal rings, since it offers greater solvating power for poorly water-soluble drugs compared with aqueous media, such as simulated vaginal fluid [2–5,17]. The volumes of media for release testing were selected based on previously measured solubility values in 1:1 IPA/water (LNG – 0.75 mg/mL at 25°C; DPV – 0.80 mg/mL at 37°C). The flasks were sealed and placed in an orbital shaking incubator (Infors HT AGCH-4103; 37°C, 60 rpm, throw 25 mm). After 24 h (± 15 min), each flask was removed from the incubator and a sample (2 mL) of the release medium was retained for HPLC analysis. The remaining release medium was discarded and replaced with fresh medium (100 mL IPA:H_2O). This sampling procedure was continued on a daily basis, except weekends when 200 mL of release medium were added to the flasks each Friday so as to maintain sink conditions through the following Monday.

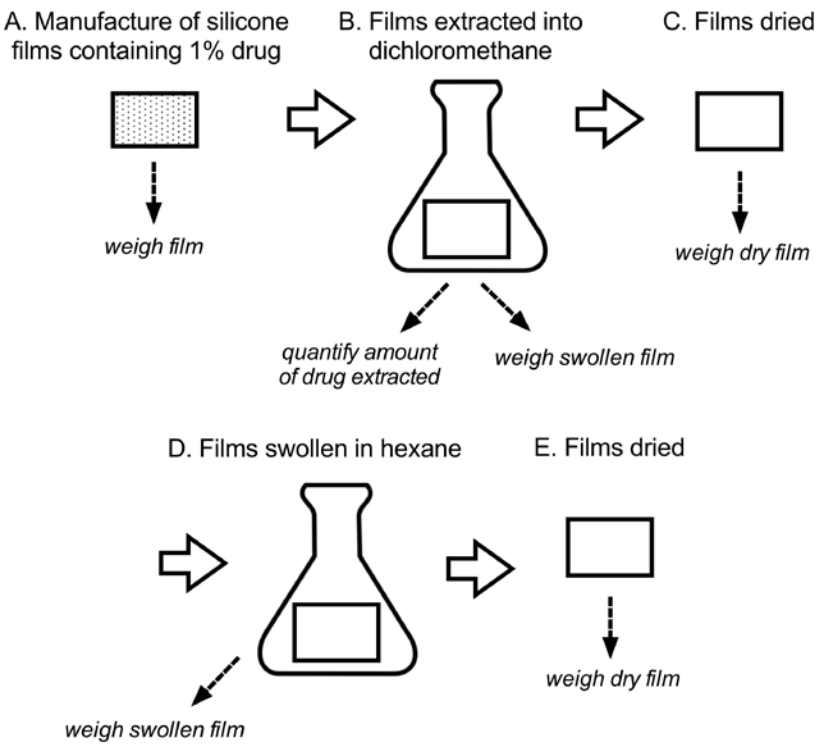
2.5. Levonorgestrel content analysis of manufactured rings and slabs

The total amount of levonorgestrel recoverable from silicone elastomer rings and slabs was determined using a solvent extraction method followed by quantification using HPLC. Each device was weighed, cut into approximately 2 mm sections, and placed in a 250 mL screw-top glass flask. 5 mL of a 2.5 mg/mL solution of 19-norethindrone in methanol (internal standard)

was added along with 95 mL of dichloromethane (extraction solvent). The flasks were placed in an orbital shaking incubator (37°C, 60 rpm, throw 25 mm) for 72 h. A 2 mL aliquot of the solution was evaporated to dryness and the residue reconstituted in 10 mL of methanol before being diluted ten-fold in a mixture of methanol and deionised water prior to analysis by HPLC. Control flasks containing known amounts of a levonorgestrel standard solution were also tested in each set of experiments.

2.6. Drug extraction and swelling studies

Cured silicone elastomer slabs containing 1% w/w loading of either dapivirine, micronised levonorgestrel or non-micronised levonorgestrel were manufactured, extracted with dichloromethane (100 mL), and then the resulting drug-depleted slabs swollen in n-hexane to assess cross-linking density [27]. A pictorial representation of the experimental method is shown in Figure 2. Briefly, drugs were weighed into the appropriate amounts of part A and part B silicone elastomer and speedmixed for 2 min. Part A and part B active mixes were then hand mixed (10 s), speedmixed (30 s), and then injection molded at 90 °C for 3 min. These slabs were individually weighed and the weights were recorded (W_0). These slabs were then placed into screw-top glass flasks containing 45 mL of dichloromethane. 5 mL of 500 µg/mL methanolic solution containing norethindrone was added as an internal standard. The flasks were placed in a shaking orbital shaker (37 °C, 60 rpm, throw 25 mm) and the release medium was sampled (~5 mL) after a period of 72 h. Quantification of drug concentrations in the samples was performed using HPLC-UV.



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194 **Figure 2.** Experimental protocol for hexane swelling experiments to assess crosslinking density
195 in drug-loaded silicone silicone silicone slabs.

196

197 Immediately after drug sampling, the swollen slabs were removed from the dichloromethane,
198 wiped with tissue paper to remove any excess solvent, placed in a tightly closed flasks and
199 weighed (W_1). These slabs were then dried overnight in a fume hood at room temperature,
200 followed by drying at 60°C for 1 h. The dry weights of the slabs were recorded (W_2). The dried
201 slabs were then placed in 50 mL of n-hexane. As before, the swollen slabs were weighed (W_3),
202 dried, and weighed again (W_4). The amount of drug extracted, swelling ratio and total mass
203 extracted from these silicone elastomer slabs in both solvents were calculated (Equations 1 –
204 4).

205 $$Swelling\ ratio\ in\ CH_2Cl_2 = \frac{(W_1 - W_2)}{W_2} \times 100 \qquad (1)$$

206 $$Mass\ extraction\ in\ CH_2Cl_2 = \frac{(W_0 - W_2)}{W_2} \times 100 \qquad (2)$$

207 $$Swelling\ ratio\ in\ n-hexane = \frac{(W_3 - W_4)}{W_4} \times 100 \qquad (3)$$

208 $$Swelling\ ratio\ in\ n-hexane = \frac{(W_2 - W_4)}{W_2} \times 100 \qquad (4)$$

209

210 ## *2.7. Oscillatory rheology*

211 Silicone elastomer samples containing 1, 5, 10, 15 and 20% w/w of dapivirine, micronised or
212 non-micronised levonorgestrel were prepared by adding appropriate quantities of each drug
213 powder to Parts A and B of silicone elastomer DDU-4320. Following mixing with a Speedmixer
214 for 30 s, a sample of each silicone elastomer active mix (1.0 g) was placed onto the lower
215 stationary plate of a TA instruments AR 1500 rotational rheometer, maintained at 25°C, and the
216 upper plate (40 mm cross-hatch plate) was lowered to produce a gap between the plates of
217 1000 µm. The time taken from loading of the sample to commencement of the experiment was
218 typically less than 30 s. Each sample was heated to 80^{o}C and maintained at that temperature
219 for 15 min at 10 Hz oscillation frequency and the storage modulus monitored over 900 s,
220 corresponding to the initial cure period for the silicone elastomer [28].

3. Results and Discussion

3.1. Particle size analysis

Representative digital microscopy images of the four different levonorgestrel materials used in this study (one micronized material, two non-micronized and sieved fractions, and one non-micronized and non-sieved material) are presented in Figure 3 and clearly illustrate visual differences in the particle size distributions. Quantitative measures of particle size (d10, d50 and d90 values) for each of the four materials are also presented in Table 2.

3.2. In vitro release testing of rings demonstrating bound levonorgestrel

The mean daily release vs. time and mean cumulative release vs. root time profiles for MED-4870 silicone elastomer matrix-type vaginal rings containing 200 mg micronized dapivirine and 32 mg of either micronised levonorgestrel or non-micronised levonorgestrel and cured at 160 °C for 90 s are presented in Figure 4. Dapivirine and non-micronised levonorgestrel are effectively released from the vaginal rings, with relatively high quantities of each drug released on Day 1 (~6000 µg and ~750 µg for dapivirine and levonorgestrel, respectively) followed by steadily declining release rates on subsequent days. Dapivirine release was very similar irrespective of the particle size of the levonorgestrel incorporated into the vaginal rings. The lower rate of release observed of non-micronised levonorgestrel from the vaginal rings compared with dapivirine is attributed to both its lower initial loading and its unique molecular permeability characteristics in the silicone elastomer. In general, the daily release profiles are typical of matrix-type vaginal rings containing solid drug dispersed within a non-degradable polymer matrix. The linear cumulative release vs. root time profiles (Figure 4C and 4D) confirm

243 root time ($t^{1/2}$) kinetics, and are indicative of a permeation-controlled mechanism [29,30].
244 However, surprisingly, vaginal rings containing micronised levonorgestrel showed no drug
245 release (Figures 4B and 4D). Repeat experiments confirmed that the correct quantity of
246 micronised levonorgestrel was added to the silicone elastomer premixes and that no
247 levonorgestrel release was observed (repeat data not shown). Based on scant evidence from
248 the literature [24], we hypothesized that all 32 mg micronised levonorgestrel initially
249 incorporated into the vaginal ring formulation had dissolved in the ring matrix and
250 subsequently covalently bonded to the silicone elastomer via a hydrosilylation reaction (Figure
251 1D) between the alkynyl (i.e. ethinyl) group in levonorgestrel (Figure 1A) and the Si–H groups in
252 the silicone elastomer system (Figure 1B). This drug-binding hypothesis was supported by
253 attempting to solvent-extract the levonorgestrel content from the vaginal rings; only 0.5%
254 levonorgestrel was recovered for vaginal rings containing micronised levonorgestrel compared
255 to 57.3% for non-micronised levonorgestrel vaginal rings. Dapivirine does not contain alkenyl or
256 alkynyl functional groups (Figure 1A), and therefore is incapable of undergoing hydrosilylation
257 reaction. 96.7% and 97.7% of incorporated dapivirine content was recovered from vaginal rings
258 containing 200 mg dapivirine + 32 mg non-micronised levonorgestrel and 200 mg dapivirine +
259 32 mg micronised levonorgestrel, respectively. In previous studies, complete recovery of
260 dapvirine was also achieved with rings containing just 25 mg dapvirine, further confirming that
261 dapvirine does not have the chemical functionality required to take part in the hydrosilylation
262 reaction [4,18].

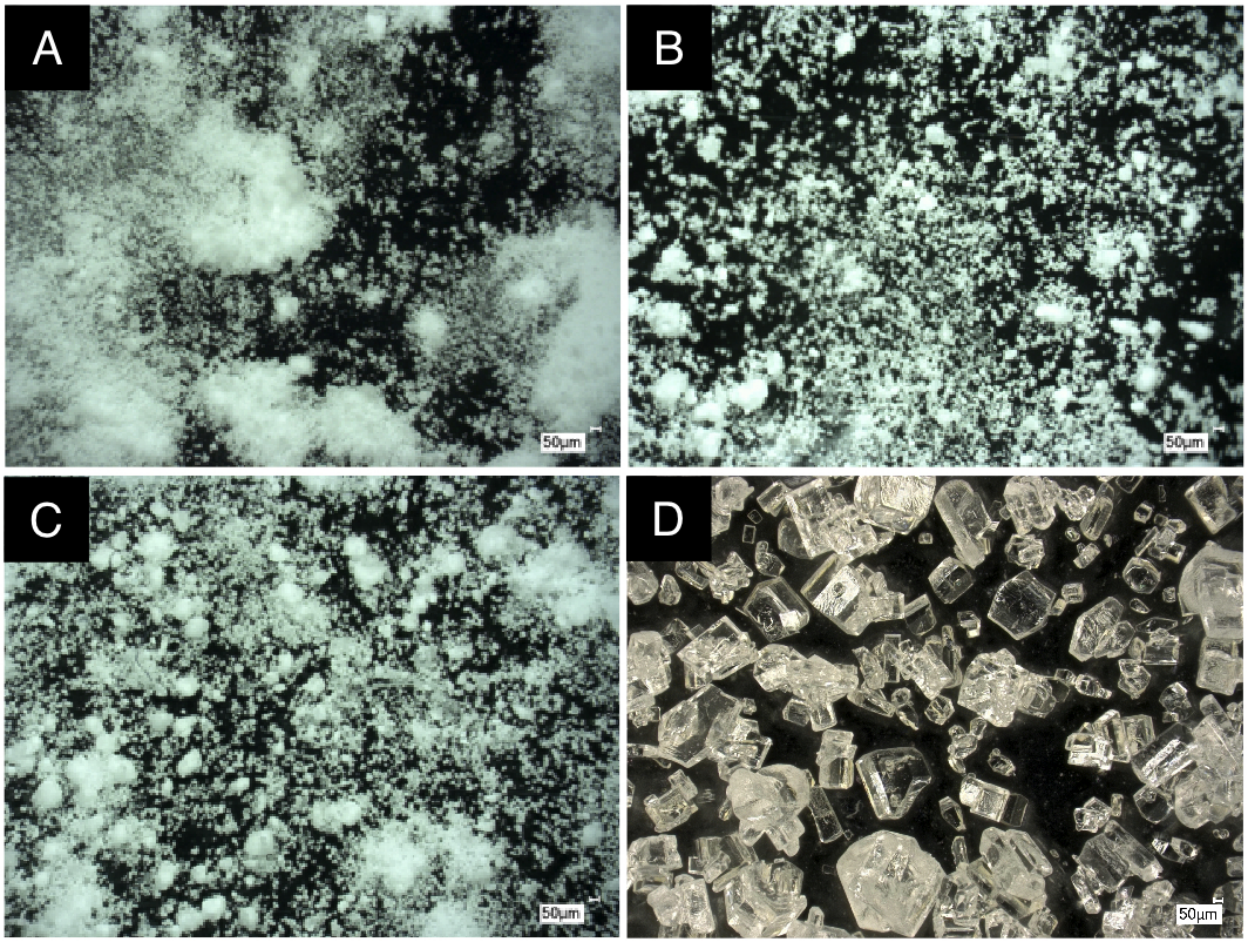


Figure 3. Digital microscopy images at x 100 magnification of different particle size batches of levonorgestrel. A – $d_{90} \leq 6.11$ µm, B – $d_{90} \leq 294$ µm, C – $d_{90} \leq 384$ µm and D – $d_{90} \leq 542$ µm. 50 µm size bars (⊢⊣) are presented bottom right in each photograph. Particle size values are also summarized in Table 2.

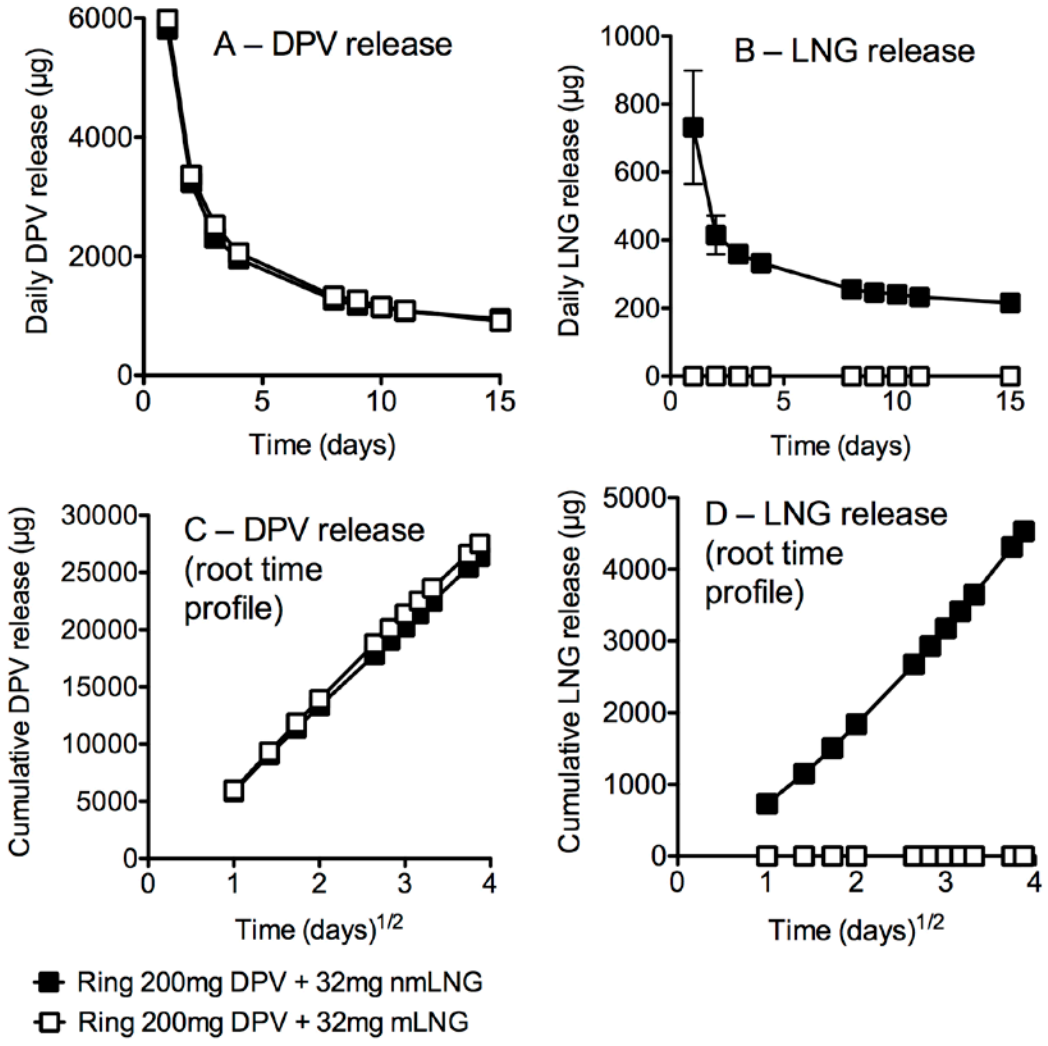
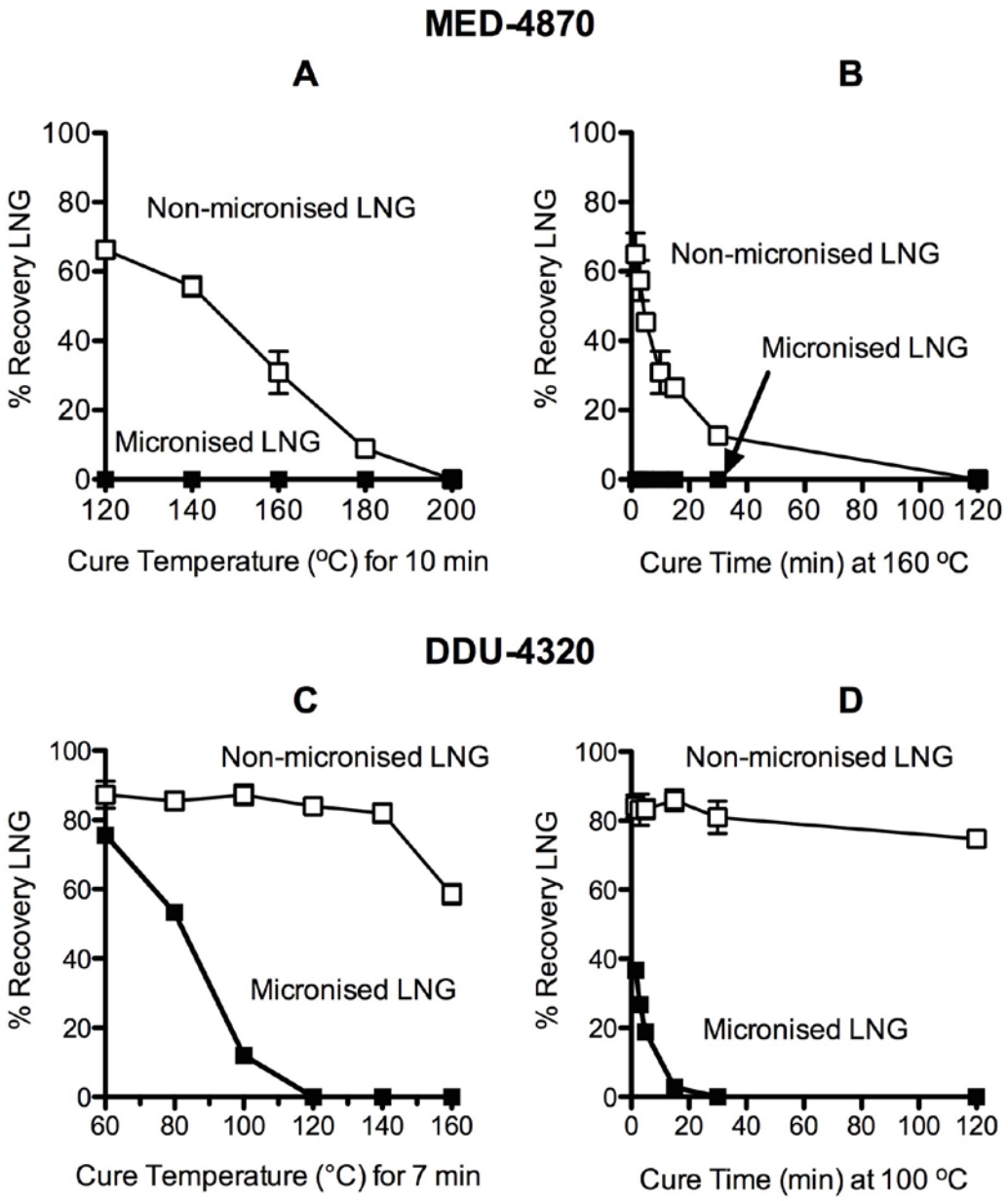


Figure 4. Mean daily release versus time and mean cumulative release versus root time profiles for matrix-type MED-4870 silicone elastomer vaginal rings containing 200 mg micronized dapivirine and 32 mg of either micronised levonorgestrel or non-micronised levonorgestrel (n=4). A – Daily dapivirine release vs. time plots. B – Daily levonorgestrel release vs time plots. C – Cumulative dapivirine release vs. root time plots. D – Cumulative levonorgestrel vs. root time plots. DPV – dapivirine. LNG – levonorgestrel. All rings were manufactured by injection molding at 160 °C for 90 s.

In order for effective drug release to occur from a silicone elastomer vaginal ring device, incorporated drug substances must permeate through the bulk of the silicone elastomer matrix. This permeation process can be considered as two discrete steps – dissolution of the drug in the silicone elastomer, followed by molecular diffusion of the solvated drug molecules through the silicone elastomer [30]. Both the degree of solubility and the rate of diffusion of drugs incorporated into silicone elastomers are temperature-dependent, and are expected to increase significantly at the high temperatures commonly used in injection molding manufacture of silicone elastomer vaginal rings. Solubility of the drug in the silicone elastomer matrix also increases the proportion of levonorgestrel molecules available for chemical binding via the hydrosilylation reaction. Thus, a rational explanation for the differences observed in release between micronised levonorgestrel and non-micronised levonorgestrel is that the micronized form of the drug exhibits faster rate of solubilization in the silicone elastomer at the 160 °C manufacturing temperature (levonorgestrel melting point is 240 °C), such that a significant proportion of the levonorgestrel molecules subsequently react with the hydrosilane groups within the silicone elastomer. By contrast, the observation that vaginal rings containing 32 mg non-micronised levonorgestrel exhibited significant levonorgestrel release was attributed to the reduced rate of dissolution associated with the larger levonorgestrel particles and, in turn, reduced levonorgestrel binding to the silicone elastomer. This observation concurs with the well-established principle of increased surface area leading to increased rate of dissolution, as expressed in the Noyes-Whitney equation [31].

3.3. Levonorgestrel binding studies

To explore this levonorgestrel-binding hypothesis further, additional solvent extraction experiments were conducted using levonorgestrel-loaded silicone elastomer slabs manufactured under different cure conditions. No levonorgestrel could be recovered by solvent extraction from MED-4870 silicone elastomer slabs containing 0.4% w/w micronised levonorgestrel manufactured at (i) various cure temperatures (120–200 °C) and a fixed cure time (10 min) (Figure 5A) and (ii) various cure times (1.5–120 min) and a fixed cure temperature (160 °C) (Figure 5B). By contrast, MED-4870 silicone elastomer slabs containing 0.4% w/w non-micronised levonorgestrel produced measurable recovery of levonorgestrel by solvent extraction at all but the most extreme cure conditions (i.e. 200 °C at 10 min and 120 min at 160 °C; Figures 6A and 6B, respectively). In fact, the non-micronised levonorgestrel MED-4870 slabs showed a clear trend of decreasing levonorgestrel recovery values as a function of both increasing cure temperature (Figure 5A) and increasing cure time (Figure 5B). These data and trends strongly support the hypothesis that levonorgestrel binding to the silicone elastomer system is dependent on its solubilization in the elastomer.



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314 **Figure 5.** Influence of cure temperature and cure time on percentage recovery of micronized
315 and non-micronised levonorgestrel from MED-4870 and DDU-4320 silicone elastomer slabs.
316 Levonorgestrel loading for all samples was 0.4% w/w (equivalent to 32 mg in a human-sized
317 vaginal ring device). Each data point represents the mean ± SD of 4 replicates. Error bars are
318 often smaller than the plot symbols.

319

For the MED-4870 system, the highest recovery of levonorgestrel (66.2%) was measured for the non-micronised levonorgestrel slabs prepared at the 120 °C / 10 min cure condition (Figure 5A). This value is significantly below the range of acceptable assay values (85–115%) commonly specified for a Phase 1 product. Although higher values for levonorgestrel recovery could be achieved for the MED-4870 system by curing at lower temperatures, this would lead to significantly increased process cycle times such that scaled manufacture would be impractical. For example, in additional experiments, we demonstrated that MED-4870 silicone elastomer containing 0.4% w/w non-micronised levonorgestrel can be cured at 80 °C for 2.5 h to give a levonorgestrel recovery value of 86.2%. However, injection molding cycle times less than 2 min are preferable. Reducing cure time is also not an option, since 90 s at 160 °C was the minimum cure condition required to produce a ring or slab device having sufficient mechanical properties for demolding.

An alternative medical grade addition-cure silicone elastomer system, DDU-4320, was selected for testing based on its recommended low cure temperature characteristics. For silicone elastomer slabs containing micronised levonorgestrel and prepared at 60 °C for 7 min, levonorgestrel recovery was 75.6%, with values decreasing as cure temperature was increased (Figure 5C). However, no levonorgestrel recovery was obtained for cure temperatures 120 °C and above (Figure 5C). For the non-micronised levonorgestrel DDU-4320 samples, levonorgestrel recovery values were significantly higher (87.3% at 60 °C declining to 58.5% at 160 °C; Figure 5C) compared to those for micronised levonorgestrel, mimicking the trend observed previously with the high temperature cure MED-4870 system. Selecting an

intermediate cure temperature of 100 °C for this DDU-4320 system, the effect of varying cure time has a greater influence on the micronised levonorgestrel compared with the non-micronized material (Figure 5D). Importantly, the DDU-4320 slabs containing non-micronised levonorgestrel offered levonorgestrel recovery values close to the specified range for content assay / uniformity (90–110% label claim).

For comparison, incorporation of 0.4% w/w micronised levonorgestrel and non-micronised levonorgestrel into MED-6382 silicone elastomer (a medical grade tin-catalysed condensation cure system) produced levonorgestrel recovery values of 98.9 and 100.8%, respectively. However, this condensation-cure silicone elastomer produces isopropanol as a by-product of the curing reaction. Both levonorgestrel and dapivirine are highly soluble in isopropanol, and therefore these condensation cure silicone elastomers are not preferred in order to avoid drug migration to the ring surface as isopropanol migrates to the vaginal ring surface and evaporates [17].

Initially, the influence of levonorgestrel particle size on the extent of binding was tested using a single batch of micronised levonorgestrel and non-micronised levonorgestrel (Batches 1 and 4, respectively; Table 2; Figure 3; supplied by CHEMO). Two additional levonorgestrel materials (Batches 2 and 3) were sieved at source so that particles intermediate in size could be tested (Table 2). Silicone elastomer DDU-4320 slabs containing 0.4% w/w levonorgestrel were prepared at 100 ˚C for 90 s for each levonorgestrel batch. As expected, the levonorgestrel recovery values for these sieved levonorgestrel materials (56.7% and 55.6%; Table 2) lie

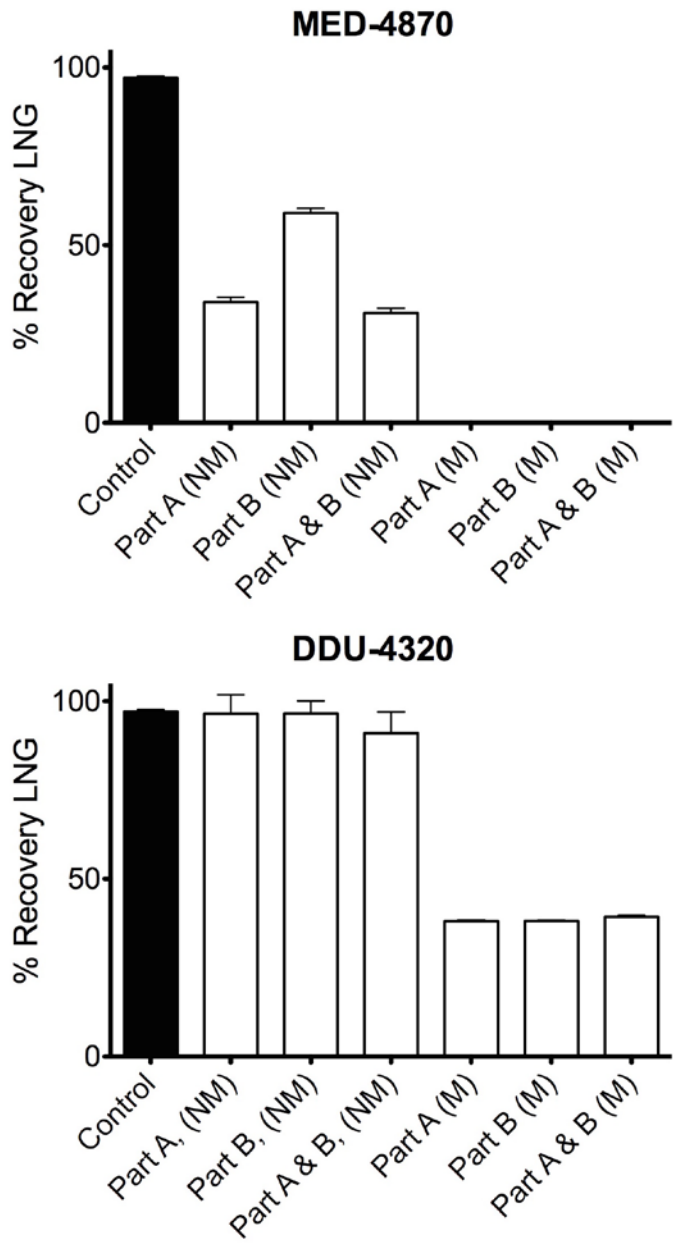
364 between the values for the previously tested non-micronised levonorgestrel and micronised
365 levonorgestrel (98.4% and 41.3%, respectively; Table 2), further supporting the solubilisation
366 hypothesis. The similarity in levonorgestrel recovery values for Batches 2 and 3 is most likely
367 due to the equivalence in d10 values (despite the very different d90 and d50 values), since the
368 fines are expected to contribute disproportionately to the overall surface area of the material.
369
370 The percentage recovery of levonorgestrel from DDU-4320 silicone elastomer vaginal rings
371 cured at 90˚C for 3 min increased (55.0 – 84.9%) as the loading amount of micronised
372 levonorgestrel incorporated into the device was increased from 25 to 100 and 400 mg (Table 3).
373 This trend is consistent with the proposed solubility hypothesis, since progressively smaller
374 fractions of the total levonorgestrel loading are expected to dissolve and bind to the silicone
375 elastomer as the levonorgestrel loading is increased. For the vaginal rings containing non-
376 micronised levonorgestrel, recoveries were close to 100% irrespective of levonorgestrel loading
377 (Table 3). These recovery values are also slightly higher than those predicted based on the
378 previous slab data (Figure 5). This is likely attributed to a temperature-insulating effect for drug
379 particles located in the bulk of the vaginal rings, attributed to the larger volume and/or the
380 smaller surface area-to-volume ratio associated with the vaginal ring devices compared with
381 slabs. This insulation effect is further illustrated by the data presented in Supplementary
382 Information in which reported non-micronised levonorgestrel recovery values were lower (but
383 not significantly) for slabs compared with vaginal rings, and where both are manufactured
384 under the same cure conditions.
385

Table 3. Percentage recovery levonorgestrel from matrix-type DDU-4320 silicone elastomer vaginal rings cured at 90 ˚C for 3 min and containing different amounts of micronized and non-micronised levonorgestrel. Each levonorgestrel recovery value is the mean of four replicates and reported errors denote standard deviations.

Levonorgestrel loading (mg, *% w/w*)	% Levonorgestrel Recovery	
	Non-micronized	Micronized
25, *0.3125*	99.5 ± 2.3	55.0 ± 5.8
100, *1.25*	96.1 ± 6.9	71.1 ± 1.3
400, *5.00*	100.6 ± 8.0	84.9 ± 4.8

MED-4870 and DDU-4320 addition-cure silicone elastomer systems are supplied as two-part kits. Both parts contain the silicone elastomer base material, which comprises various vinyl-functionalised (Figure 1B) and hydroxy-terminated polydimethylsiloxane molecules. Part A also includes the platinum catalyst, while Part B also includes the poly(methylhydrosiloxane) component that ultimately reacts with the vinyl-functionalised polydimethylsiloxane molecules (Figure 1B). (Both parts also contain other components that are not pertinent to this discussion.) Therefore, it is conceivable that addition of levonorgestrel to only one of the parts might impact its propensity to bind when the two parts are subsequently mixed and cured. Percentage levonorgestrel recovery values for slabs made with either MED-4870 or DDU-4320 and in which micronised levonorgestrel or non-micronised levonorgestrel is added to Part A only, Part B only or to both Parts are reported in Figure 6. For DDU-4320 slabs, the addition of levonorgestrel to one, other or both parts of the silicone elastomer system had no impact on

levonorgestrel recovery, although, as expected, differences in levonorgestrel recovery were observed for the micronized versus non-micronized materials (Figure 6B). However, addition of non-micronised levonorgestrel to Part B only of MED-4870 resulted in increased levonorgestrel recovery (57%) compared to adding it to Part A only (33%) or to both parts (30%) (Figure 6A). As previously observed, levonorgestrel recovery values were significantly higher for DDU-4320 compared to MED-4870, and for non-micronised levonorgestrel compared to micronised levonorgestrel.



411

412 **Figure 6.** Influence of order of addition of levonorgestrel upon levonorgestrel recovery from

413 MED-4870 and DDU-4320 silicone elastomer slabs (n=4). MED-4870 slabs were cured at 160 ˚C

414 for 90 s. DDU-4320 slabs were cured at 100 ˚C for 90 s. Part A – levonorgestrel added only to

415 Part A of the silicone elastomer prior to curing; Part B – levonorgestrel added only to Part B of

416 the silicone elastomer only prior to curing; Part A & B – equal amounts of levonorgestrel added

417 to both parts prior to curing; NM – non-micronised levonorgestrel, M – micronised
418 levonorgestrel.

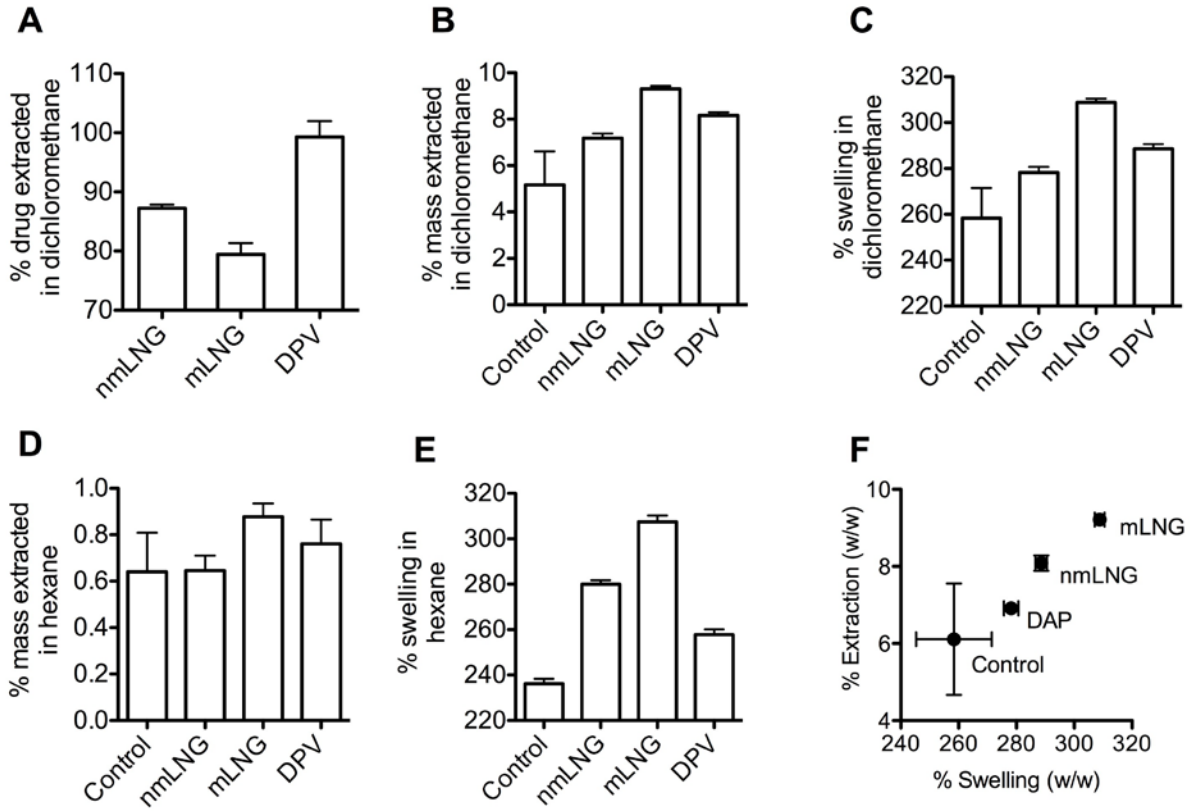
419

420 Silicone oils are sometimes used as dispersing agents for addition of drug powders to silicone
421 elastomer systems. The impact of dispersing levonorgestrel in MED-360 silicone oil prior to
422 manufacture of DDU-4320 silicone elastomer slabs was investigated for selected cure
423 conditions. The results, presented in Supplementary Information, show that levonorgestrel
424 recovery was typically 4–10% higher with use of the silicone oil. However, at the highest cure
425 temperature tested (160 ˚C), no significant difference in levonorgestrel recovery was observed
426 for either non-micronised or micronised levonorgestrel.

427

428 In an additional set of experiments, silicone elastomer DDU-4320 slabs containing 1% w/w
429 dapivirine, non-micronised levonorgestrel or micronised levonorgestrel and prepared via
430 injection molding at 90 °C for 3 min were subjected to drug extraction via dichloromethane to
431 measure drug recovery followed by swelling in hexane to determine the silicone elastomer
432 crosslinking density. Similar to previous results, percentage drug recovery decreased in the
433 order dapivirine (99.3%) > non-micronised levonorgestrel (87.6%) > micronised levonorgestrel
434 (79.4%), reflecting the extent of reaction of each drug with the hydrosilane groups in the
435 silicone elastomer system under the conditions of cure (Figure 7A). Following drug extraction,
436 the slabs were dried and reweighed; the values for total percentage mass extracted are
437 presented in Figure 7B. The ~5% mass loss observed for the control sample (containing no drug)
438 was attributed to the extraction of non-reactive silicone oil components in the silicone

elastomer formulation. The same mass loss due to extraction of these oils was presumably also observed in each the drug-loaded samples, with any additional mass loss due to extraction of either non-bound drug or unreacted polydimethylsiloxane components. Interestingly, the highest extraction mass was observed for the micronised levonorgestrel slab, which is counter-intuitive if only the propensity for the micronised levonorgestrel crystalline material to rapidly solubilize and undergo hydrosilylation reaction within the silicone elastomer is considered. However, these levonorgestrel molecules compete with the vinyl-functionalised polydimethylsiloxane molecules in the silicone elastomer system for reaction with the hydride-functionalised polydimethylsiloxane molecules. In this way, the overall crosslink density is reduced in the silicone elastomer and there is increased opportunity for extraction of non-reactive polydimethylsiloxane molecules. This effectively explains the trends in percentage mass extraction values (Figure 7B). An entirely similar trend is also observed for the percentage swelling values (in dichloromethane; Figure 7C), adding further support to the different impact of the various drug molecules on the silicone elastomer crosslinking density. Conventionally, crosslinking densities in silicone elastomer systems are measured by swelling samples in hexane rather than dichloromethane. Subsequent placement of the silicone elastomer slabs into hexane resulted in minimal additional mass loss (Figure 7D), and confirmed the previous trend in crosslinking density obtained with dichloromethane (compare Figures 8C and 7E). The plot of mean percentage mass extraction verses mean percentage swelling for the various silicone elastomer slab formulations showed a linear positive correlation ($r^2 = 0.972$) (Figure 7F).



460

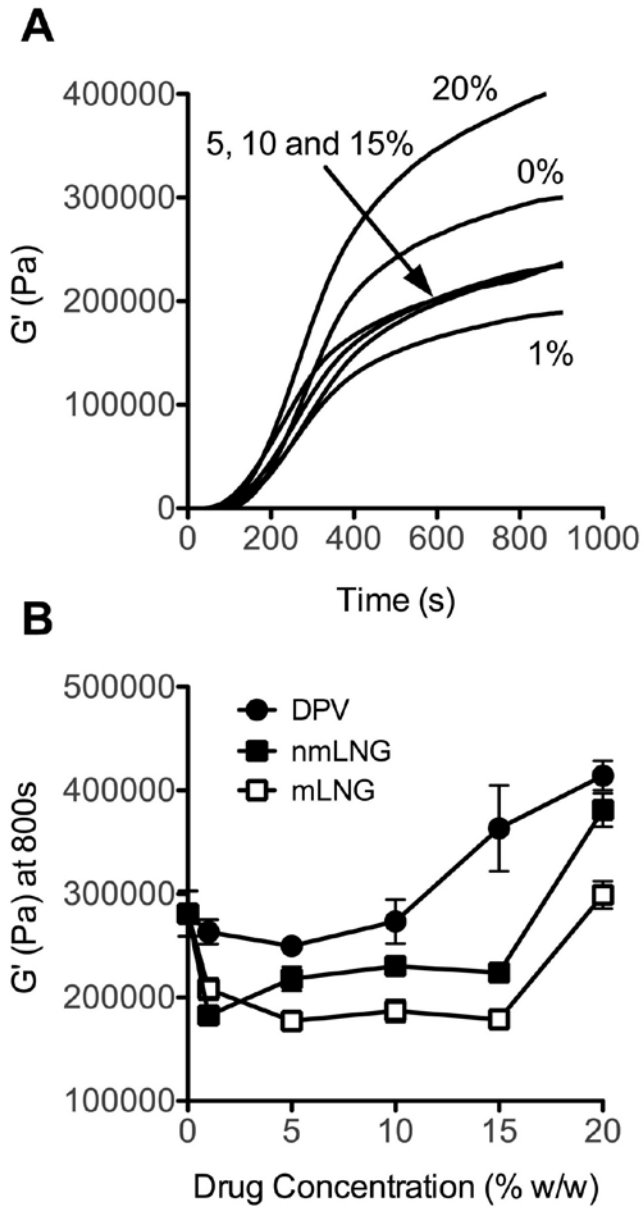
461 **Figure 7.** Results of hexane swelling experiments to assess degree of crosslinking in silicone
462 elastomer slabs after extraction of non-bound drug (n=4). nmLNG – non-micronised
463 levonorgestrel; mLNG – micronised levonorgestrel; DPV – dapivirine. A – Percentage drug
464 extraction in dichloromethane. B – Percentage mass extraction in dichloromethane. C –
465 Percentage swelling in dichloromethane. D – Percentage mass extraction in dichloromethane. E
466 – Percentage swelling in hexane. F - % Extraction (w/w) vs. swelling (w/w) in hexane.

467

468 Given the observation that levonorgestrel covalently binds to the silicone elastomer during
469 cure, oscillatory rheology was used to monitor changes in storage modulus (a measure of the
470 stored energy, representing the elastic portion) of different non-micronised levonorgestrel,

micronised levonorgestrel and dapivirine-loaded DDU-4320 silicone elastomer systems during the curing process. Consistent with previous reports [28], the storage modulus for each formulation increased with time, as exemplified by the rheograms for the non-micronised levonorgestrel formulations presented in Figure 8A and reflecting the increase in viscosity as cure progresses. However, the relationship between storage modulus and drug concentration is not simple. For example, for the non-micronised levonorgestrel formulations, storage modulus increased in the concentration rank order 1% < 5% ≅ 10% ≅ 15% < 0% < 20% (Figure 8B). This trend reflects the interplay between levonorgestrel's ability to inhibit cure (via dissolution and reaction with the silicone elastomer) and to act as mechanical filler. For silicone elastomer formulations having relatively low levonorgestrel concentrations (e.g. 1% w/w), cure inhibition predominates and the storage modulus is reduced significantly compared to the control formulation (0% levonorgestrel). However, at higher levonorgestrel loadings (e.g. 20% w/w), the cure inhibition effect is masked by the mechanical filler effect, as evidenced by storage modulus values significantly greater than the control formulation (Figure 8A). By plotting the value of the storage modulus at 800 s cure time versus drug concentration (Figure 8B), it is apparent that this complex interplay exists for both micronised levonorgestrel and non-micronised levonorgestrel. Unsurprisingly, the extent of cure inhibition is generally lower for the slower-dissolving (less prone to binding) non-micronised levonorgestrel samples. Dapivirine, on the other hand, lacks the chemical functionality to react with the silicone elastomer components and therefore does not exhibit the initial decline in storage modulus at low dapivirine concentrations compared with the non-medicated control. Instead, dapivirine acts solely as

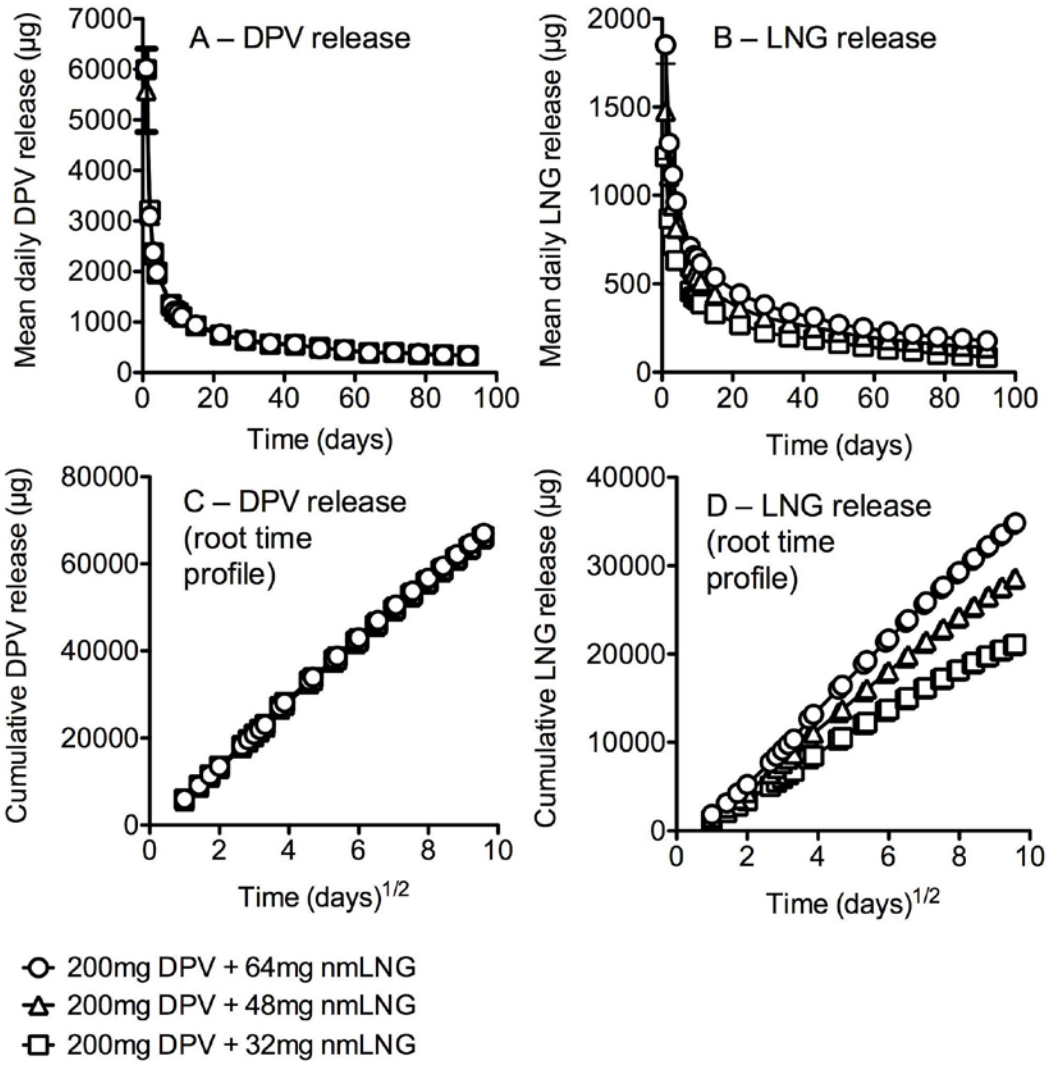
493 a mechanical filler by increasing the storage modulus at relatively high dapivirine
494 concentrations (Figure 8B).
495



496
497 **Figure 8.** A – Oscillatory rheograms (storage modulus versus time) following the cure process
498 for silicone elastomer samples containing different concentrations of non-micronised
499 levonorgestrel. Plot symbols and error bars represent the mean and standard deviation of four

500 replicates. Similar rheograms were obtained for samples containing dapivirine and micronised
501 levonorgestrel. B – Graph showing storage modulus at 800 s versus concentration of dapivirine,
502 non-micronised levonorgestrel and micronised levonorgestrel.
503
504 *3.4. In vitro release of dapivirine and levonorgestrel from rings manufactured under optimised*
505 *cure conditions*
506 Finally, armed with the knowledge that silicone elastomer type, cure temperature, cure time,
507 levonorgestrel loading and levonorgestrel particle size all have a role to play in determining the
508 extent of levonorgestrel binding, additional dapivirine+levonorgestrel matrix-type DDU-4320
509 silicone elastomer vaginal rings were fabricated under processing conditions (94 ˚C cure
510 temperature and 90 s cure time) selected to minimise levonorgestrel binding. The vaginal rings,
511 all containing 200 mg micronized dapivirine and either 32, 48 or 64 mg non-micronised
512 levonorgestrel were then tested for *in vitro* release and content assay. The 92-day *in vitro*
513 dapivirine and levonorgestrel release profiles are presented in Figure 9. Dapivirine release
514 characteristics over the first 15 days were entirely similar to those measured for the original
515 MED-4870 vaginal rings (Figure 4). Dapivirine release on day 90 was in the range 325–342 µg for
516 all vaginal ring formulations (Figure 9A) and dapivirine content assay values matched the label
517 claim (99.3–100.4 %). For DDU-4320 vaginal rings manufactured using the optimised cure
518 conditions, the mean day 1 amount of levonorgestrel released from the 200 mg dapivirine + 32
519 mg non-micronised levonorgestrel vaginal ring was 1219 ± 37 µg (Figure 9B), a significant
520 increase over the 732 ± 166 mean day 1 value for the MED-4870 rings (Figure 4B) and a
521 consequence of a reduction in the extent of levonorgestrel binding. For the same DDU-4320

522 vaginal ring formulation, day 90 levonorgestrel release was 85 ± 5 μg (Figure 9B). The total
523 percentage DPV release was approximately 33% (66 mg from initial 200 mg loading), while total
524 percentage LNG release ranged from 54% – 64% depending on initial drug loading.
525
526 These 92-day release values for both dapivirine and levonorgestrel bode well for a viability of a
527 3-month MPT vaginal ring product offering both HIV prevention and contraception. As
528 expected, increasing the non-micronised levonorgestrel loading within the DDU-4320 vaginal
529 ring from 32 mg to either 48 mg or 64 mg produced increases in the levonorgestrel release rate
530 proportional to the loading (Figure 9B and 9D). Critically, the percentage levonorgestrel
531 recovery values in the content assay (93.0%, 94.4% and 95.9% for the 200/32, 200/48 and
532 200/64 vaginal rings, respectively) now fall within the 90–110% label claim range.



533

534 **Figure 9.** *In vitro* daily release vs. time graph for release of micronised dapivirine and non-
535 micronised levonorgestrel from human-type, silicone elastomer, matrix vaginal rings into 1:1
536 isopropanol/water. Cure conditions for these rings were 94 °C for 90 s. Daily release plot
537 symbols and error bars (mostly not visible due to being smaller in size than the plot symbol)
538 represent the mean and standard deviation of six replicates.

539

540 # 4. Conclusions

Chemical reaction between the ethynyl functional group in levonorgestrel and the hydrosilane functional groups in addition-cure silicone elastomers takes place via the same hydrosilylation reaction used to cure the silicone elastomer, and leads to irreversible covalent binding of levonorgestrel molecules to the elastomer. The extent of levonorgestrel binding depends upon the processing conditions, including cure temperature, cure time, levonorgestrel particle size, levonorgestrel loading and the type of silicone elastomer. This API-binding phenomenon raises challenges for the future development of certain silicone elastomer drug delivery devices that incorporate drug molecules with similar chemically reactive functional groups, including a significant number of other steroid molecules.

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Author Contributions

All authors contributed to the design of experiments and analysis of the data. D.J.M, P.B, C.F.M and S.K conducted the experimental work. The manuscript was drafted by R.K.M and D.J.M, with input from other authors.

Notes

The authors declare no competing financial interest.

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