Intravaginal rings for continuous low-dose administration of cervical ripening agents

Yujing Wang[1], Peter Boyd[1], Alyson Hunter[2], R. Karl Malcolm[1]*

[1]*School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK;*

[2]*Royal Jubilee Maternity Service, Belfast Health and Social Care Trust, Belfast BT12 6BA, UK*

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

Abstract

Intravaginal rings (VRs) have been widely reported for administration of pharmaceutical drugs – most notably estrogens, progestogens and antiretrovirals – to the vagina for clinical benefit. Here, for the first time, we describe the design, manufacture and preclinical testing of VRs for sustained/controlled release of the cervical ripening agents isosorbide mononitrate (ISMN) and misoprostol (MP), either singly or in combination. Matrix-type silicone elastomer VRs containing ISMN showed declining daily release rates, ranging from 31–168 mg (Day 1) to 3–25 mg (Day 11). Novel orifice-type rings, in which a MP-containing silicone elastomer core is partially exposed to the external environment by overmolding with a non-medicated silicone elastomer sheath containing orifices, provided relatively constant daily MP release rates over 14 days (~20 or 60 μg/day depending on the formulation type). Combination vaginal rings offered simultaneous release of both ISMN and MP over 14 days, with an almost constant MP release rate (60 μg/day) and steadily declining daily ISMN release (295 mg on Day 1 and 24 mg on Day 11). The VR design can be readily tailored to provide sustained or controlled release of ISMN and MP at rates potentially useful for cervical ripening.

Keywords

induction of labour; silicone elastomer drug delivery device; vaginal drug administration; controlled release; sustained release; out-patient.

1. Introduction

Flexible polymeric ring-shaped devices have been investigated since the 1970s for sustained or controlled release of drug substances to the human vagina. To date, six vaginal ring (VR) products have reached market, each offering release of one or more steroid molecules for estrogen replacement therapy (Estring® and Femring®), contraception (Nuvaring®, Progering® and Ornibel®) or hormone supplementation and pregnancy maintenance during *in vitro* fertilization (Fertiring®) (Brache et al., 2013; Friend, 2011; Malcolm et al., 2015, 2012). Since 2002, great progress has been made in the design and development of various antiretroviral-releasing VRs for reducing HIV acquisition (Kiser et al., 2012; Malcolm et al., 2015, 2010; Thurman et al., 2013), with a dapivirine-releasing silicone elastomer ring having recently completed late-stage clinical testing and currently under regulatory review by the European Medicines Agency (Baeten et al., 2016; Devlin et al., 2013; Nel et al., 2016b). A major impetus for continued innovation in VR design has been the growing interest in combination HIV microbicide and multipurpose prevention technology products for which release needs to be individually tailored for each drug molecule (Baum et al., 2012; Boyd et al., 2016; Derby et al., 2017; Fetherston et al., 2013; Friend et al., 2013; Malcolm et al., 2015, 2014; Moss et al., 2016; Murphy et al., 2014; Smith et al., 2017; Shweta R. Ugaonkar et al., 2015; Shweta R Ugaonkar et al., 2015). However, other clinical indications within women's health are also likely to benefit from enhanced drug administration using existing or new VR technologies.

Induction of labour (IOL) is a widely-used practice in obstetrics to prevent maternal and fetal morbidity and mortality. However, a major complication of IOL is hyperstimulation of the

uterus, which can lead to the sudden onset of powerful, frequent and painful contractions which women may find unbearable or frightening. These uterine contractions can damage the fetus due to hypoxia as the placenta cannot be fully revascularised if the uterus is contracting too frequently. Despite these concerns, there is increasing demand to deliver IOL in an outpatient setting where women can be more comfortable and relaxed and reduce their length of stay in hospital. Here, we describe a potential new IOL method involving slow and continuous administration of cervical ripening agents from VRs. This approach has the potential to more closely mimic the natural cervical ripening process and spontaneous onset of labour and reduce the incidence of hyperstimulation, making it safer and more acceptable to women, particularly in an out-patient setting.

Cervicovaginal administration of pharmacological agents – most notably dinoprostone and misoprostol – has been widely used to ripen the cervix as a method for initiating IOL (Agarwal et al., 2012; Alfirevic et al., 2015; Calder et al., 2008; MacKenzie, 2006; Vogel et al., 2017; Zhang et al., 2015). Two forms of vaginal dinoprostone are already used routinely. Prepidil® is a non-aqueous gel containing 0.5 mg dinoprostone in 3 g triacetin which is administered to the endocervix every 6 h up to a maximum of three doses in 24 h. Cervidil®/Propress® is a pessary-type dosage form comprising 10 mg dinoprostone dispersed within a hydrogel copolymer inserted in a mesh pouch. The insert is placed in the posterior fornix of the vagina and provides sustained release of dinoprostone over 12 h, after which the pouch is removed. Thus, use of modified release formulations for cervical ripening is already well established and offers certain advantages over more conventional immediate-release products (Embrey et al., 1980; Lyrenäs et al., 2001).

77

78 Vaginal administration of the synthetic prostaglandin misoprostol, often in the form of
79 fractionated tablet doses (25 μg) of the anti-ulcer product Cytotec®, has also been widely
80 reported for cervical ripening (Furukan et al., 2007; Rodrigues et al., 1998; Sanchez-Ramos
81 et al., 1998; Sharma et al., 2005; Zieman et al., 1997). As with dinoprostone, a hydrogel insert
82 for sustained-release vaginal administration of misoprostol has also been developed, marketed
83 under the brand names Mysodelle®/Myspess®/Misodel® and offering administration rates of
84 approximately 7 μg/h misoprostol (Miller et al., 2016; Powers et al., 2008; Stephenson and
85 Wing, 2015; Wing, 2008; Wing et al., 2013).
86

87 Although more commonly used for the treatment of angina pectoris, there has also been
88 interest in the use of the nitric oxide donor isosorbide mononitrate (ISMN) for cervical
89 ripening (Agarwal et al., 2014, 2012; Bollapragada et al., 2009; Bullarbo et al., 2007;
90 Chanrachakul et al., 2002, 2000; Chwalisz et al., 1997; Habib et al., 2008; Ledingham et al.,
91 2000; Norman, 1996; Tschugguel et al., 1999; Väisänen-Tommiska et al., 2003). In contrast
92 to prostaglandins, nitric oxide donors induce cervical ripening without causing uterine
93 contractions, making them particularly interesting for use in out-patient settings where fetal
94 monitoring is not possible.
95

96 Here, for the first time, we report that silicone elastomer VRs can offer sustained/controlled
97 release of misoprostol and ISMN at rates likely to be clinically effective for cervical ripening
98 (Amorosa and Stone, 2015; Leopold and Sciscione, 2017; Sharp et al., 2016). A combination

VR device offering release of both agents at significantly different release rates is also described.

2. Materials and methods

2.1. Materials

ISMN (70% w/w in lactose) and pure ISMN (98%) were purchased from Clariant (Leeds, UK) and iChemical Technologies Inc. (Shanghai, China), respectively. Misoprostol (MP, 1% w/w dispersion in HPMC) was purchased from Sequoia Research Products Ltd. (Pangbourne, UK). DDU-4320 silicone elastomer kits were purchased from NuSil Technology LLC (Carpinteria, CA, US). HPLC-grade acetonitrile, HPLC-grade acetone, sodium chloride, potassium hydroxide, calcium hydroxide, bovine serum albumin, lactic acid, acetic acid and glucose were bought from Sigma (Gillingham, UK). Urea and hydrogen chloride (37% w/w in water) were obtained from VWR International Ltd. (Dublin, Ireland). Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK) was used to obtain HPLC-grade water.

2.2. Manufacture of vaginal rings

Silicone elastomer matrix-type VRs (Fig. 1A) containing various loadings of ISMN (100, 500 and 1000 mg per ring) were manufactured on a laboratory-scale injection molding machine, according to well-established and previously reported manufacturing protocols (Boyd et al., 2016; Fetherston et al., 2013). Briefly, the required amounts of ISMN were added to both Parts A and B of the MED-4320 addition-cure silicone elastomer system contained in custom screw-cap polypropylene containers, and the contents of each container individually mixed using a Dual Asymmetric Centrifuge (DAC) mixer (SpeedMixerTM DAC 150 FVZ-K, Hauschild, Germany) for 30 s at 3000 rpm. The Part A and B premixes were combined in a

1:1 ratio in a polypropylene container, hand-mixed using a spatula, and then speedmixed (30 s, 3000 rpm) to produce the active silicone elastomer mixture. The active mix was manually injected into a heated (60 °C) matrix ring mold assembly fitted to the molding machine using a SEMCO® injection cartridge and cured for 10 min producing matrix-type rings (dimensions: 7.6 mm cross-sectional diameter and 55.0 mm external diameter).

Silicone elastomer reservoir-type VRs (Fig. 1B) – comprising ISMN (7% w/w) or MP (0.2% w/w) in a central core, with the core encapsulated with a silicone elastomer sheath – were similarly manufactured by injection molding via a previously described three-step process (Boyd et al., 2016). Briefly, active silicone elastomer mixtures, prepared as described for the matrix-type rings, were injected into a core ring mold assembly (Fig. 1D) and cured at 60 ºC for 10 min to produce ISMN-loaded cores with 4.5 mm cross-section diameter and 51.9 mm external diameter. Full-length or half-length cores were then placed in a custom mold assembly and overmolded with non-medicated MED-4320 silicone elastomer in two (top and bottom) additional injection molding steps. The resulting reservoir-type rings had the following dimensions: 4.5 mm core diameter, 7.6 mm cross-sectional diameter, 55.0 mm external diameter and 1.55 mm membrane thickness.

Silicone elastomer orifice-type VRs (Fig. 1C) – comprising ISMN or MP in a central silicone elastomer core and partially encapsulated with a silicone elastomer sheath containing various orifices exposing the underlying core – were manufactured in a similar manner to reservoir rings except using custom-designed injection molds with pins designed to partially expose the

drug-loaded central core to the external environment via orifices in the membrane (Figure 1E, 1).

2.3. Differential scanning calorimetry

Thermal analysis of ISMN, MP and samples taken from the drug-loaded rings was performed using differential scanning calorimetry (DSC; TA Instruments Q100; 50 mL min^{-1} nitrogen flow; –70 ºC to 150 ºC at a rate of 10 ºC min^{-1}) to probe the nature of the drugs within the rings, following established methods (Boyd et al., 2016; Fetherston et al., 2013).

2.4. Quantification of ISMN in the rings

Individual VRs were cut into 2 mm slices and placed in a 250 mL glass bottle (Duran® GLS 80®) containing 100 mL acetone. The bottles were placed in a shaking orbital incubator (Infors HT Unitron, Switzerland; 37 ºC, 60 rpm, 25 mm orbital throw). After 7 d, the supernatants were collected and diluted 20-fold with water-acetonitrile (90:10, v/v). The amount of ISMN in each VR was analysed by ultra-performance liquid chromatography (UPLC).

2.5. In vitro release testing

Individual VRs were placed into 250 mL glass bottles (Duran® GLS 80®) containing 50 mL pH 4.2 simulated vaginal fluid (SVF). SVF mimics the chemical composition of vaginal fluid (Owen and Katz, 1999). The bottles were placed in the shaking orbital incubator (Infors HT Unitron, Switzerland; 37 ºC, 60 rpm, 25 mm orbital throw) for 14 days. Sampling (1 mL) followed by complete replacement of the release medium was performed daily except on Fridays when the flask was refilled with 100 mL SVF and no sampling was performed again until the following Monday. The amount of drug in each sample was quantified by UPLC,

and the data used to construct release vs time plots. All release testing experiments were conducted n=4.

2.6. Quantification of ISMN and MP using UPLC

ISMN and MP were quantified using a Waters ACQUITY UPLC® system fitted with an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) and an in-line filter (0.2 μm). ISMN samples were injected (1.0 μL) onto the column (40 ºC) and isocratic elution was performed with a mobile phase of water-acetonitrile (90/10, v/v) at flow rate of 0.6 mL/min. ISMN was detected at 210 nm with a 0.76 min retention time. MP samples were injected (2.0 μL) onto the column (40 ºC) and isocratic elution was performed using a water-acetonitrile (50/50, v/v) mobile phase at 0.6 mL/min flow rate. MP was detected at 210 nm with a 0.69 min retention time. Linear ranges for ISMN and MP were 25–800 μg/mL and 0.5–25 μg/mL, respectively.

2.7. Statistical analyses

Statistical analyses were performed using one-way ANOVA, followed by post-hoc analysis using the Tukey-Kramer multiple comparisons test. Statistical significance is defined as $p < 0.05$. Analysis was conducted using GraphPad Prism.

3. Results

3.1. Design and manufacture of rings

Matrix-type rings containing ISMN (F1–F4, Table 1), reservoir and orifice-type rings containing ISMN (F5–F8, Table 1), and matrix-type and orifice-type rings containing MP (F9–F13, Table 1) were manufactured successfully; Fig. 2 shows representative photographs.

185 The different ring designs are intended to offer different drug release rates. Matrix-type rings
186 contain the drug distributed uniformly throughout the entire volume of the ring (Fig. 1A), and
187 typically exhibit declining release rates with time (Boyd et al., 2016; Fetherston et al., 2013;
188 Malcolm et al., 2016). Reservoir rings contain a drug-loaded core and a non-medicated
189 membrane (Fig. 1B) that controls the drug release rate from the core via a permeation
190 mechanism, and typically results in constant release rates (Boyd et al., 2016; Malcolm et al.,
191 2016, 2005). Orifice-type rings (Fig. 1C) – reported here for the first time – are similar in
192 construction to reservoir-type rings except that the core containing the drug active(s) is
193 partially exposed to the external environment by orifices extending through the rate-
194 controlling membrane, intended to further modulate the drug release rate.

195 *3.2. Thermal analysis of rings*

196 DSC analysis was performed to investigate the physical state of the drugs within the rings,
197 since this impacts the release mechanism. The thermograms of the non-medicated silicone
198 elastomer sample and each of the representative ring formulations F1, F2, F3, F6, and F8 (Fig.
199 3A) show an endothermic peak close to –45ºC, due to crystalline melting of the silicone
200 elastomer. Ring samples F1, F2, F3 and F6 show an additional endothermic peak at ~90°C,
201 attributed to melting of crystalline ISMN. Thus, these ring devices are shown to contain solid
202 crystalline ISMN (i.e. the drug is incorporated above its solubility limit in the silicone
203 elastomer). The peak size associated with the ISMN melting transition correlates with the
204 ISMN loading in the ring. Melting enthalpies associated with the ISMN were linearly
205 correlated with the mass fraction of ISMN in the rings (Fig. 3B). From the x-intercept of the
206 fitted line, the solubility of ISMN at its melting point in the silicone elastomer ring was
207 determined to be 0.62% w/w (Gramaglia et al., 2005; Woolfson et al., 2003). This is important,

208 as diffusion of drugs through silicone elastomers (and therefore release of drugs from VRs)
209 requires at least some of the incorporated drug to be in the dissolved state (Malcolm et al.,
210 2003, 2016).
211
212 No discernible thermal transition related to crystalline melting of MP was observed for either
213 the supplied MP material (a 1% dispersion in HPMC) or the ring loaded with 0.2% w/w MP
214 (F8, Table 1, Fig. 3A). This is due to the very low concentration of MP in the supplied
215 material, which is significantly below the DSC detection limit. However, a very broad
216 endothermic peak (30–130 ^{o}C) was observed for MP (1% in HPMC) (Fig. 3A), due to
217 dehydration of HPMC during heating (Chandak and Verma, 2008). This broad peak was
218 greatly suppressed in the thermogram of the MP-containing ring (F8) due to dilution of the
219 MP material within the silicone elastomer.

220 *3.3. Release of ISMN from VRs*
221 ISMN was effectively released from matrix-type rings F1–F4 into the SVF release medium,
222 displaying typical 'burst and decline' release profiles (Fig. 4A). Release rates were dependent
223 on the initial ISMN loading in the rings, with Day 1 values ranging between 31 and 168 mg.
224 By Day 4, the daily quantities of ISMN released from the rings had declined to between 8–57
225 mg, and to 3–25 mg by Day 11. These daily release quantities administered from the rings are
226 of an entirely similar order of magnitude to doses reported previously for vaginal
227 administration of ISMN tablets in both out-patient and in-patient settings (Agarwal et al.,
228 2014, 2012; Bollapragada et al., 2009; Bullarbo et al., 2007; Chanrachakul et al., 2002; Habib
229 et al., 2008; Vidanagamage and Goonewardene, 2011). The continuous dosing of ISMN
230 offered by the rings may be clinically more effective and enhance compliance compared to

231 the periodic dosing regimen used for the tablets (48, 32 and 16 h prior to the scheduled time
232 of admission for induction).
233
234 Release of ISMN from the VRs obeys root time ($t^{0.5}$) kinetics, as evidenced by linear
235 cumulative release versus root-time graphs (Fig. 4B). Increasing the ISMN loading from 100
236 (F1) to 1000 mg (F3) significantly increased the ISMN release rate from 24.6 to 176.0
237 mg/day$^{0.5}$ (Table 1).
238
239 Rings F3 and F4 both contained 1000 mg ISMN per ring, However, F3 contained ISMN
240 diluted with 30% lactose (as supplied), while F4 contained pure ISMN (i.e. no lactose). The
241 presence of the lactose diluent serves to significantly increase the rate of release of ISMN
242 from the rings (176.0 vs. 121.8 mg/day$^{0.5}$; Table 1 and Fig. 4B).
243
244 Release of ISMN from reservoir and orifice-type rings is presented in Figs. 4C and 4D. Here,
245 daily ISMN release is relatively constant over the study period (Fig. 3C), at least compared to
246 the matrix-type rings (Fig. 3A), resulting in linear cumulative release vs. time profiles (Fig.
247 4D). Daily ISMN release from the reservoir ring containing a full-length ISMN-loaded core
248 (F6) decreased from 7.2 mg on Day 1 to 4.4 mg on Day 11. The half-core ring (F5) provided
249 Day 1 release of 3.7 mg and 2.2 mg on Day 11, approximately half the values for the full-core
250 ring (F6). Compared with Ring F6, the orifice-type ring (F7) had a relatively large amount of
251 ISMN release on Day 1 (10.3 vs. 7.2 mg) and a higher daily ISMN release rate (6.29 vs. 5.22
252 mg/day), clearly illustrating the impact of partly exposing the drug-loaded core to the SVF.
253

Unlike the matrix-type ring design, formulating the reservoir-type ring to contain pure ISMN (F8; no lactose diluent) produced ISMN release rates identical to the reservoir ring containing ISMN+lactose (F6) (Figs 4C and 4D). The water-soluble lactose is unable to modulate the ISMN release from reservoir rings, since the lactose is contained only in the core of the device and is not directly exposed to the SVF release medium. After 14 days, cumulative ISMN release from the reservoir rings was 37.5, 74.2, 92.0 and 73.8 mg, F5–F8 respectively, equivalent to 39.7%, 39.3%, 48.7% and 39.0% of the initial loadings (Table 1).

3.4. Release of MP from VRs

Ring F9 is the core component used to manufacture the orifice-type MP rings (F10–F13). Not surprisingly, it displayed release behaviour typical of a matrix-type ring, including relatively high Day 1 release (779 μg), declining amounts of MP released on subsequent days (233 μg on Day 4 and only 26 μg on Day 11, Fig. 4E), and root-time kinetics (Fig. 4F). The quantities of MP released during Day 1 and Day 4 were much higher than the safe clinical doses (25–200 μg) reported for vaginal administration of MP in IOL (Ambika et al., 2017; Calder et al., 2008; Chaudhuri et al., 2011; Frohn, 2002; Oboro and Tabowei, 2005).

When this F9 core was incorporated into a conventional reservoir-type ring, no release of MP was observed. However, rings having either small circular orifices (F10, F11, F13) or larger window-style orifices (F12) exposing an underlying full-length (F11 and F13) or a partial-length (F10 and F12) MP-loaded core showed significant MP release (Fig. 4G and 4H, Table 1). In general, the daily release profiles (Fig. 4G) do not show the burst-and-decline behaviour of matrix-type rings (Fig. 4E); instead, greater constancy of release is observed, similar to a conventional reservoir-type ring (Fig. 4C). Moreover, MP release is clearly shown to depend

upon the length of the core, the type of orifices (circular vs. elongated), and the number of orifices. Inevitably, given that only part of the core in these rings is exposed to the SVF, the release rates are reduced compared to ring F9. However, daily MP release rates tend to hover around either 20 or 60 μg/day (Fig. 3G), conveniently straddling the dosing range currently used in vaginal administration of MP for cervical ripening (Ambika et al., 2017; Calder et al., 2008; Chaudhuri et al., 2011; Frohn, 2002; Oboro and Tabowei, 2005).

Finally, ring formulation F13 – containing 5.4 mg MP in the core and 1209.8 mg ISMN in the sheath (Table 1) – effectively demonstrates the utility of vaginal ring technology in offering simultaneous and independent release of multiple cervical ripening agents over extended time periods. For this combination drug ring, MP is released from the core of the device at an almost constant rate of 60 μg/day (Figs. 4G and 4H), while the ISMN incorporated into the sheath component provides Day 1 release of 295 mg followed by declining daily release on each subsequent day and reaching 24 mg on Day 11 (Fig. 4I). After 14 days, the cumulative amount of MP and ISMN released was 780 μg and 870 mg (Fig. 4J), respectively, equivalent to 14.4% and 72.2% of the initial loadings, respectively (Table 1).

4. Discussion

In general, the silicone elastomer matrix-type rings are best suited to administration of daily IMSN doses in the relatively high 1–200 mg range. The administered dose of ISMN was readily modulated by adjusting the initial drug loading. Matrix rings provided an initial burst release followed by declining release on subsequent days; as such, the release range would need to carefully tailored for optimal clinical efficacy. However, constant low milligram-per-day release of ISMN was achieved with silicone elastomer reservoir rings, with the daily

300 release rate proportional to the length of the IMSN-loaded core. According to well-established
301 design principles for reservoir-type rings, the thickness of the rate-controlling membrane
302 could also be adjusted to further modulate release; for example, adjusting the membrane
303 thickness of the F5 and F6 rings to 0.78 mm (half of the current 1.55 mm) would result in a
304 two-fold increase in the daily ISMN release rate. Incorporating orifices in the membrane also
305 significantly increased the ISMN release rate and offered release kinetics intermediate
306 between conventional matrix and reservoir rings.
307
308 Much lower microgram-per-day doses of MP are required for IOL. Since conventional
309 reservoir-type VRs containing MP provided no release, we focused our attention on ring
310 designs including orifices in the membrane so as to expose, to varying extents, the MP-loaded
311 core to the release medium. By controlling the number of orifices, the size of orifices and the
312 length of the MP-loaded core, near-constant release rates in the range 20–60 μg/day could be
313 easily achieved.
314
315 This is the first study reporting VRs for sustained or controlled delivery cervical ripening
316 agents. The release rates of ISM and MP from VRs could be readily tailored by drug loading
317 and ring designs to meet the clinical requirements for IOL. This study is based on *in vitro*
318 testing of VRs, and therefore the release data may not correlate with performance *in vivo*.
319 However, for other VRs – most notably the dapivirine-releasing silicone elastomer VR for
320 prevention of sexual transmission of the human immunodeficiency virus (HIV) – *in vitro*
321 testing of dapivirine release using SVF correlated closely with *in vivo* release, based on

residual drug analysis content post-use (Baeten et al., 2016; Malcolm et al., 2016; McCoy et al., 2017; Nel et al., 2016a).

VRs are already used successfully for estrogen replacement therapy and hormonal contraception, and an antiretroviral-releasing VR will likely soon be available in Africa for HIV prevention (Baeten et al., 2016; Nel et al., 2016a). The ring devices described in this study are of similar size and fabricated from similar materials as other marketed vaginal ring products (most notably Estring®, Femring® and the dapivirine-releasing ring). In general, VRs are user-friendly, are easily inserted and removed by the woman, and are highly acceptable (Mulders and Dieben, 2001; Novák et al., 2003).

As with other sustained/controlled release drug formulations, increased user acceptability and compliance/adherence with VRs is often mooted as a potential advantage over more conventional immediate release vaginal dosage forms (Malcolm et al., 2015). Previous studies have reported high user acceptability and/or adherence of VRs for contraception and estrogen replacement therapy (Ayton et al., 1996; Barentsen et al., 1997; Brache et al., 2000; Casper and Petri, 1999; Dieben et al., 2002; Faundes et al., 1981; Nachtigall, 1995; Novák et al., 2003; Roumen et al., 2001; Roumen and Dieben, 1999; Stifani et al., 2018; Vartiainen et al., 1993; Weisberg et al., 1995). Yet, lower-than-expected adherence has been reported in late-stage clinical trials of the dapivirine ring, particularly among young women (Baeten et al., 2016; Montgomery et al., 2017; Nel et al., 2016b, 2016a; Spence et al., 2016). However, the observed low adherence with the dapivirine may reflect various sociocultural issues particularly pertinent to Sub-Saharan Africa and HIV infection (Montgomery et al., 2017).

345 The relatively short use regime intended for a VR for cervical ripening coupled with its use in
346 a managed primary care health setting should lead to high levels of user adherence.
347
348 The combination ISMN+MP ring described in this study is particular interesting, since
349 synergistic effects have previously been reported when administering the combination of
350 ISMN and MP intravaginally for cervical ripening (Abdellah et al., 2011; Elsokary et al.,
351 2015; Soliman, 2013).
352

353 # 4. Conclusions

354 The results of this proof-of-concept study highlight the potential for development of long-
355 acting sustained or controlled release drug delivery devices for low dose vaginal
356 administration of cervical ripening agents for IOL. The continuous dosing of small amounts
357 of these agents might be particularly useful in out-patient settings, reducing the time spent in
358 hospital, reducing health service costs, and offering increased user compliance compared to
359 periodic dosing regimens. The results strongly support progress to clinical testing.
360

361 # Transparency declarations

362 The authors declare no conflicts of interest.
363

364 # Acknowledgements

365 This study was funded through the UK Medical Research Council's 'Confidence in Concept'
366 scheme (MC-PC-15035).

References

Abdellah, M.S., Hussien, M., Aboalhassan, A., 2011. Intravaginal administration of isosorbide mononitrate and misoprostol for cervical ripening and induction of labour: a randomized controlled trial. Arch. Gynecol. Obstet. 284, 25–30. https://doi.org/10.1007/s00404-010-1572-4

Agarwal, K., Batra, A., Batra, A., Aggarwal, A., 2014. Randomized Comparison of Isosorbide Mononitrate and PGE2 Gel for Cervical Ripening at Term including High Risk Pregnancy. Int. J. Reprod. Med. 2014, 1–5. https://doi.org/10.1155/2014/147274

Agarwal, K., Batra, A.A., Batra, A.A., Dabral, A., Aggarwal, A., 2012. Evaluation of isosorbide mononitrate for cervical ripening prior to induction of labor for postdated pregnancy in an outpatient setting. Int. J. Gynecol. Obstet. 118, 205–209. https://doi.org/10.1016/j.ijgo.2012.04.017

Alfirevic, Z., Keeney, E., Dowswell, T., Welton, N.J., Dias, S., Jones, L. V., Navaratnam, K., Caldwell, D.M., 2015. Labour induction with prostaglandins: a systematic review and network meta-analysis. Bmj 350, h217–h217. https://doi.org/10.1136/bmj.h217

Ambika, H.E., Bhat, S., Lepakshi, B.G., Savitha, C.S., 2017. Comparative study of efficacy and safety of vaginal misoprostol versus oral misoprostol in induction of labour when used in equal doses 6, 4–7.

Amorosa, J.M.H., Stone, J.L., 2015. Outpatient cervical ripening. Semin. Perinatol. 39, 488–494. https://doi.org/10.1053/j.semperi.2015.07.014

Ayton, R.A., Darting, G.M., Murkies, A.L., Farrell, E.A., Weisberg, E., Statistician, I.S., Fraser, I.S., 1996. A comparative study of safety and efficacy of continuous low dose oestradiol released from a vaginal ring compared with conjugated equine oestrogen vaginal cream in the treatment of postmenopausal urogenital atrophy. BJOG An Int. J. Obstet. Gynaecol. 103, 351–358. https://doi.org/10.1111/j.1471-0528.1996.tb09741.x

Baeten, J.M., Palanee-Phillips, T., Brown, E.R., Schwartz, K., Soto-Torres, L.E., Govender, V., Mgodi, N.M., Matovu Kiweewa, F., Nair, G., Mhlanga, F., Siva, S., Bekker, L.-G., Jeenarain, N., Gaffoor, Z., Martinson, F., Makanani, B., Pather, A., Naidoo, L., Husnik, M., Richardson, B.A., Parikh, U.M., Mellors, J.W., Marzinke, M.A., Hendrix, C.W., van der Straten, A., Ramjee, G., Chirenje, Z.M., Nakabiito, C., Taha, T.E., Jones, J., Mayo, A., Scheckter, R., Berthiaume, J., Livant, E., Jacobson, C., Ndase, P., White, R., Patterson, K., Germuga, D., Galaska, B., Bunge, K., Singh, D., Szydlo, D.W., Montgomery, E.T., Mensch, B.S., Torjesen, K., Grossman, C.I., Chakhtoura, N., Nel, A., Rosenberg, Z., McGowan, I., Hillier, S., 2016. Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women. N. Engl. J. Med. 375, 2121–2132. https://doi.org/10.1056/NEJMoa1506110

Barentsen, R., van de Weijer, P.H., Schram, J.H., 1997. Continuous low dose estradiol released from a vaginal ring versus estriol vaginal cream for urogenital atrophy. Eur. J. Obstet. Gynecol. Reprod. Biol. 71, 73–80.

Baum, M.M., Butkyavichene, I., Gilman, J., Kennedy, S., Kopin, E., Malone, A.M., Nguyen, C., Smith, T.J., Friend, D.R., Clark, M.R., Moss, J.A., 2012. An intravaginal

ring for the simultaneous delivery of multiple drugs. J. Pharm. Sci. 101, 2833–43. https://doi.org/10.1002/jps.23208

Bollapragada, S.S., Mackenzie, F., Norrie, J.D., Eddama, O., Petrou, S., Reid, M., Norman, J.E., 2009. Randomised placebo-controlled trial of outpatient (at home) cervical ripening with isosorbide mononitrate (IMN) prior to induction of labour - Clinical trial with analyses of efficacy and acceptability. the IMOP Study. BJOG An Int. J. Obstet. Gynaecol. 116, 1185–1195. https://doi.org/10.1111/j.1471-0528.2009.02216.x

Boyd, P., Fetherston, S.M., Mccoy, C.F., Major, I., Murphy, D.J., Kumar, S., Holt, J., Brimer, A., Blanda, W., Devlin, B., Malcolm, R.K., 2016. Matrix and reservoir-type multipurpose vaginal rings for controlled release of dapivirine and levonorgestrel. Int. J. Pharm. 511, 619–629. https://doi.org/10.1016/j.ijpharm.2016.07.051

Brache, V., Alvarez-Sanchez, F., Faundes, a, Jackanicz, T., Mishell, D.R., Lähteenmäki, P., 2000. Progestin-only contraceptive rings. Steroids 65, 687–91.

Brache, V., Payán, L.J., Faundes, A., 2013. Current status of contraceptive vaginal rings. Contraception 87, 264–272. https://doi.org/10.1016/j.contraception.2012.08.037

Bullarbo, M., Orrskog, M.E., Andersch, B., Granström, L., Norström, A., Ekerhovd, E., 2007. Outpatient vaginal administration of the nitric oxide donor isosorbide mononitrate for cervical ripening and labor induction postterm: a randomized controlled study. Am. J. Obstet. Gynecol. 196, 1–5. https://doi.org/10.1016/j.ajog.2006.08.034

Calder, A.A., Loughney, A.D., Weir, C.J., Barber, J.W., 2008. Induction of labour in nulliparous and multiparous women: A UK, multicentre, open-label study of intravaginal misoprostol in comparison with dinoprostone. BJOG An Int. J. Obstet. Gynaecol. 115, 1279–1288. https://doi.org/10.1111/j.1471-0528.2008.01829.x

Casper, F., Petri, E., 1999. Local Treatment of Urogenital Atrophy with an Estradiol-Releasing Vaginal Ring: A Comparative and a Placebo-Controlled Multicenter Study. Int. Urogynecol. J. Pelvic Floor Dysfunct. 10, 171–176. https://doi.org/10.1007/s001920050040

Chandak, A.R., Verma, P.R.P., 2008. [Design and development of hydroxypropyl methycellulose (HPMC) based polymeric films of methotrexate: physicochemical and pharmacokinetic evaluations]. Yakugaku Zasshi 128, 1057–66. https://doi.org/10.1248/yakushi.128.1057

Chanrachakul, B., Herabutya, Y., Punyavachira, P., 2002. Randomized trial of isosorbide mononitrate versus misoprostol for cervical ripening at term. Int. J. Gynecol. Obstet. 78, 139–145. https://doi.org/10.1016/S0020-7292(02)00128-5

Chanrachakul, B., Herabutya, Y., Punyavachira, P., 2000. Potential efficacy of nitric oxide for cervical ripening in pregnancy at term. Int. J. Gynecol. Obstet. 71, 217–219. https://doi.org/10.1016/S0020-7292(00)00284-8

Chaudhuri, S., Mitra, S.N., Banerjee, P.K., Biswas, P.K., Bhattacharyya, S., 2011. Comparison of vaginal misoprostol tablets and prostaglandin E2 gel for the induction of labor in premature rupture of membranes at term: A randomized comparative trial. J. Obstet. Gynaecol. Res. 37, 1564–1571. https://doi.org/10.1111/j.1447-

0756.2011.01575.x

Chwalisz, K., Shao-Qing, S., Garfield, R.E., Beier, H.M., 1997. Cervical ripening in guinea-pigs after a local application of nitric oxide. Hum. Reprod. 12, 2093–2101. https://doi.org/10.1093/humrep/12.10.2093

Derby, N., Aravantinou, M., Kenney, J., Ugaonkar, S.R., Wesenberg, A., Wilk, J., Kizima, L., Rodriguez, A., Zhang, S., Mizenina, O., Levendosky, K., Cooney, M.L., Seidor, S., Gettie, A., Grasperge, B., Blanchard, J., Piatak, M., Lifson, J.D., Fern?ndez-Romero, J., Zydowsky, T.M., Robbiani, M., 2017. An intravaginal ring that releases three antiviral agents and a contraceptive blocks SHIV-RT infection, reduces HSV-2 shedding, and suppresses hormonal cycling in rhesus macaques. Drug Deliv. Transl. Res. 2. https://doi.org/10.1007/s13346-017-0389-0

Devlin, B., Nuttall, J., Wilder, S., Woodsong, C., Rosenberg, Z., 2013. Development of dapivirine vaginal ring for HIV prevention. Antiviral Res. 100 Suppl, S3-8. https://doi.org/10.1016/j.antiviral.2013.09.025

Dieben, T.O.M., Roumen, F.J.M.E., Apter, D., 2002. Efficacy, cycle control, and user acceptability of a novel combined contraceptive vaginal ring. Obstet. Gynecol. 100, 585–93.

Elsokary, M., Abdelhamed, M., Mohamad, E., 2015. Isosorbide Mononitrate and Misoprostol for Cervical Ripening in Induction of Labor 15.

Embrey, M.P., Graham, N.B., McNeill, M.E., 1980. Induction of labour with a sustained-release prostaglandin E2 vaginal pessary. Br. Med. J. 281, 901–902.

Faundes, A., Hardy, E., Reyes, C., Pastene, L., Portes-Carrasco, R., 1981. Acceptability of the contraceptive vaginal ring by rural and urban population in two Latin American countries. Contraception 24, 393–414.

Fetherston, S.M., Boyd, P., McCoy, C.F., McBride, M.C., Edwards, K., Ampofo, S., Malcolm, R.K., 2013. A silicone elastomer vaginal ring for HIV prevention containing two microbicides with different mechanisms of action. Eur. J. Pharm. Sci. 48, 406–415. https://doi.org/10.1016/j.ejps.2012.12.002

Friend, D.R., 2011. Intravaginal rings: controlled release systems for contraception and prevention of transmission of sexually transmitted infections. Drug Deliv. Transl. Res. 1, 185–193. https://doi.org/10.1007/s13346-011-0024-4

Friend, D.R., Clark, J.T., Kiser, P.F., Clark, M.R., 2013. Multipurpose prevention technologies: products in development. Antiviral Res. 100 Suppl, S39-47. https://doi.org/10.1016/j.antiviral.2013.09.030

Frohn, W., 2002. Prostaglandin E2 gel vs misoprostol for cervical ripenings in patinets with premature rupture of membranes after34 weeks 99, 206–210.

Furukan, M., Rameez, M., Malik, I., Goonewardene, R., 2007. Nitric oxide donor isosorbide mononitrate for pre-induction cervical ripening at 41 weeks ' gestation : A randomized controlled trial 33, 452–456. https://doi.org/10.1111/j.1447-0756.2007.00573.x

Gramaglia, D., Conway, B.R., Kett, V.L., Malcolm, R.K., Batchelor, H.K., 2005. High speed DSC (hyper-DSC) as a tool to measure the solubility of a drug within a solid or semi-solid matrix. Int. J. Pharm. 301, 1–5.

492 https://doi.org/10.1016/j.ijpharm.2005.04.038
493 Habib, S.M., Emam, S.S., Saber, A.S., 2008. Outpatient cervical ripening with nitric oxide
494 donor isosorbide mononitrate prior to induction of labor. Int. J. Gynecol. Obstet. 101,
495 57–61. https://doi.org/10.1016/j.ijgo.2007.09.027
496 Kiser, P.F., Johnson, T.J., Clark, J.T., 2012. State of the art in intravaginal ring technology
497 for topical prophylaxis of HIV infection. AIDS Rev. 14, 62–77.
498 Ledingham, M., Thomson, A.J., Young, A., Macara, L.M., Greer, I.A., Norman, J.E., 2000.
499 Changes in the expression of nitric oxide synthase in the human uterine cervix during
500 pregnancy and parturition 6, 1041–1048.
501 Leopold, B., Sciscione, A., 2017. Is There a Place for Outpatient Preinduction Cervical
502 Ripening? Obstet. Gynecol. Clin. North Am. 44, 583–591.
503 https://doi.org/10.1016/j.ogc.2017.08.010
504 Lyrenäs, S., Clason, I., Ulmsten, U., 2001. In vivo controlled release of PGE2 from a
505 vaginal insert (0.8 mm, 10 mg) during induction of labour. BJOG 108, 169–78.
506 https://doi.org/10.1016/S0306-5456(00)00039-5
507 MacKenzie, I.Z., 2006. Induction of labour at the start fo the new millennium. Reproduction
508 131, 989–998. https://doi.org/10.1530/rep.1.00709
509 Malcolm, K., Woolfson, D., Russell, J., Tallon, P., Mcauley, L., Craig, D., 2003. Influence
510 of silicone elastomer solubility and diffusivity on the in vitro release of drugs from
511 intravaginal rings. J. Control. Release 90, 217–25.
512 Malcolm, R.K., Boyd, P., McCoy, C.F., Murphy, D.J., 2014. Beyond HIV microbicides:
513 multipurpose prevention technology products. BJOG An Int. J. Obstet. Gynaecol. 121,
514 62–69. https://doi.org/10.1111/1471-0528.12852
515 Malcolm, R.K., Boyd, P.J., McCoy, C.F., Murphy, D.J., 2016. Microbicide vaginal rings:
516 Technological challenges and clinical development. Adv. Drug Deliv. Rev. 103, 33–56.
517 https://doi.org/10.1016/j.addr.2016.01.015
518 Malcolm, R.K., Boyd, P.J., McCoy, C.F., Murphy, D.J., 2015. Microbicide vaginal rings:
519 Technological challenges and clinical development. Adv. Drug Deliv. Rev. 103, 33–56.
520 https://doi.org/10.1016/j.addr.2016.01.015
521 Malcolm, R.K., Edwards, K.-L., Kiser, P., Romano, J., Smith, T.J., 2010. Advances in
522 microbicide vaginal rings. Antiviral Res. 88 Suppl 1, S30-9.
523 https://doi.org/10.1016/j.antiviral.2010.09.003
524 Malcolm, R.K., Fetherston, S.M., McCoy, C.F., Boyd, P., Major, I., 2012. Vaginal rings for
525 delivery of HIV microbicides. Int. J. Womens. Health 4, 595–605.
526 https://doi.org/10.2147/IJWH.S36282
527 Malcolm, R.K., Woolfson, A.D., Toner, C.F., Morrow, R.J., Mccullagh, S.D., 2005. Long-
528 term, controlled release of the HIV microbicide TMC120 from silicone elastomer
529 vaginal rings. J. Antimicrob. Chemother. 56, 954–6. https://doi.org/10.1093/jac/dki326
530 McCoy, C.F., Murphy, D.J., Boyd, P., Derrick, T., Spence, P., Devlin, B., Malcolm, R.K.,
531 2017. Packing Polymorphism of Dapivirine and Its Impact on the Performance of a
532 Dapivirine-Releasing Silicone Elastomer Vaginal Ring. J. Pharm. Sci. 106, 2015–2025.

https://doi.org/10.1016/j.xphs.2017.04.026

Miller, H., Goetzl, L., Wing, D.A., Powers, B., Rugarn, O., 2016. Optimising daytime deliveries when inducing labour using prostaglandin vaginal inserts. J. Matern. Neonatal Med. 29, 517–522. https://doi.org/10.3109/14767058.2015.1011117

Montgomery, E.T., van der Straten, A., Chitukuta, M., Reddy, K., Woeber, K., Atujuna, M., Bekker, L.-G., Etima, J., Nakyanzi, T., Mayo, A.J., Katz, A., Laborde, N., Grossman, C.I., Soto-Torres, L., Palanee-Phillips, T., Baeten, J.M., MTN-020/ASPIRE Study, 2017. Acceptability and use of a dapivirine vaginal ring in a phase III trial. AIDS 31, 1159–1167. https://doi.org/10.1097/QAD.0000000000001452

Moss, J.A., Butkyavichene, I., Churchman, S.A., Gunawardana, M., Fanter, R., Miller, C.S., Yang, F., Easley, J.T., Marzinke, M.A., Hendrix, C.W., Smith, T.J., Baum, M.M., 2016. Combination Pod-Intravaginal Ring Delivers Antiretroviral Agents for HIV Prophylaxis: Pharmacokinetic Evaluation in an Ovine Model. Antimicrob. Agents Chemother. 60, 3759–3766. https://doi.org/10.1128/AAC.00391-16

Mulders, T.M.T., Dieben, T.O.M., 2001. Use of the novel combined contraceptive vaginal ring NuvaRing for ovulation inhibition. Fertil. Steril. 75, 865–870. https://doi.org/10.1016/S0015-0282(01)01689-2

Murphy, D.J., Desjardins, D., Dereuddre-Bosquet, N., Brochard, P., Perrot, L., Pruvost, A., Le Grand, R., Lagatie, O., Vanhooren, L., Feyaerts, M., van Roey, J., Malcolm, R.K., 2014. Pre-clinical development of a combination microbicide vaginal ring containing dapivirine and darunavir. J. Antimicrob. Chemother. 1–12. https://doi.org/10.1093/jac/dku160

Nachtigall, L.E., 1995. Clinical trial of the estradiol vaginal ring in the U.S. Maturitas 22, S43–S47. https://doi.org/10.1016/0378-5122(95)00963-9

Nel, A., Bekker, L., Bukusi, E., Hellström, E., Kotze, P., Louw, C., Martinson, F., Masenga, G., Montgomery, E., Ndaba, N., van der Straten, A., van Niekerk, N., Woodsong, C., 2016a. Safety, Acceptability and Adherence of Dapivirine Vaginal Ring in a Microbicide Clinical Trial Conducted in Multiple Countries in Sub-Saharan Africa. PLoS One 11, e0147743. https://doi.org/10.1371/journal.pone.0147743

Nel, A., van Niekerk, N., Kapiga, S., Bekker, L.-G., Gama, C., Gill, K., Kamali, A., Kotze, P., Louw, C., Mabude, Z., Miti, N., Kusemererwa, S., Tempelman, H., Carstens, H., Devlin, B., Isaacs, M., Malherbe, M., Mans, W., Nuttall, J., Russell, M., Ntshele, S., Smit, M., Solai, L., Spence, P., Steytler, J., Windle, K., Borremans, M., Resseler, S., Van Roey, J., Parys, W., Vangeneugden, T., Van Baelen, B., Rosenberg, Z., 2016b. Safety and Efficacy of a Dapivirine Vaginal Ring for HIV Prevention in Women. N. Engl. J. Med. 375, 2133–2143. https://doi.org/10.1056/NEJMoa1602046

Norman, J., 1996. Nitric oxide and the myometrium. Pharmacol. Ther. 70, 91–100. https://doi.org/https://doi.org/10.1016/0163-7258(96)00004-6

Novák, A., de la Loge, C., Abetz, L., van der Meulen, E.., 2003. The combined contraceptive vaginal ring, NuvaRing®: an international study of user acceptability. Contraception 67, 187–194. https://doi.org/10.1016/S0010-7824(02)00514-0

Oboro, V.O., Tabowei, T.O., 2005. Outpatient misoprostol cervical ripening without

575 subsequent induction of labor to prevent post-term pregnancy. Acta Obstet. Gynecol.
576 Scand. 84, 628–631. https://doi.org/10.1111/j.0001-6349.2005.00655.x
577 Owen, D.H., Katz, D.F., 1999. A vaginal fluid simulant. Contraception 59, 91–5.
578 Powers, B.L., Wing, D. a, Carr, D., Ewert, K., Di Spirito, M., 2008. Pharmacokinetic
579 profiles of controlled-release hydrogel polymer vaginal inserts containing misoprostol.
580 J. Clin. Pharmacol. 48, 26–34. https://doi.org/10.1177/0091270007309707
581 Rodrigues, R., Nunes, F., Tiago, D., Avillez, T., Vieira, a., Meirinho, M., 1998. Induction
582 of labor with intravaginal administration of misoprostol. Int. J. Gynecol. Obstet. 60,
583 233–237. https://doi.org/10.1016/S0020-7292(97)00248-8
584 Roumen, F.J., Apter, D., Mulders, T.M., Dieben, T.O., 2001. Efficacy, tolerability and
585 acceptability of a novel contraceptive vaginal ring releasing etonogestrel and ethinyl
586 oestradiol. Hum. Reprod. 16, 469–75.
587 Roumen, F.J., Dieben, T.O., 1999. Clinical acceptability of an ethylene-vinyl-acetate
588 nonmedicated vaginal ring. Contraception 59, 59–62.
589 Sanchez-Ramos, L., Peterson, D.E., Delke, I., Gaudier, F.L., Kaunitz, a M., 1998. Labor
590 induction with prostaglandin E1 misoprostol compared with dinoprostone vaginal
591 insert: a randomized trial. Obstet. Gynecol. 91, 401–5.
592 Sharma, Y., Kumar, S., Mittal, S., Misra, R., Dadhwal, V., 2005. Evaluation of glyceryl
593 trinitrate, misoprostol, and prostaglandin E 2 gel for preinduction cervical ripening in
594 term pregnancy. J. Obstet. Gynaecol. Res. 31, 210–215. https://doi.org/10.1111/j.1447-
595 0756.2005.00271.x
596 Sharp, A.N., Stock, S.J., Alfirevic, Z., 2016. Outpatient induction of labour in the UK: a
597 survey of practice. Eur. J. Obstet. Gynecol. Reprod. Biol. 204, 21–23.
598 https://doi.org/10.1016/j.ejogrb.2016.06.023
599 Smith, J.M., Moss, J.A., Srinivasan, P., Butkyavichene, I., Gunawardana, M., Fanter, R.,
600 Miller, C.S., Sanchez, D., Yang, F., Ellis, S., Zhang, J., Marzinke, M.A., Hendrix,
601 C.W., Kapoor, A., Baum, M.M., 2017. Novel multipurpose pod-intravaginal ring for
602 the prevention of HIV, HSV, and unintended pregnancy: Pharmacokinetic evaluation in
603 a macaque model. PLoS One 12, e0185946.
604 https://doi.org/10.1371/journal.pone.0185946
605 Soliman, A., 2013. A comparison of isosorbide mononitrate, misoprostol, and combination
606 therapy for preinduction cervical ripening at term: a randomized controlled trial. Tanta
607 Med. J. 41, 310. https://doi.org/10.4103/1110-1415.126184
608 Spence, P., Nel, A., van Niekerk, N., Derrick, T., Wilder, S., Devlin, B., 2016. Post-Use
609 Assay of Vaginal Rings (VRs) as a Potential Measure of Clinical Trial Adherence. J.
610 Pharm. Biomed. Anal. 125, 94–100. https://doi.org/10.1016/j.jpba.2016.03.023
611 Stephenson, M.L., Wing, D.A., 2015. A novel misoprostol delivery system for induction of
612 labor: Clinical utility and patient considerations. Drug Des. Devel. Ther. 9, 2321–2327.
613 https://doi.org/10.2147/DDDT.S64227
614 Stifani, B.M., Plagianos, M., Vieira, C.S., Merkatz, R.B., 2018. Factors associated with
615 nonadherence to instructions for using the Nestorone®/ethinyl estradiol contraceptive
616 vaginal ring. Contraception 97, 415–421.

https://doi.org/10.1016/j.contraception.2017.12.011

Thurman, A.R., Clark, M.R., Hurlburt, J.A., Doncel, G.F., 2013. Intravaginal rings as delivery systems for microbicides and multipurpose prevention technologies. Int. J. Womens. Health 5, 695–708. https://doi.org/10.2147/IJWH.S34030

Tschugguel, W., Schneeberger, C., Lass, H., Stonek, F., Zaghlula, M.B., Czerwenka, K., Schatten, C., Kaider, a, Husslein, P., Huber, J.C., 1999. Human cervical ripening is associated with an increase in cervical inducible nitric oxide synthase expression. Biol. Reprod. 60, 1367–72.

Ugaonkar, S.R., Clark, J.T., English, L.B., Johnson, T.J., Buckheit, K.W., Bahde, R.J., Appella, D.H., Buckheit, R.W., Kiser, P.F., 2015. An Intravaginal Ring for the Simultaneous Delivery of an HIV-1 Maturation Inhibitor and Reverse-Transcriptase Inhibitor for Prophylaxis of HIV Transmission. J. Pharm. Sci. n/a-n/a. https://doi.org/10.1002/jps.24551

Ugaonkar, S.R., Wesenberg, A., Wilk, J., Seidor, S., Mizenina, O., Kizima, L., Rodriguez, A., Zhang, S., Levendosky, K., Kenney, J., Aravantinou, M., Derby, N., Grasperge, B., Gettie, A., Blanchard, J., Kumar, N., Roberts, K., Robbiani, M., Fernández-Romero, J.A., Zydowsky, T.M., 2015. A novel intravaginal ring to prevent HIV-1, HSV-2, HPV, and unintended pregnancy. J. Control. Release 213, 57–68. https://doi.org/10.1016/j.jconrel.2015.06.018

Väisänen-Tommiska, M., Nuutila, M., Aittomäki, K., Hiilesmaa, V., Ylikorkala, O., 2003. Nitric oxide metabolites in cervical fluid during pregnancy: Further evidence for the role of cervical nitric oxide in cervical ripening. Am. J. Obstet. Gynecol. 188, 779–785. https://doi.org/10.1067/mob.2003.161

Vartiainen, J., Wahlström, T., Nilsson, C.G., 1993. Effects and acceptability of a new 17 beta-oestradiol-releasing vaginal ring in the treatment of postmenopausal complaints. Maturitas 17, 129–37.

Vidanagamage, R.S., Goonewardene, I.M.R., 2011. The efficacy of two different doses of vaginal isosorbide mononitrate in pre induction cervical ripening : a double blind randomised controlled trial 56, 91–100.

Vogel, J.P., Osoti, A.O., Kelly, A.J., Livio, S., Norman, J.E., Alfirevic, Z., 2017. Pharmacological and mechanical interventions for labour induction in outpatient settings. Cochrane Database Syst. Rev. https://doi.org/10.1002/14651858.CD007701.pub3

Weisberg, E., Fraser, I.S., Mishell, D.R., Lacarra, M., Barding, C.W., Bardin, C.W., 1995. The acceptability of a combined oestrogen/progestogen contraceptive vaginal ring. Contraception 51, 39–44.

Wing, D.A., 2008. Misoprostol vaginal insert compared with dinoprostone vaginal insert: a randomized controlled trial. Obstet. Gynecol. 112, 801–812. https://doi.org/10.1097/AOG.0b013e318187042e

Wing, D.A., Brown, R., Plante, L.A., Miller, H., Rugarn, O., Powers, B.L., 2013. Misoprostol Vaginal Insert and Time to Vaginal Delivery. Obstet. Gynecol. 122, 201–209. https://doi.org/10.1097/AOG.0b013e31829a2dd6

659 Woolfson, A.D., Malcolm, R.K., Gallagher, R.J., 2003. Design of a silicone reservoir
660 intravaginal ring for the delivery of oxybutynin. J. Control. Release 91, 465–476.
661 https://doi.org/10.1016/S0168-3659(03)00277-3
662 Zhang, Y., Wang, J., Yu, Y., Xie, C., Xiao, M., Ren, L., 2015. Misoprostol versus
663 prostaglandin E2 gel for labor induction in premature rupture of membranes after
664 34weeks of pregnancy. Int. J. Gynecol. Obstet. 130, 214–218.
665 https://doi.org/10.1016/j.ijgo.2015.04.031
666 Zieman, M., Fong, S.K., Benowitz, N.L., Banskter, D., Darney, P.D., 1997. Absorption
667 kinetics of misoprostol with oral or vaginal administration. Obstet. Gynecol. 90, 88–92.
668 https://doi.org/10.1016/S0029-7844(97)00111-7
669

Table and figure captions

Table 1. Parameters for DDU-4320 VRs loaded with cervical ripening agents. Standard deviation values were calculated based on four replicates.

Fig. 1. Cross-sectional views of (A) matrix rings, (B) reservoir rings and (C) orifice rings. D – core ring mold assembly. E – orifice ring mold assembly, showing the protruding pins for incorporation of orifices in the ring structure.

Fig. 2. Representative images of DDU-4320 VRs loaded with cervical ripening agents. F1-F4: matrix-type rings loaded with ISMN. F5-F6: reservoir-type or orifice-type rings loaded with ISMN; F9-F13: matrix-type or orifice-type rings containing MP.

Fig. 3. A: DSC thermographs of the non-medicated silicone elastomer (SE), ISMN (70% w/w dispersion in lactose), silicone elastomers loaded with ISMN, MP (1% w/w dispersion in HPMC) and silicone elastomers loaded with MP. Each concentration of ISMN or MP in the silicone elastomer is equivalent to the drug concentration in the ring formulation listed in the brackets. B: melting enthalpy of the crystalline ISMN as a function of the mass fraction of ISMN in silicone elastomer. The dotted line represents the result of the curve fitting.

Fig. 4. Daily release and cumulative release profiles for DDU-4320 VRs loaded with cervical ripening agents over 14 days in SVF. A and B: daily release versus time and cumulative release versus root time profiles of ISMN released from matrix-type rings loaded with 100

693 (F1), 500 (F2), 1000 (F3) and 1000 mg ISMN (without lactose, F4). C and D: daily release
694 and cumulative release of ISMN from Ring F5 (reservoir-type ring with half-length core), F6
695 (reservoir-type ring with full-length core), F7 (orifice-type ring with full-length core) and F8
696 (reservoir-type ring with full-length core, formulated with pure ISMN). The plot of F8 in
697 Graph B is not visible due to overlapping with the plot of F6. E and F: daily MP release versus
698 time plots and cumulative MP release versus root time plots for Ring F9. G and H: daily and
699 cumulative MP release versus time plots for Ring F10-13. I and J: daily ISMN release versus
700 time plots and cumulative ISMN release versus root time plots for Ring F13.