

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

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1	Controlling Levonorgestrel Binding and Release in a Multi-
2	Purpose Prevention Technology Vaginal Ring Device
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14	implantable devices

#### 15 **ABSTRACT**

16 Despite a long history of incorporating steroids into silicone elastomers for drug delivery 17 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 18 19 chemically with hydrosilane groups in addition-cure silicone elastomers has been thoroughly 20 investigated. Cure time, cure temperature, levonorgestrel particle size, initial levonorgestrel 21 loading and silicone elastomer type were demonstrated to be key parameters impacting the 22 extent of levonorgestrel binding, each through their influence on the solubility of levonorgestrel 23 in the silicone elastomer. Understanding and overcoming this levonorgestrel binding 24 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 25 26 levonorgestrel and dapivirine – a lead candidate antiretroviral microbicide – for combination 27 HIV prevention and hormonal contraception.

# 28 1. Introduction

29 Silicone elastomers have been widely used in controlled release drug delivery applications since 30 Dzuik and Cook first demonstrated in 1966 that various steroid molecules were capable of 31 effectively permeating and releasing from silicone rubber capsules subcutaneously implanted in 32 ewes [1]. Numerous steroid-releasing silicone elastomer devices, including subdermal implants, 33 vaginal rings and intrauterine systems, have since reached market (Table 1). The past ten years 34 have seen considerable interest in silicone elastomer vaginal ring technology for controlled 35 release of antiretroviral (ARV) drug molecules for prevention of sexual transmission of human 36 immunodeficiency virus (HIV) (Table 1) [2–9]. The International Partnership for Microbicides 37 (IPM) and the Microbicide Trial Network (MTN) are currently in Phase III clinical trials in Africa 38 with a matrix-type silicone elastomer vaginal ring developed by IPM. This ring device provides 39 controlled release of dapivirine (Figure 1A), a non-nucleoside reverse transcriptase inhibitor, 40 over 28 days and has already been shown to be safe and well tolerated in vivo. If successful, the 41 dapivirine ring will likely provide both further impetus and a viable technology platform for 42 development of multi-purpose prevention technologies (MPTs) aimed at combining HIV 43 prevention with prevention of unintended pregnancy and prevention/treatment of other 44 sexually transmitted infections (STIs) through use of a single formulation or drug-device 45 combination product [10–13]. Many of the MPT products currently undergoing development have prioritised use of levonorgestrel (Figure 1A) as the contraceptive hormone component, 46 47 based on its historical record of safety and efficacy [12–14].

49 **Table 1.** Controlled release drug delivery devices for humans that use silicone elastomer as the rate controlling material. Marketed

- 50 products (current or previous), discontinued development products and products presently undergoing clinical testing are included.
- 51 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 <sup>®</sup> (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

53 Silicone elastomers for use in medical and pharmaceutical applications are prepared through 54 the chemical crosslinking of functionalised, linear, polydimethylsiloxane molecules. The most 55 important chemical crosslinking mechanisms involve either condensation-cure or addition-cure chemistries. Condensation-cure systems involve the tin-catalysed reaction between hydroxy-56 57 terminated polydimethylsiloxane molecules and a tetraalkoxysilane, resulting in the formation 58 of the cured elastomer and an alcohol by-product [15,16]. Although the chemistry of this 59 silicone elastomer crosslinking reaction is generally compatible with a very wide range of 60 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 61 62 systems relies on the platinum-catalysed hydrosilylation reaction between hydride- and vinylfunctionalised polydimethylsiloxane molecules (Figure 1B). No by-product is formed with this 63 64 reaction. However, the platinum catalyst is particularly sensitive to poisoning by certain 65 chemical functional groups, most notably organotin, organosulfur and certain amine containing 66 compounds.



hydrosilane residue in a poly(methyl hydrogen)siloxane

٠H

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.

73

74 It is well established that small molecules containing ethylenic (C=C) and acetylenic (C=C) 75 functional groups can undergo hydrosilylation reaction with molecules containing hydrosilane 76 (Si–H) groups (Figures 1C and 1D, respectively) [19–23]. In general, the alkyne hydrosilylation 77 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 78 79 [19]. Given the large number of steroid molecules containing ethylenic or acetylenic functional 80 groups that have been previously formulated in silicone elastomers, it is rather surprising that 81 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 82 83 that reacts with the silicone elastomer is "negligible for the sustained drug release rate". On the 84 contrary, here we report that levonorgestrel, a common contraceptive progestin, reacts with 85 addition-cure silicone elastomer systems such that a very significant fraction of the 86 incorporated levonorgestrel can be irreversibly bonded to the silicone elastomer impacting 87 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. 88 patent applications by IPM [25,26], this issue has not been reported previously for 89

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

# 92 2. Methods and Materials

#### 93 2.1. Materials

94 Medical grade, addition-cure silicone elastomers DDU-4320 and MED-4870, condensation-cure 95 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 96 OmniChem N.V. (Wetteren, Belgium). Micronised levonorgestrel was supplied by CHEMO 97 98 Group (Saronno, Italy). Non-micronized and sieved fractions of non-micronized levonorgestrel 99 (non-micronised levonorgestrel) were supplied by Tecoland (Irvine, CA, US) and CHEMO Group 100 (Saronno, Italy); except where explicitly stated, non-micronised levonorgestrel in the text refers 101 to material sourced from Tecoland. Particle size data (d10, d50 and d90; measured via laser 102 diffraction) for each of the levonorgestrel materials was provided by the suppliers (Table 2). 103 HPLC-grade acetonitrile, isopropanol and dichloromethane, and phosphoric acid (85% w/w in 104 water) were purchased from Sigma Aldrich (Gillingham, UK). HPLC-grade water was obtained 105 using a Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK). 19-norethindrone was 106 supplied by LGM Pharma (Boca Raton, Florida, USA) and used as an internal standard for HPLC. 107 Analytical grade potassium dihydrogen orthophosphate was obtained from VWR (Dublin, 108 Ireland).

- 109 **Table 2.** Influence of levonorgestrel particle size on its recovery from 0.4% w/w levonorgestrel-
- 110 loaded DDU-4320 silicone elastomer slabs cured at 100 °C for 90 s. Each levonorgestrel
- 111 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

112 \* This non-micronized material was supplied by CHEMO.

113

### 114 *2.2. Manufacture of silicone rings and slabs*

115 Matrix-type, silicone elastomer vaginal rings containing 200 mg micronized dapivirine and 32 116 mg levonorgestrel (either micronized or non-micronized) and measuring 57 mm overall 117 diameter x 7.8 mm cross-sectional diameter were manufactured by reaction injection molding 118 of active silicone elastomer mixes using a Babyplast 6/10P injection-molding machine in semi-119 automatic mode fitted with custom, stainless steel, single-cavity injection molds. These rings 120 were cured at 160°C for 90 s. Briefly, the appropriate quantities of dapivirine and levonorgestrel 121 powders were added to both Parts A and B of the addition-cure silicone elastomer system MED-122 4870 and mixed at 3000 rpm for 3 min using a SpeedMixer DAC 150 FVZ-K (Synergy Devices, 123 UK). These active premixes were stored at 4°C until use. Prior to combining the premixes, they 124 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, 125 126 speedmixed for 30 s at 3000 rpm, and then transferred to a 65 cc low-density polyethlyene 127 Semco 220316 cartridge system (Polymer Systems Technology Ltd., Buckinghamshire, UK) that 128 operates with the dosing meter fitted to the Babyplast injection molder.

129

130 Silicone elastomer slabs ( $20.0 \times 30.0 \times 2.0$  mm) or vaginal rings containing levonorgestrel or 131 dapivirine were also manufactured in a similar fashion using a custom, aluminium, multi-cavity 132 mold fitted to a electrically-heated, laboratory-scale injection molding machine. For rings and 133 slabs prepared using the DDU-4320 silicone elastomer (a low temperature cure system), cure 134 time and temperature were varied between 1.5–120 min and 60–160°C, respectively. For slabs 135 prepared with the MED-4870 silicone elastomer (a high temperature cure system), cure time 136 and temperature were varied between 1.5-120 min and 120-200°C, respectively. 137 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 138 139 slabs, the drug powder was first dispersed in MED-360 silicone oil prior to preparing the silicone 140 elastomer premixes.

141

## 142 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

150 were processed using the supplied Waters Breeze software.

151

## 152 2.4. In vitro release testing of rings

153 In vitro levonorgestrel release from vaginal rings (n=6) was assessed over a 15-day period. Each 154 vaginal ring was placed into a glass flask containing 200 mL of isopropanol (IPA)/water mixture (1:1 volume ratio). This IPA/H<sub>2</sub>O mixture has been widely used for in vitro release testing of 155 156 silicone elastomer vaginal rings, since it offers greater solvating power for poorly water-soluble 157 drugs compared with aqueous media, such as simulated vaginal fluid [2–5,17]. The volumes of 158 media for release testing were selected based on previously measured solubility values in 1:1 159 IPA/water (LNG – 0.75 mg/mL at 25°C; DPV – 0.80 mg/mL at 37°C). The flasks were sealed and 160 placed in an orbital shaking incubator (Infors HT AGCH-4103; 37°C, 60 rpm, throw 25 mm). After 161 24 h (± 15 min), each flask was removed from the incubator and a sample (2 mL) of the release 162 medium was retained for HPLC analysis. The remaining release medium was discarded and 163 replaced with fresh medium (100 mL IPA:H<sub>2</sub>O). This sampling procedure was continued on a 164 daily basis, except weekends when 200 mL of release medium were added to the flasks each 165 Friday so as to maintain sink conditions through the following Monday.

166

## 167 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.

178

### 179 2.6. Drug extraction and swelling studies

180 Cured silicone elastomer slabs containing 1% w/w loading of either dapivirine, micronised 181 levonorgestrel or non-micronised levonorgestrel were manufactured, extracted with 182 dichloromethane (100 mL), and then the resulting drug-depleted slabs swollen in n-hexane to 183 assess cross-linking density [27]. A pictorial representation of the experimental method is 184 shown in Figure 2. Briefly, drugs were weighed into the appropriate amounts of part A and part 185 B silicone elastomer and speedmixed for 2 min. Part A and part B active mixes were then hand 186 mixed (10 s), speedmixed (30 s), and then injection molded at 90 °C for 3 min. These slabs were 187 individually weighed and the weights were recorded (W<sub>0</sub>). These slabs were then placed into 188 screw-top glass flasks containing 45 mL of dichloromethane. 5 mL of 500 µg/mL methanolic 189 solution containing norethindrone was added as an internal standard. The flasks were placed in 190 a shaking orbital shaker (37 °C, 60 rpm, throw 25 mm) and the release medium was sampled 191 (~5 mL) after a period of 72 h. Quantification of drug concentrations in the samples was 192 performed using HPLC-UV.



194 Figure 2. Experimental protocol for hexane swelling experiments to assess crosslinking density195 in drug-loaded silicone silicone silicone slabs.

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<sub>1</sub>). 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<sub>2</sub>). The dried 201 slabs were then placed in 50 mL of n-hexane. As before, the swollen slabs were weighed (W<sub>3</sub>), 202 dried, and weighed again (W<sub>4</sub>). 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 
$$CH_2Cl_2 = \frac{(W_1 - W_2)}{W_2} \times 100$$
 (1)

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

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

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

## 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  $\mu$ m. 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°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].

# 221 3. Results and Discussion

#### 222 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 nonmicronized 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.

228

### 229 3.2. In vitro release testing of rings demonstrating bound levonorgestrel

230 The mean daily release vs. time and mean cumulative release vs. root time profiles for MED-231 4870 silicone elastomer matrix-type vaginal rings containing 200 mg micronized dapivirine and 232 32 mg of either micronised levonorgestrel or non-micronised levonorgestrel and cured at 160 233 °C for 90 s are presented in Figure 4. Dapivirine and non-micronised levonorgestrel are 234 effectively released from the vaginal rings, with relatively high quantities of each drug released 235 on Day 1 (~6000 µg and ~750 µg for dapivirine and levonorgestrel, respectively) followed by 236 steadily declining release rates on subsequent days. Dapivirine release was very similar 237 irrespective of the particle size of the levonorgestrel incorporated into the vaginal rings. The 238 lower rate of release observed of non-micronised levonorgestrel from the vaginal rings 239 compared with dapivirine is attributed to both its lower initial loading and its unique molecular 240 permeability characteristics in the silicone elastomer. In general, the daily release profiles are 241 typical of matrix-type vaginal rings containing solid drug dispersed within a non-degradable 242 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].



263

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





272 dapivirine and 32 mg of either micronised levonorgestrel or non-micronised levonorgestrel

273 (n=4). A – Daily dapivirine release vs. time plots. B – Daily levonorgestrel release vs time plots. C

- 274 Cumulative dapivirine release vs. root time plots. D Cumulative levonorgestrel vs. root time
- 275 plots. DPV dapivirine. LNG levonorgestrel. All rings were manufactured by injection molding

276 at 160 °C for 90 s.

268

277 In order for effective drug release to occur from a silicone elastomer vaginal ring device, 278 incorporated drug substances must permeate through the bulk of the silicone elastomer matrix. 279 This permeation process can be considered as two discrete steps – dissolution of the drug in 280 the silicone elastomer, followed by molecular diffusion of the solvated drug molecules through 281 the silicone elastomer [30]. Both the degree of solubility and the rate of diffusion of drugs 282 incorporated into silicone elastomers are temperature-dependent, and are expected to 283 increase significantly at the high temperatures commonly used in injection molding 284 manufacture of silicone elastomer vaginal rings. Solubility of the drug in the silicone elastomer 285 matrix also increases the proportion of levonorgestrel molecules available for chemical binding 286 via the hydrosilylation reaction. Thus, a rational explanation for the differences observed in release between micronised levonorgestrel and non-micronised levonorgestrel is that the 287 288 micronized form of the drug exhibits faster rate of solubilization in the silicone elastomer at the 289 160 °C manufacturing temperature (levonorgestrel melting point is 240 °C), such that a 290 significant proportion of the levonorgestrel molecules subsequently react with the hydrosilane 291 groups within the silicone elastomer. By contrast, the observation that vaginal rings containing 292 32 mg non-micronised levonorgestrel exhibited significant levonorgestrel release was 293 attributed to the reduced rate of dissolution associated with the larger levonorgestrel particles 294 and, in turn, reduced levonorgestrel binding to the silicone elastomer. This observation concurs 295 with the well-established principle of increased surface area leading to increased rate of 296 dissolution, as expressed in the Noyes-Whitney equation [31].

297

#### 298 3.3. Levonorgestrel binding studies

299 To explore this levonorgestrel-binding hypothesis further, additional solvent extraction 300 experiments were conducted using levonorgestrel-loaded silicone elastomer slabs 301 manufactured under different cure conditions. No levonorgestrel could be recovered by solvent 302 extraction from MED-4870 silicone elastomer slabs containing 0.4% w/w micronised 303 levonorgestrel manufactured at (i) various cure temperatures (120-200 °C) and a fixed cure 304 time (10 min) (Figure 5A) and (ii) various cure times (1.5–120 min) and a fixed cure temperature 305 (160 °C) (Figure 5B). By contrast, MED-4870 silicone elastomer slabs containing 0.4% w/w non-306 micronised levonorgestrel produced measurable recovery of levonorgestrel by solvent 307 extraction at all but the most extreme cure conditions (i.e. 200 °C at 10 min and 120 min at 160 308 °C; Figures 6A and 6B, respectively). In fact, the non-micronised levonorgestrel MED-4870 slabs 309 showed a clear trend of decreasing levonorgestrel recovery values as a function of both 310 increasing cure temperature (Figure 5A) and increasing cure time (Figure 5B). These data and 311 trends strongly support the hypothesis that levonorgestrel binding to the silicone elastomer 312 system is dependent on its solubilization in the elastomer.



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

320 For the MED-4870 system, the highest recovery of levonorgestrel (66.2%) was measured for the 321 non-micronised levonorgestrel slabs prepared at the 120 °C / 10 min cure condition (Figure 5A). 322 This value is significantly below the range of acceptable assay values (85–115%) commonly 323 specified for a Phase 1 product. Although higher values for levonorgestrel recovery could be 324 achieved for the MED-4870 system by curing at lower temperatures, this would lead to 325 significantly increased process cycle times such that scaled manufacture would be impractical. 326 For example, in additional experiments, we demonstrated that MED-4870 silicone elastomer 327 containing 0.4% w/w non-micronised levonorgestrel can be cured at 80 °C for 2.5 h to give a 328 levonorgestrel recovery value of 86.2%. However, injection molding cycle times less than 2 min 329 are preferable. Reducing cure time is also not an option, since 90 s at 160 °C was the minimum 330 cure condition required to produce a ring or slab device having sufficient mechanical properties 331 for demolding.

332

333 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 334 335 elastomer slabs containing micronised levonorgestrel and prepared at 60 °C for 7 min, 336 levonorgestrel recovery was 75.6%, with values decreasing as cure temperature was increased 337 (Figure 5C). However, no levonorgestrel recovery was obtained for cure temperatures 120 °C 338 and above (Figure 5C). For the non-micronised levonorgestrel DDU-4320 samples, 339 levonorgestrel recovery values were significantly higher (87.3% at 60 °C declining to 58.5% at 340 160 °C; Figure 5C) compared to those for micronised levonorgestrel, mimicking the trend 341 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 nonmicronized 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).

347

348 For comparison, incorporation of 0.4% w/w micronised levonorgestrel and non-micronised 349 levonorgestrel into MED-6382 silicone elastomer (a medical grade tin-catalysed condensation 350 cure system) produced levonorgestrel recovery values of 98.9 and 100.8%, respectively. 351 However, this condensation-cure silicone elastomer produces isopropanol as a by-product of 352 the curing reaction. Both levonorgestrel and dapivirine are highly soluble in isopropanol, and 353 therefore these condensation cure silicone elastomers are not preferred in order to avoid drug 354 migration to the ring surface as isopropanol migrates to the vaginal ring surface and evaporates 355 [17].

356

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

between the values for the previously tested non-micronised levonorgestrel and micronised levonorgestrel (98.4% and 41.3%, respectively; Table 2), further supporting the solubilisation hypothesis. The similarity in levonorgestrel recovery values for Batches 2 and 3 is most likely due to the equivalence in d10 values (despite the very different d90 and d50 values), since the 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 fractions of the total levonorgestrel loading are expected to dissolve and bind to the silicone 374 375 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 previous slab data (Figure 5). This is likely attributed to a temperature-insulating effect for drug 378 379 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

387 vaginal rings cured at 90 °C for 3 min and containing different amounts of micronized and non-

388 micronised levonorgestrel. Each levonorgestrel recovery value is the mean of four replicates

389 and reported errors denote standard deviations.

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

390

391

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

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



Figure 6. Influence of order of addition of levonorgestrel upon levonorgestrel recovery from
MED-4870 and DDU-4320 silicone elastomer slabs (n=4). MED-4870 slabs were cured at 160 °C
for 90 s. DDU-4320 slabs were cured at 100 °C for 90 s. Part A – levonorgestrel added only to
Part A of the silicone elastomer prior to curing; Part B – levonorgestrel added only to Part B of
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

Silicone oils are sometimes used as dispersing agents for addition of drug powders to silicone elastomer systems. The impact of dispersing levonorgestrel in MED-360 silicone oil prior to manufacture of DDU-4320 silicone elastomer slabs was investigated for selected cure conditions. The results, presented in Supplementary Information, show that levonorgestrel recovery was typically 4–10% higher with use of the silicone oil. However, at the highest cure temperature tested (160 °C), no significant difference in levonorgestrel recovery was observed 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 order dapivirine (99.3%) > non-micronised levonorgestrel (87.6%) > micronised levonorgestrel 433 434 (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

439 elastomer formulation. The same mass loss due to extraction of these oils was presumably also 440 observed in each the drug-loaded samples, with any additional mass loss due to extraction of 441 either non-bound drug or unreacted polydimethylsiloxane components. Interestingly, the 442 highest extraction mass was observed for the micronised levonorgestrel slab, which is counter-443 intuitive if only the propensity for the micronised levonorgestrel crystalline material to rapidly 444 solubilize and undergo hydrosilylation reaction within the silicone elastomer is considered. 445 However, these levonorgestrel molecules compete with the vinyl-functionalised 446 polydimethylsiloxane molecules in the silicone elastomer system for reaction with the hydride-447 functionalised polydimethylsiloxane molecules. In this way, the overall crosslink density is 448 reduced in the silicone elastomer and there is increased opportunity for extraction of non-449 reactive polydimethylsiloxane molecules. This effectively explains the trends in percentage 450 mass extraction values (Figure 7B). An entirely similar trend is also observed for the percentage 451 swelling values (in dichloromethane; Figure 7C), adding further support to the different impact 452 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 453 454 rather than dichloromethane. Subsequent placement of the silicone elastomer slabs into 455 hexane resulted in minimal additional mass loss (Figure 7D), and confirmed the previous trend 456 in crosslinking density obtained with dichloromethane (compare Figures 8C and 7E). The plot of 457 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). 458

459



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

467

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

471 micronised levonorgestrel and dapivirine-loaded DDU-4320 silicone elastomer systems during 472 the curing process. Consistent with previous reports [28], the storage modulus for each 473 formulation increased with time, as exemplified by the rheograms for the non-micronised 474 levonorgestrel formulations presented in Figure 8A and reflecting the increase in viscosity as 475 cure progresses. However, the relationship between storage modulus and drug concentration is 476 not simple. For example, for the non-micronised levonorgestrel formulations, storage modulus increased in the concentration rank order  $1\% < 5\% \approx 10\% \approx 15\% < 0\% < 20\%$  (Figure 8B). 477 478 This trend reflects the interplay between levonorgestrel's ability to inhibit cure (via 479 dissolution and reaction with the silicone elastomer) and to act as mechanical filler. For 480 silicone elastomer formulations having relatively low levonorgestrel concentrations (e.g. 481 1% w/w), cure inhibition predominates and the storage modulus is reduced significantly 482 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 483 mechanical filler effect, as evidenced by storage modulus values significantly greater than 484 485 the control formulation (Figure 8A). By plotting the value of the storage modulus at 800 s 486 cure time versus drug concentration (Figure 8B), it is apparent that this complex interplay 487 exists for both micronised levonorgestrel and non-micronised levonorgestrel. 488 Unsurprisingly, the extent of cure inhibition is generally lower for the slower-dissolving 489 (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 490 491 therefore does not exhibit the initial decline in storage modulus at low dapivirine 492 concentrations compared with the non-medicated control. Instead, dapivirine acts solely as 493 a mechanical filler by increasing the storage modulus at relatively high dapivirine494 concentrations (Figure 8B).

495



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

replicates. Similar rheograms were obtained for samples containing dapivirine and micronised
 levonorgestrel. B – Graph showing storage modulus at 800 s versus concentration of dapivirine,
 non-micronised levonorgestrel and micronised levonorgestrel.

503

3.4. In vitro release of dapivirine and levonorgestrel from rings manufactured under optimised
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  $325-342 \mu g$  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  $1219 \pm 37 \mu g$  (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

These 92-day release values for both dapivirine and levonorgestrel bode well for a viability of a 3-month MPT vaginal ring product offering both HIV prevention and contraception. As expected, increasing the non-micronised levonorgestrel loading within the DDU-4320 vaginal ring from 32 mg to either 48 mg or 64 mg produced increases in the levonorgestrel release rate proportional to the loading (Figure 9B and 9D). Critically, the percentage levonorgestrel recovery values in the content assay (93.0%, 94.4% and 95.9% for the 200/32, 200/48 and 200/64 vaginal rings, respectively) now fall within the 90–110% label claim range.



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

# 540 4. Conclusions

541 Chemical reaction between the ethynyl functional group in levonorgestrel and the hydrosilane 542 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 543 544 levonorgestrel molecules to the elastomer. The extent of levonorgestrel binding depends upon 545 the processing conditions, including cure temperature, cure time, levonorgestrel particle size, 546 levonorgestrel loading and the type of silicone elastomer. This API-binding phenomenon raises 547 challenges for the future development of certain silicone elastomer drug delivery devices that 548 incorporate drug molecules with similar chemically reactive functional groups, including a 549 significant number of other steroid molecules.

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554

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556 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,
- 558 with input from other authors.

559

- 560 **Notes**
- 561 The authors declare no competing financial interest.

562

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