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Packing polymorphism of dapivirine and its impact on the performance of a dapivirine-releasing silicone elastomer vaginal ring

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N.B. Red text in this document highlights changes made to the manuscript since the original submission.

Abstract

A silicone elastomer vaginal ring device providing sustained release over 28 days of the antiretroviral microbicide dapivirine has recently completed Phase III clinical testing and showed moderate protection against HIV acquisition. Here, for the first time, and in support of the product licensure program, we report the impact of dapivirine packing polymorphism on the *in vitro* performance of the 25 mg dapivirine ring product. Thermal, particle size, powder x-ray diffraction and thermodynamic solubility analyses of dapivirine polymorphic forms I and IV, both of which are persistent at room temperature and with form I being the thermodynamically stable form, were conducted for micronized and non-micronized materials. Matrix-type silicone elastomer vaginal rings were manufactured and the impact of dapivirine polymorphism on key *in vitro* parameters (compression and tensile behaviour; content assay; in vitro release; residual content assay) was investigated. The data demonstrate that dapivirine packing polymorphism has no significant impact on *in vitro* performance of the 25 mg dapivirine vaginal ring.

1. Introduction

Many solid drug substances exist in different crystalline forms – known as packing polymorphs – that differ in their physical properties.[1] In some cases, these different crystalline forms of the drug substance can significantly affect the pharmacological performance of the drug product. One of the most widely reported examples is the influence of polymorphism on the oral bioavailability of the antiretroviral drug ritonavir.[2,3] Ritonavir exists in two major crystalline forms – forms I and II. In 1998, the unexpected appearance of the more stable (and therefore less soluble) form II during routine testing of the drug led to compromised oral bioavailability of the drug and ultimately removal of the oral capsule formulation from the market. Since this incident, the U.S. Food and Drug Administration (FDA) has focused increased attention on the potential impact of drug polymorphism on the performance of drug products and the measures taken to ensure that physical properties to not change during shelf life. It is therefore imperative that polymorphism is investigated during the drug product development process. Both the FDA and the International Council for Harmonisation (ICH) have published regulatory documents addressing pharmaceutical polymorphism.[4–6]

Dapivirine (DPV) is an experimental non-nucleoside reverse transcriptase inhibitor (NNRTI) that is currently being developed as a vaginal microbicide for prevention of sexual transmission of human immunodeficiency virus type 1 (HIV-1).[7–12] A wide range of formulation strategies have been reported for vaginal administration of DPV,[13–18] the most advanced and the most promising of which are silicone elastomer vaginal rings.[19–33] Two Phase III efficacy studies – The Ring Study (IPM027) and APSIRE (MTN-020) –

involving more than 4,500 women volunteers across southern and eastern Africa have recently been completed, designed to support licensure of a monthly matrix-type silicone elastomer vaginal ring containing 25 mg micronized DPV intended for 28-day continuous use (DPV Ring-004). The studies showed approximately 30% reduced incidence of HIV infection in women compared to a placebo, the first time two studies have confirmed statistically significant efficacy for a HIV microbicide.[27,33] The lower than anticipated protection rates were attributed to poor user adherence, an ongoing problem that has adversely affected clinical testing of HIV microbicides.[33–42] Post-hoc analyses of the DPV ring clinical data in The Ring Study and ASPIRE have revealed that rates of protection are very significantly increased (>60%) in sub-groups demonstrating increased adherence.[27,33]

Three crystalline polymorphic forms of DPV have been identified – forms I, II and IV (Figure 1).[22] A dichloromethane hemi-solvate stable up to 130 °C was originally identified as polymorphic form III. However, dichloromethane is no longer used in the DPV manufacturing process. Therefore, further work with this form was no longer relevant and was not pursued. The current method for chemical synthesis of DPV reproducibly produces the drug in packing polymorphic form I, which is the most stable form at room temperature [22]. To date, form I has been confirmed for all manufactured batches of micronized DPV used in clinical development [22].

DPV form I undergoes a solid-solid transition to form II at ~100 °C (Figure 2; can range from 96.9 to 110.3 °C), as evidenced by a small endothermic transition in the differential scanning calorimetry (DSC) trace.[20,23,26] The variation in solid-solid transition temperature

between form I and form II has been observed for different lots of form I; however, it could not be attributed to a single phenomenon. Upon further heating, DPV form II undergoes crystalline melting at ~220 °C (ranges from 217.9 to 226.9 °C), and then, upon cooling below 100 °C, form II instantaneously reverts to form I. Form I and form II are therefore related enantiotropically with a transition temperature close to 100 °C. The same polymorphic interconversion and crystalline melt transitions are also observed when DPV is incorporated into the silicone elastomer matrix of the Dapivirine Ring-004, indicating that there are no significant drug-polymer interactions.[23]

During development, DPV has also been observed in crystalline polymorphic form IV, which is stable at room temperature and forms when dapivirine is recrystallized from methanol at higher temperatures (Figure 1). Upon heating, it exhibits two endothermic transitions at 212 and 221 °C corresponding to transformation of form IV to form II and crystalline melting of form II, respectively (Figure 1).

In order to meet the requirements of the regulatory agencies, it is important to assess how polymorphism affects drug product performance. Surprisingly, this issue seems not to have been reported – at least in the scientific literature – for other vaginal ring products, despite an explicit understanding that different polymorphic forms of a drug can exhibit significantly different solubilities in the polymeric matrix and potentially result in different drug permeation rates. Since forms I and IV are the only DPV polymorphs stable at room temperature (which is the desired storage temperature of the vaginal ring product), this

study was conducted to evaluate the thermal properties and *in vitro* performance of vaginal rings containing 25mg DPV as either the form I or the form IV polymorph.

2. Materials and methods

2.1. Materials

Non-micronized DPV form I and form IV and micronized form I were supplied by S.A. Ajinomoto OmniChem n.v. (Wetteren, Belgium). DPV form IV was micronized by JetPharma (Balerna, Switzerland). MED-4870 addition-cure silicone elastomer (Parts A and B) and MED-360 silicone oil were purchased from NuSil Technology (Carpinteria, CA, USA). Potassium dihydrogen orthophosphate, potassium hydroxide and urea (AnalaR, analytical reagent grade) were purchased from VWR International Ltd. (Dublin, Ireland). Norethindrone was purchased from LGM Pharma, (Nashville, TN, USA). HPLC-grade 2-propanol (IPA) and acetonitrile, phosphoric acid (85% w/w in water), Tween 80, sodium chloride, calcium hydroxide, bovine serum albumin, lactic acid, acetic acid and glucose were all purchased from Sigma-Aldrich (Gillingham, UK). A Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK) was used to obtain HPLC-grade water. Simulated vaginal fluid + 0.2% (w/v) Tween 80 (SVF+Tween) release media was prepared according to a previously described method followed by addition of the Tween 80 component.[43]

2.2 Thermal analysis

The thermal stability of DPV forms I and IV were analysed by thermogravimetric analysis (TGA) using a TA Instruments Q50™ Thermogravimetric Analyser and a TA Instruments Differential Scanning Calorimeter Q20™ (TA Instruments, UK). For these experiments,

5–10 mg of sample was heated from 25 to 300 °C at 10 °C /min in an open aluminium pan under a nitrogen atmosphere. For differential scanning calorimetry (DSC) experiments, 5–7 mg of sample (either pure polymorph or 10% w/w DPV-loaded silicone elastomer) underwent heat-cool-heat cycles between 20 and 235 °C using a heating rate of 10 °C per min. The temperature range was selected to encompass the molding temperatures commonly used to fabricate DPV matrix-type rings via injection molding processes (160–180 °C). For each sample, onset temperature (°C), peak temperature (°C) and enthalpy (ΔH, J/g) values were recorded for each thermal transition observed.

2.3. Particle size analysis

The particle size distributions (PSDs) of micronized and non-micronized forms of both polymorphs were characterised using a Mastersizer 3000 (Malvern , UK) instrument fitted with an AERO S accessory. Approximately 1 g of material was weighed and added to the Venturi. Using an air pressure of 2 Bar(g), the hopper gap was sequentially raised in 0.5 mm steps from 0.5 mm and the feed rate increased to between 30 and 60% to provide a reasonable flow of powder into the instrument. The target obscuration range was 1–4 %. A minimum of six measurements of each sample were performed to give an estimate of the variability about the measurement.

2.4. Powder X-ray diffraction

Powder X-ray diffraction (PXRD) patterns of non-micronized and micronized DPV form I and IV powders were obtained using a X'PERT Pro MPR X-ray diffractometer (PANalytical Ltd., UK). Samples were pressed onto a zero background holder so that a

smooth, flat surface was achieved and mounted in a rotating sample holder. Samples were exposed to CuKα radiation (40 kV, 40 mA), scanned in continuous mode across the 2θ angular range of 3.0–90.0° with a step size of 0.016°.

2.5. Microscopy analysis

Digital microscopy was performed using a Keyence VHX-700F series Digital Microscope (Keyence Limited, UK) fitted with an RZ 20–200x wide-range zoom lens. A small sample was dusted onto a section of adhesive tape to provide a thin layer of powder for particle morphology (shape and size) evaluation.

2.6. Ring manufacture

Matrix-type vaginal rings containing 25 mg micronized and non-micronized DPV form I or form IV dispersed in MED-4870 silicone elastomer were manufactured using a Babyplast™ 6/10P injection molding machine (Chronoplast, Spain). DPV MED-4870 Part A premixes (100 g) were prepared by accurately weighing appropriate quantities of MED-4870 (97.5% w/w), MED-360 silicone oil (2.1875% w/w) and DPV (0.3125% w/w) into a sealed polypropylene container before mixing at 3000 rpm for 3 min in a DAC-150 FVZ-K Speedmixer™ (Hauschild, Germany). Part B premixes were manufactured using the same protocol. Four 100 g portions of premix A and premix B were prepared (800 g in total) for each DPV polymorph formulation. Premixes were stored at 4 °C until use. Immediately prior to injection molding, ~100 g portions each of Part A premix and Part B premix were sequentially added to a large plastic Speedmixer™ container until ~400 g in total had been transferred. The material was handmixed for 30 s, speedmixed at 2350 rpm

for 30 s and further speedmixed for 60 s at 1800 rpm. The silicone elastomer mix was transferred to a Babyplast™ cartridge which was then fitted into the Babyplast™ injection molding machine. Rings were manufactured at 185 °C for 60 s.

2.7. Ring appearance and weight

Ring weight, colour, external diameter (ExD) and cross-sectional diameter (CSD) were recorded in order to assess the consistency of ring physical parameters. Ten rings from each DPV polymorph formulation were randomly selected and evaluated. CSD and ExD were measured using digital callipers (RS Components, UK). Care was taken not to compress the ring during measurement.

2.8. Mechanical testing

In the absence of a ratified international standard on the mechanical testing of vaginal rings, the Food and Drug Administration's (FDA) Center for Drug Evaluation and Research (CDER) have published nonbinding recommendations to industry in respect of tests for vaginal microbicide drug product specification, which include the mechanical testing of ring devices.[44] Here, as part of ongoing efforts to establish practical test methods, we have applied mechanical test methods to vaginal rings based on the minimum requirements and test methods used for reusable silicone rubber contraceptive diaphragms, as described in ISO-8009:2014.

Shore A Hardness testing, also known as durometer testing, was performed on five rings randomly selected from each DPV polymorph production run. Measurement was carried

194 out using a Sauter HBA 100-0 graduated dial durometer (Sauter, Switzerland) calibrated
195 for Shore A hardness (arbitrary units). During testing the rings were placed on an
196 unyielding, flat surface. With the durometer held in a vertical position, the instrument's
197 indentor was pressed on the uppermost surface of the ring in a constant movement without
198 shocks until the presser foot was parallel to the ring surface. The maximum deflection on
199 the dial (0–100), representing the Shore Hardness was recorded. Four individual
200 measurements per ring were recorded.
201
202 Compression testing was performed using a TA.XTplus Texture Analyser (Stable
203 Microsystems, UK). Rings previously selected for non-destructive durometer testing were
204 placed in the appropriate holder and analysed in compression mode using a test speed of 2
205 mm/s and a target distance of 5.0 mm. Six compression cycles were performed, and the
206 last five values for the maximum compressive force exerted by the texture analyser
207 recorded. The first value is not recorded to allow the ring to stabilize in the holder during
208 the first compression cycle.
209
210 Tensile strength testing was also performed using the TA.XTplus Texture Analyser. Rings
211 were placed around upper and lower tensile grips and analysed in tension mode with a test
212 speed of 10 mm/s and a target force of 5 kg. The pass/fail criterion for tensile strength
213 testing was set at 5 kg i.e. if the ring withstood a force equivalent to 5 kg without rupture
214 then it was deemed acceptable.
215

216 ## 2.9. *In vitro* release testing

Twenty-four samples of each ring formulation were selected for *in vitro* release testing over a 30-day period – twelve rings for release into a 1:1 mixture of IPA+H_2O and twelve for release into SVF+Tween. Both media have been used routinely for *in vitro* release testing of silicone elastomer vaginal rings, and other vaginal formulations, containing highly lipophilic poorly water-soluble antiretroviral microbicides, including DPV.[17,19,20,23,25,26,31,45–47]. IPA/water is commonly used as a performance test to predict and monitor the consistency in manufacturing. SVF is intended to mimic the chemical composition of vaginal fluid, including pH and osmolarity matched to normal vaginal fluid.[43] However, solubility of DPV in SVF is impractically low (< 1 µg/mL),[22,46] and, as a result, *in vitro* release from vaginal rings into this medium does not correlate with *in vivo* release (as measured by residual drug content following clinical use). By comparison, use of SVF + 0.2% w/v Tween 80 closely mimics *in vivo* release,[27,48] and its use has been supported by regulatory authorities.

On Day 0, each ring was placed into a 250 mL glass, screw-top bottle containing 200 mL of either IPA+H_2O or SVF+Tween release medium and stored in a temperature-controlled orbital shaking incubator (37˚C, 60 rpm, 25 mm orbital throw). The release medium was sampled and completely replaced (100 mL) daily, with the exception of weekends where 200 mL was added. Drug release was quantified by reverse-phase HPLC with UV detection (Section 2.11).

2.10. Content assay and residual content testing

239 Both the total DPV content of manufactured rings and the residual content of rings after *in*
240 *vitro* release testing were assessed (n=6 per formulation per test). Rings were weighed and
241 then cut in half along the length of the ring. The ring halves were immediately transferred
242 into individually labelled 250 mL glass flasks containing 100 mL acetone. Flasks were
243 sealed and placed in a temperature-controlled orbital shaking incubator (37 °C, 60 rpm, 25
244 mm orbital throw). After 24 h, the flasks were removed and allowed to cool to room
245 temperature. A 1.00 mL aliquot of the acetone extraction solution was transferred to a 100
246 mL volumetric flask using a positive displacement pipette and diluted to volume with
247 methanol. Samples were allowed to stand at ambient temperature for 1 h before final
248 dilution to volume with methanol. Samples were transferred to HPLC vials and analysed
249 against standard solutions of known DPV concentration.

2.11. Solubility determination

Thermodynamic solubility of DPV (form 1 and form IV, micronised and non-micronised) was measured using the shake-flask method at 37 °C in both SVF+0.2% w/w Tween and 1:1 v/v IPA/water mixture. For SVF/Tween measurement, ~5 mg DPV was added to a glass vial followed by 5.00 mL SVF/Tween; for IPA/water measurement, ~40 mg DPV was added to a glass vial followed by 5.00 mL IPA/water. The sealed vials were placed in an orbital shaking incubator for 72 hr. While still in the incubator but with shaking stopped, 1.00 mL and 100 µL volumes of the saturated SVF/Tween and IPA/water solutions, respectively, were sampled from the vials using suitable micropipettes and placed in new glass vials, taking care not to sample the settled excess solid drug layer at the bottom of each vial. SVF/Tween samples were subsequently diluted twofold for HPLC analysis, while IPA/water samples were diluted by a factor of 100. Drug concentrations were subsequently quantified by HPLC. In a similar fashion, the solubilities of both DPV form 1 and form IV (micronized only) were measured in aqueous media at different pH values – 0.1M HCl, 0.01M HCl, pH2 (KCl/HCl), pH4 (acetate buffer), pH6 (phosphate buffer), pH8 (phosphate buffer). For each solubility measurement, the residual solids were analyzed by PXRD to determine the extent of form conversion during the solubility analysis and to ensure the results reflect the true solubility of each form.

2.12. HPLC Analysis

Samples for DPV content analysis in rings were analysed on a Waters HPLC system (Waters Corporation, Dublin, Ireland) consisting of a 1525 Binary HPLC pump with an in-line degasser AF unit, 1500 column heater, 717 Plus Autosampler and a 2487 dual wavelength absorbance detector. 10 µL of each content sample was injected onto a Kromasil C18 HPLC column (150 mm x 4.6 mm, 5 µm particle size). Column temperature was maintained at 25 °C and isocratic elution was performed using a mobile phase of 75% HPLC-grade methanol and 25% water with a flow rate of 0.75 mL/min and a run time of 15 min. DPV was detected at 257 nm after approximately 10.8 min.

In vitro release samples (25 µL) were injected onto a Thermo Scientific BDS Hypersil™ C18 HPLC column (150 mm x 4.6 mm, 3 µm particle size) fitted with a guard column. The column was held at 45 °C and isocratic elution was performed using a mobile phase of 45% HPLC-grade acetonitrile and 55% phosphate buffer (pH 3.0; 7.7 mM) with a run time of 8 min. DPV was detected at 240 nm after 6.1 min.

2.12. Statistical analyses

Where appropriate, data sets were analysed using a one-way ANOVA followed by post-hoc analysis using the Tukey-Kramer multiple comparisons test. Analysis was conducted using GraphPad Prism software and significance was noted for a P value of less than 0.05: * = significant ($0.01 < P < 0.05$), ** = very significant ($0.001 < P < 0.01$), *** = extremely significant ($P < 0.001$), ns = not significant ($P > 0.05$).

The similarity factor (f_2) – a logarithmic reciprocal square root transformation of one plus the mean squared (the average sum of squares) differences of drug percent dissolved between the test and the reference products – was calculated for ring dissolution data using Equation 1.[49,50] The similarity factor fits the result between 0 and 100. It is 100 when the test and reference profiles are identical and tends to 0 as the dissimilarity increases. FDA and EMEA recommend that two dissolution profiles are similar when f_2 has a value between 50 and 100 following testing of at least 12 individual dosage units.

$$f_2 = 50 \times \log\left(\left[1 + \frac{1}{n}\sum_{j=1}^{n}\left|R_j - T_j\right|^2\right]^{-0.5} \times 100\right)$$

Equation 1.

3. Results and Discussion

3.1. Thermal analysis

DPV form I and form IV polymorphs were initially tested using TGA to establish their thermal stability over the range of temperatures encountered during ring manufacture via injection molding. Both polymorphs were stable up to temperatures around 240 °C, with total percent weight loss less than 0.5% at 240 °C for both polymorphic forms (data not shown). The polymorphs were then examined by DSC using a heat-cool-heat cycle between 20 and 235 °C. Representative thermograms for the non-micronized forms of DPV form I and form IV are presented in Figure 2A and 2B, respectively. Table 1 displays the mean onset peak temperature (°C), the peak maximum temperature (°C) and the enthalpy (J/g) for each transition recorded in the thermograms.

Non-micronized DPV form I, the most stable polymorphic form of the compound at room temperature and the form produced in the synthesis of DPV, displayed characteristic melting endotherms at 101 °C and 220 °C during the first heat cycle (Figure 2A), attributed to the solid-solid I→II polymorphic transformation and the form II crystalline melt, respectively.[20,22,23] Upon cooling of this melt, an endothermic step-like shift associated with formation of amorphous DPV was observed around 80 °C. The second heat cycle then showed a glass transition (T_g) with amorphous relaxation close to 80 °C, followed by an exothermic recrystallization transition at 163 °C and the form II melt endotherm at 220 °C. Similar thermal behaviour was observed for micronized DPV form I (DSC trace not shown, but data presented in Table 1).

By comparison, the non-micronized DPV form IV showed two sharp melting endotherm transitions, one at 206 °C attributed to the solid-solid IV→II transition and the other at 220 °C due to crystalline melting of form II (Figure 2B). Micronized DPV form IV displayed a broader and smaller IV→II endothermic transition at ~190 °C compared to that observed for the non-micronized form IV material (DSC trace not shown, but data presented in Table 1), attributed to the smaller particle size of the micronized material and/or changes in crystallinity induced during the micronization process.

3.2. Particle size distribution

The PSDs of DPV form I and form II polymorphs are presented in Figure 3 for both non-micronized (nm) and micronized (m) material. The distributions were unimodal (modal particle diameters for forms $I_{(nm)}$, $IV_{(nm)}$), $I_{(m)}$ and $IV_{(m)}$ were 163, 76, 5.9 and 5.2 μm, respectively), except for an additional second smaller peak at 67 μm for the form $I_{(m)}$ material. A summary of the d_{90}, d_{50} and d_{10} values are presented in Table 2 alongside values quoted in supplied certificates of analysis (where available). The data in Table 2 indicates that the experimentally determined PSD values for non-micronized DPV form I were slightly larger than the values stated in the certificate of analysis, which may be due to slight differences in the method of analysis or powder sampling protocols. In particular, for larger particle size materials, sampling protocols can have a greater influence on the measured value. After micronization, the particle size distributions for both polymorphs were similar, with an overall tendency towards slightly smaller particles observed for the form IV sample, as evidenced both by the overlap of the distributions (Figure 3B) and the similarity of the values for d_{90}, d_{50} and d_{10} (Table 2). The other experimentally determined

particle size distribution values were in good agreement with those specified on the certificates of analysis.

3.3. Powder X-ray diffraction

The X-ray diffraction traces for non-micronized and micronized DPV form I and form IV materials are presented in Figure 4. Both DPV polymorphs are characterised by sharp diffraction peaks confirming the highly crystalline nature of the materials. No significant amorphous content was observed as indicated by the absence of broad peaks and halos. Comparison of traces obtained for the non-micronized and micronized forms of the same polymorph demonstrate a high degree of similarity with regard to peak positions, indicating that the micronization process does not significantly alter the crystal form of either polymorph. However, minor differences in peak intensities were observed, and may be due to a combination of factors including the particle (crystallite) size, orientation of the crystals (preferred or random), amount of powder applied to the background sample holder, or differences in powder packing within the sample holder. Both the non-micronized (Figure 4A) and micronized (Figure 4B) DPV form I diffraction patterns showed significant differences in diffraction peak positions when compared to the form IV materials. In particular, DPV form I traces exhibited distinct diffraction peaks at $2\theta = 5.2°$ and 10.3° not present in the form IV diffraction patterns.

366 *3.4. Microscopy*

367 Representative micrographs of micronized and non-micronized crystals of DPV form I and
368 IV are presented in Figure 5. The non-micronized materials showed large and highly
369 crystalline primary particles in the range of 50–350 μm (Figures 5A and 5B). DPV form
370 IV has a higher proportion of smaller crystals in the <100 μm range compared to DPV form
371 I, as confirmed by particle size distribution analysis (Figure 3). The micrographs of the
372 micronized DPV materials displayed particles significantly smaller in size (mostly <10
373 μm). Although the majority of the micronized material was present as small primary
374 particles, some larger agglomerations of particles were also visible.

375

376 *3.5. Ring appearance and weight*

377 All manufactured rings were free from visible foreign matter and had an off-white opaque
378 appearance consistent with uniform distribution of the white DPV powder throughout the
379 otherwise transparent silicone elastomer matrix. Mean ring weight, ExD and CSD for each
380 form I and form IV ring manufacturing batch (n=5 per batch) are recorded in Table 3. All
381 rings had weights ~8.0 g, CSDs ~7.6 mm and ExDs ~56.4 mm.

382

383 *3.6. Mechanical Testing*

384 Shore A Hardness measurements, recorded for sample rings from each manufacturing
385 batch and presented in Table 4, are close to 65. The product profile for MED-4870 states a
386 Shore A Hardness value of 70 for samples cured at 165°C (ASTM D2240). The differences
387 observed here are attributed to differences in the cure time temperature profile and the other
388 ingredients included in the formulation, which can have an effect on the mechanical

389 performance of the silicone elastomer. Although Shore A hardness measurement is
390 commonly used in the rubber industry as a standard indicator of mechanical performance,
391 it is regarded as a basic test and can provide only limited information regarding changes to
392 the mechanical properties of the rings. Since the ring surface is curved, the test performed
393 does not conform to ASTM D2240 or ISO 868:2003 testing standards for shore hardness,
394 which require test specimens to have a flat surface and be at least 6 mm (1/4 in) thick.
395
396 Compression testing to measure the maximum force required to compress a ring a distance
397 of 5 mm vertically was also performed for each ring formulation batch (n=5). The results,
398 presented in Figure 6, show that the mean maximum force required for compression of the
399 $DPV_{(m)}$ form I and form IV rings was similar for all manufacturing batches. No significant
400 batch-to-batch variability between rings of the same formulation was observed. Statistical
401 analysis confirmed that all ring batches tested had statistically similar mechanical
402 properties.
403
404 Tensile strength analysis was performed to assess the integrity of the rings on application
405 of a force equivalent to 5 kg. Ten rings of each formulation were analysed. All rings were
406 able to withstand a force equivalent to 5 kg without rupture (data not shown). This arbitrary
407 5 kg value has been used in the testing of other vaginal ring products (unpublished data).
408 In clinical use, however, vaginal ring devices are not likely to undergo extensive tensile
409 deformation. Therefore, the test is primarily used as a quality performance measure for
410 comparison of different ring formulations and manufacturing processes.
411

412 *3.7. In vitro release*

413 Mean daily and cumulative release versus time plots for both DPV forms I and IV from
414 matrix-type vaginal rings into IPA+H_2O and SVF+Tween media are presented in Figure 7.
415 The declining daily release values with time (Figures 7A and 7B) are indicative of $t^{½}$
416 kinetics and typical of permeation-controlled drug delivery systems comprising non-
417 biodegradable polymers containing excess solid drug within the matrix.[20,23,51,52] Daily DPV
418 release values were greater for release into IPA+H_2O compared with SVF+Tween across
419 all time points and for both form I and form IV rings, reflecting the higher solubility of
420 DPV in the solvent/water system. Mean day 1 release values for DPV into IPA+H_2O were
421 2459 and 2564 µg for form I and IV rings, respectively, decreasing to 191 and 183 µg,
422 respectively, on day 30. Thus, the d1/d30 release ratios for this release medium were 12.9
423 and 14.0 for form I and IV rings, respectively. Use of SVF+Tween as the release medium
424 produced significantly different (p-value < .00001) day 1 mean release values for the form
425 I and IV rings (349 and 578 µg, respectively), while mean day 30 release values were more
426 similar (116 and 106 µg, respectively; p-value .000019) (Figure 7B); the corresponding
427 d1/d30 release ratios were 3.0 and 5.5, respectively. It is therefore apparent that the
428 SVF+Tween release medium blunts the day 1 *in vitro* release value relative to the day 30
429 value, compared with the IPA+H_2O release medium. In general, greater variability is
430 observed with the SVF+Tween daily release values compared to those measured using
431 IPA+H_2O, reflecting differences in solvating power between the release media.
432
433 Release rates (µg /day$^{0.5}$) and coefficients of correlation (r^2) obtained from linear regression
434 analysis of the cumulative DPV release vs. root time plots are presented in Table 5.

Comparing the release between polymorphs reveals that the profiles are similar, with almost identical release into both release media. The only difference of note is increased DPV release over the first three days into SVF+Tween for the form IV polymorph (Figure 7B). Since there is no significant difference in SVF+Tween solubility between the polymorphs (Table 6), possible explanations include differences in silicone elastomer solubility between the two forms of DPV, or differences in drug distribution at the surface of the ring devices. Given that much greater variability in drug concentrations are observed in vaginal ring pharmacokinetic studies,[28,30,53–55] it is highly unlikely that these relatively small differences in *in vitro* release over early timepoints would be clinically significant. Comparing the line equation gradients of the cumulative release lines highlights the small differences observed in terms of the release between different polymorphs. This was confirmed by calculating the similarity factor (f_2) which has been recommended by the FDA for dissolution profile comparison.[50,56] As the mean cumulative DPV release did not exceed 55% in either case, all of the available release values were included in the calculations. Based on these results, calculated f_2 values were 98.5 for release into IPA+H_2O and 94.9 for release into SVF+Tween, both well above the value of 50 often used to indicate similarity. Interestingly, the *in vitro* cumulative release levels obtained with SVF+Tween over a 30-day period for both the form I and form IV rings were similar to 28-day *in vivo* release levels observed with IPM's 25 mg DPV matrix ring 004 (~4 mg).[33,39] This indicates that the SVF+Tween release media more closely mimics the amount of drug released *in vivo* than either SVF alone or the IPA+H_2O medium.

458 *3.8. Content and residual content*

459 Initial dapivirine content in the rings post-manufacture was 24.87 ± 0.16 and 25.82 ± 0.28
460 mg for rings containing form I and form IV dapivirine, respectively (equivalent to 99.5 and
461 103.3% of the nominal content value), as measured by a solvent extraction method, and
462 highlighting the consistency of the manufacturing process. Following completion of *in*
463 *vitro* release testing, all rings were tested for residual dapivirine content. The residual
464 content values were then combined with the cumulative release values and compared to
465 initial ring content values to assess mass balance. The data presented in Table 6
466 demonstrate almost identical cumulative release between the two polymorphs of 13.1 mg
467 and 4.5 mg into IPA+H_2O and SVF+Tween over 30 days. The amounts of DPV recovered
468 after *in vitro* release testing are also consistent with the slightly higher initial loading in the
469 rings containing form IV DPV compared to those containing form I. Thus, the calculated
470 initial loadings for each polymorph are higher for form IV at approximately 25.8 mg,
471 compared to form I at approximately 25.0 mg. These values fit very well with the initially
472 calculated content values of 25.8 mg and 24.9 mg for form IV and form I respectively.

473

474 *3.9. DPV solubility*

475 DPV, with an experimental pKa value of 5.54,[46] exhibits the typical weak base behaviour
476 of increased solubility as pH is lowered (Figure 8). Moreover, the solubility vs. pH profiles
477 are very similar for polymorphic forms I and IV, within the limits of experimental error.
478 The lower solubility values at pH 1 are due to the common ion effect (i.e. chloride ions)
479 associated with increased concentration of hydrochloric acid. Based on these *in vitro*
480 solubility data, DPV solubility at vaginal pH values typical of women of reproductive age

(typically between 3.5 and 7; the higher values are common with certain vaginal infections and in the presence of semen[43,57]) would lie within the range 0–15 µg/mL, which goes some way to explaining the wide variation in DPV pharmacokinetics measured in women during ring use.[14,28,53–55]

Experimentally determined values of thermodynamic solubility for DPV forms I and IV – micronised and non-micronised materials, and measured in both 1:1 v/v IPA/water and SVF+0.2% w/v Tween 80 – are presented in Table 8. As expected, DPV solubility in IPA/water (~1200 µg/mL) is significantly greater (by a factor of ~75) compared with solubility measured in SVF/Tween (~16 µg/mL). That in vitro DPV release from vaginal rings into these two release media does not differ by a similar factor is a consequence of the permeation-controlled release kinetics that apply to silicone elastomer vaginal rings, wherein molecular diffusion of drug through the silicone matrix is rate controlling.[51] The data also clearly illustrate that neither DPV particle size nor the polymorphic form of DPV influence the thermodynamic solubility value, irrespective of the release medium tested. PXRD analysis of the residual DPV material after preparation of saturated solutions confirmed the no form conversion was observed during the solubility analysis and indicating that the results reflect the true solubility of each form (Table 8).

4. Conclusions

This is the first report of the impact of drug polymorphism on the performance characteristics of a vaginal ring device. DPV form I and form IV polymorphs were distinguished using DSC, PXRD and solubility analyses. TGA demonstrated that both

polymorphs were thermally stable over the range of processing temperatures likely to be encountered during manufacture. Particle size analysis revealed a similar size distribution for micronized versions of both polymorphs whereas the non-micronized form I average particle size was slightly larger than form IV. Manufacture of silicone elastomer rings nominally containing 25 mg DPV produced rings with a mean content with 5% of the nominal value for both polymorphs. *In vitro* release testing of rings showed a very similar release profile for both polymorphs with similarity factor f_2 values greater than 90. An increase in the day 1 to day 3 release for the form IV polymorph compared to the form I polymorph was observed. Possible explanations for this difference include variations in dissolution rates between the two polymorphs and or different surface distributions from manufacture. DPV mass balance was achieved from residual content values plus the cumulative release values recorded into each media. Release of DPV into SVF+Tween over 30 days more closely matches the amount of DPV released *in vivo* over a similar time period than either IPA+H_2O or SVF only. Finally, no significant differences in thermodynamic solubility were observed for the various particle size and polymorphic forms of DPV.

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Declaration of interests

All authors declare no any actual or potential conflicts of interest.

Author contribution to manuscript

All authors contributed to the design of the study and drafting of the manuscript for submission. CFM, DJM, PB and KM performed the experimental work. All authors approved submission of the manuscript.

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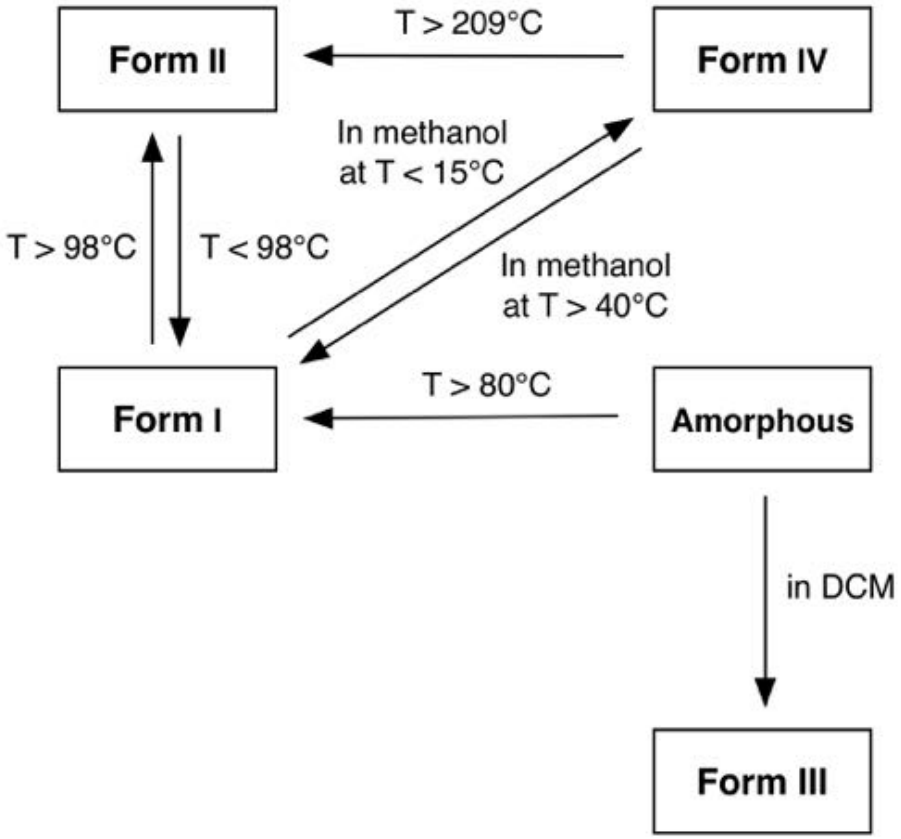
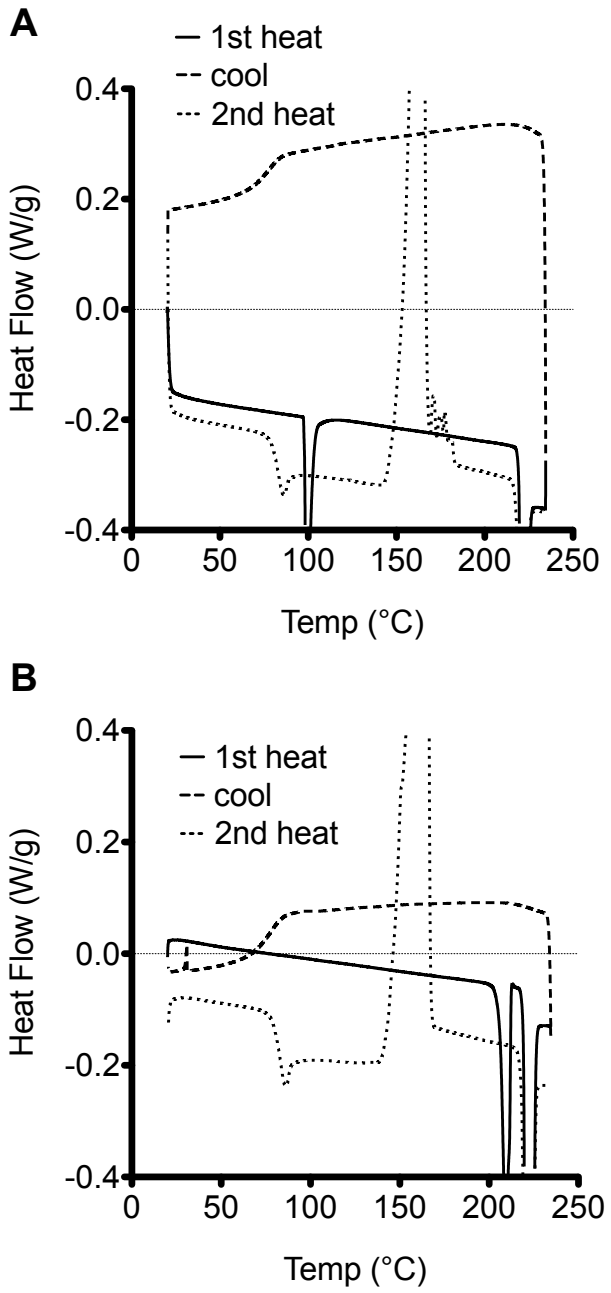
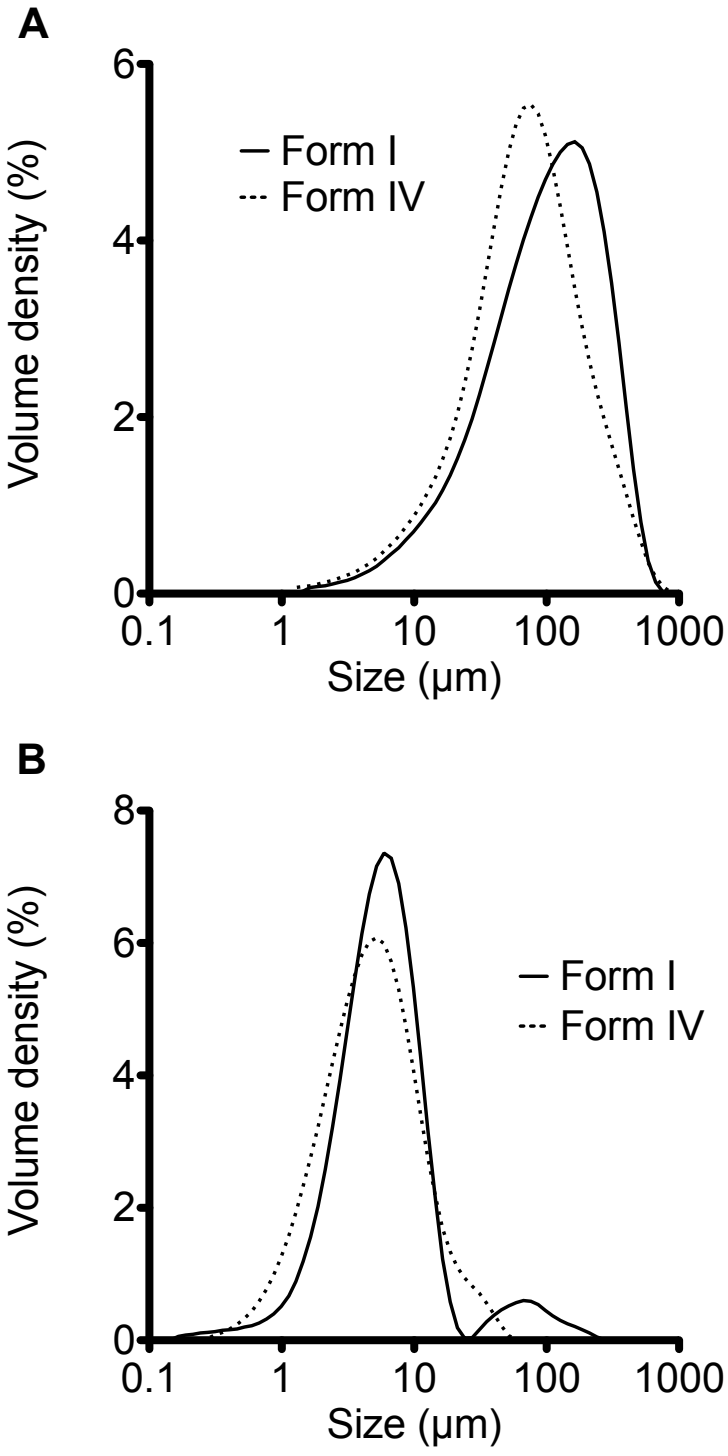


Figure 1. Summary of the relationships between the crystalline and amorphous polymorphic forms of dapivirine. DCM = dichloromethane. Forms I and IV were characterized by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), polarized light microscopy, hot stage microscopy, x-ray powder diffraction (XRPD), variable temperature XRPD (VT-XRPD) and single crystal x-ray diffraction. Both forms were also tested by gravimetric vapour sorption (GVS) to assess hygroscopicity, as well as solubility in common aqueous and organic solvents. [Unpublished data; IPM].



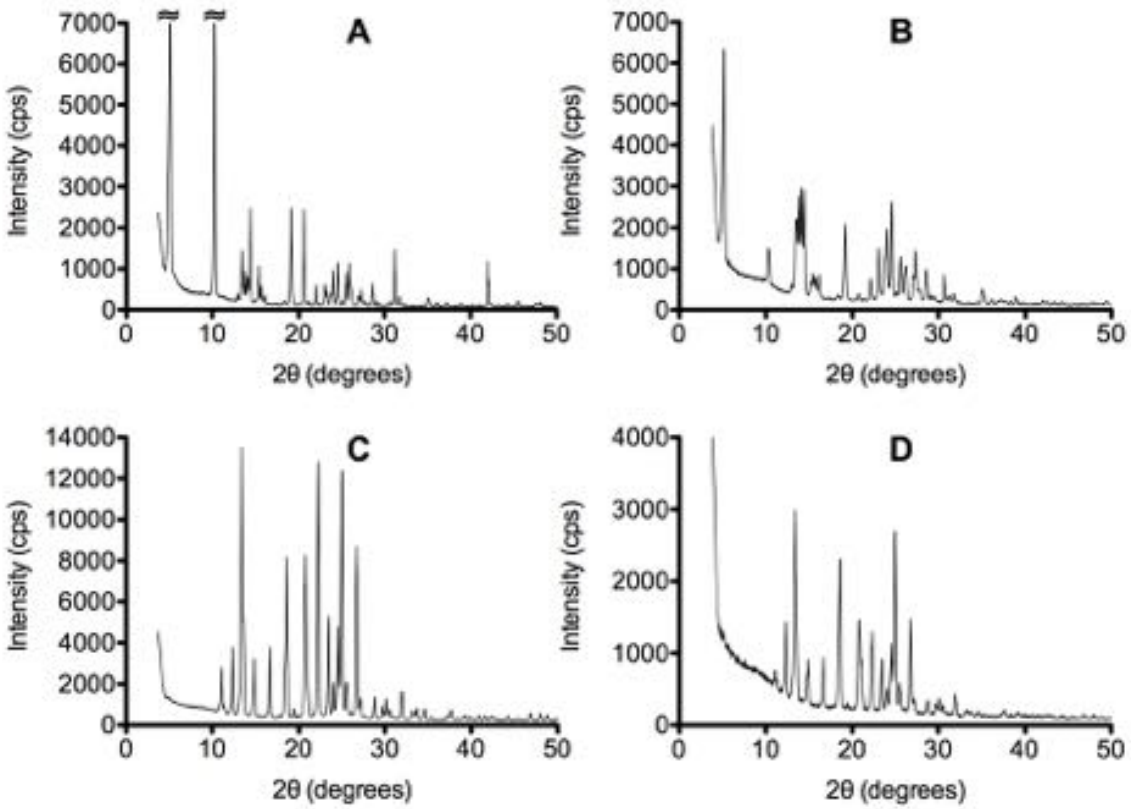
733

734 **Figure 2.** Representative DSC traces of non-micronized DPV (A) form I and (B) form IV.
735 For clarity, heat flow values between -0.4 and 0.4 are displayed, such that some peaks are
736 truncated. Values of the enthalpies associated with each endotherm and exotherm are
737 presented in Table 1. The second heat cycle for form IV has been offset by -0.1 W/g to aid
738 visualisation.



739

740 **Figure 3.** Measured particle size distribution of DPV form I and form IV. (A) non-
741 micronized powders; (B) micronized powders.



742

743 **Figure 4**. Powder XRD traces for (A) non-micronized DPV form I, (B) micronized DPV form I,
744 (C) non-micronized DPV form IV, and (D) micronized DPV form IV. Data is presented in the 2θ
745 angular range of 3 to 50°. Two peaks in A, at 2θ = 5.2 and 10.3 degrees, have been truncated to
746 allow better comparison of the traces.

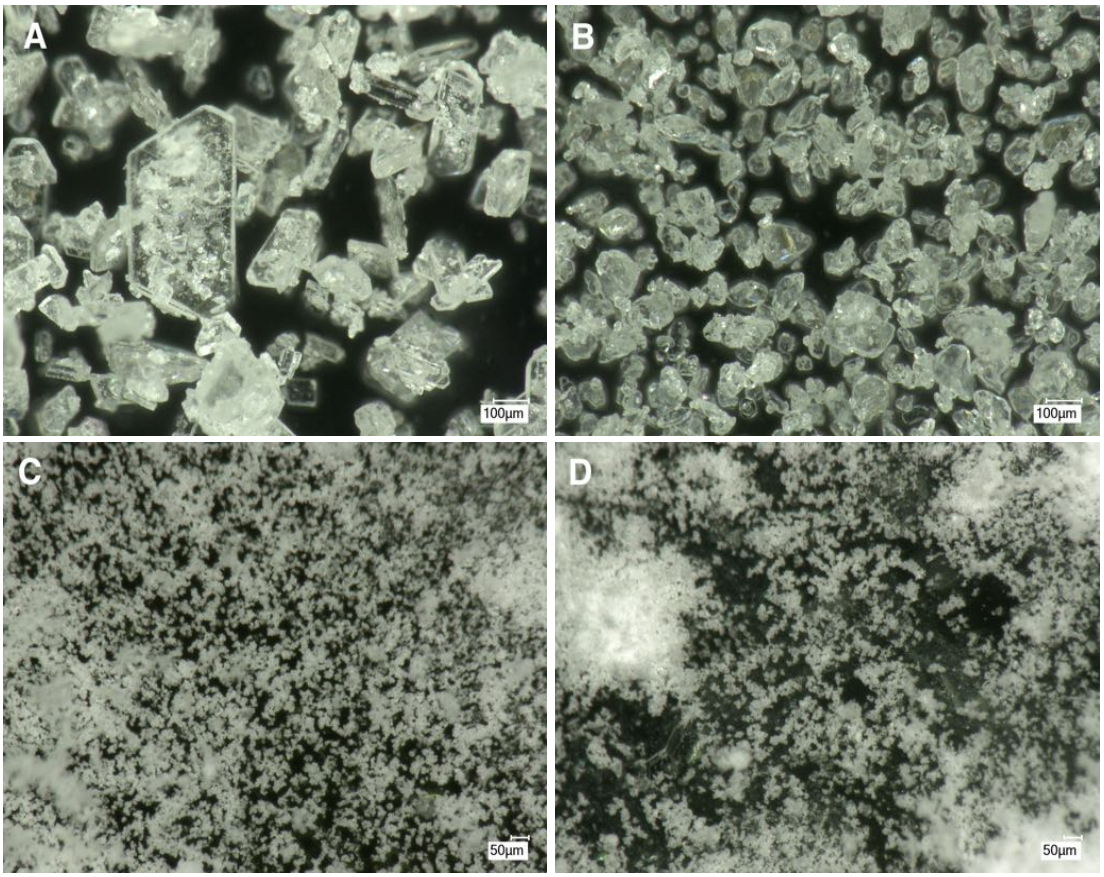
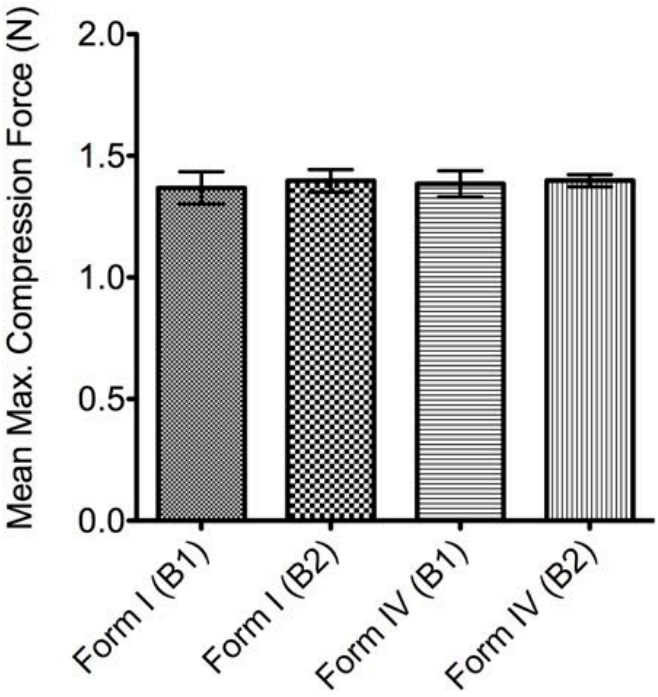


Figure 5. Representative micrographs recorded at 200x magnification of non-micronized DPV form I (A), form IV (B), and micronized DPV form I (C) and form IV (D).



753

754 **Figure 6.** Mean maximum force required to compress each ring formulation (n=5 per batch).

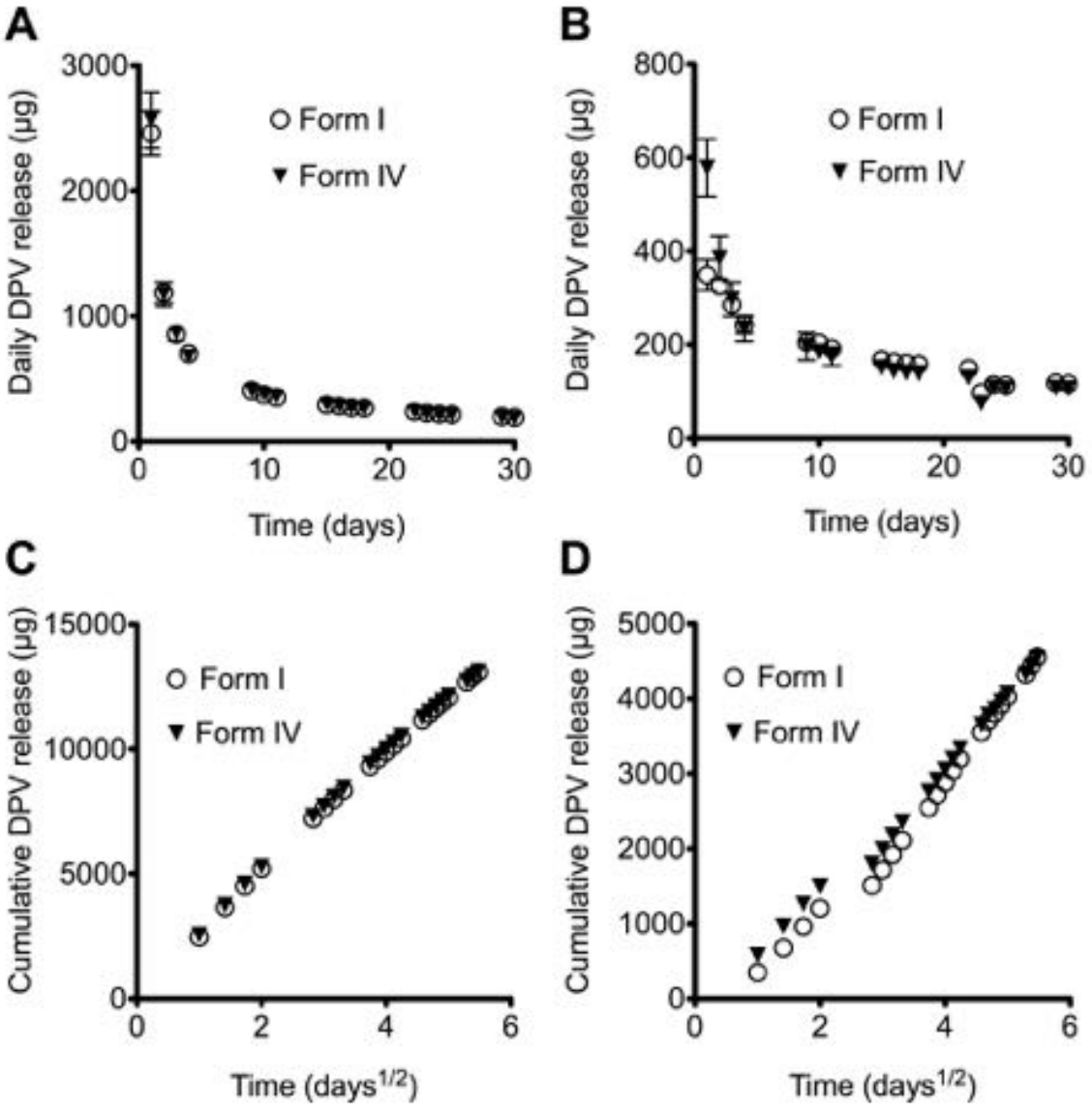
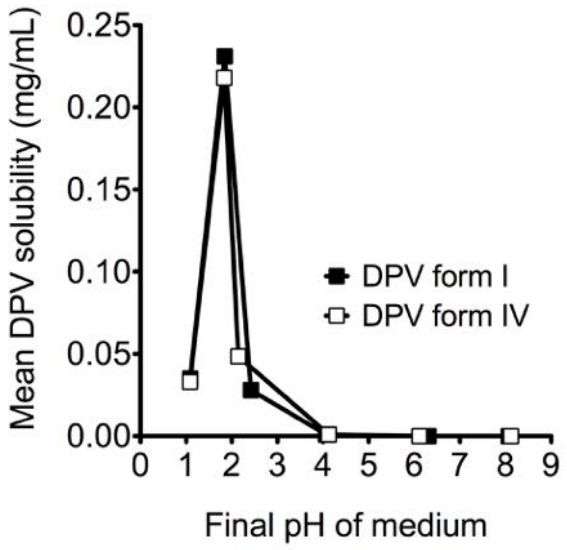


Figure 7. Mean daily release versus time profiles for release into (A) IPA+H_2O and (B) SVF+Tween, and cumulative release versus root time profiles for release into (C) IPA+H_2O and (D) SVF+Tween, of DPV from MED-4870 matrix-type vaginal rings containing DPV (either form I or form IV, 25 mg per ring) over 30 days. Error bars in graphs A and B represent standard deviation of twelve replicates; error bars were often smaller than the plot symbol. A small deviation from the otherwise very consistent drug release profile is present on day 22 of the release into SVF+Tween (B and D). This was due to an extended weekend release period without replacement of release medium.



765

766 **Figure 8.** pH versus solubility profiles for DPV forms I and IV. Plot symbols represent the mean
767 of four replicates; error bars representing ± standard deviation are smaller than the plot symbols.

768 **Table 1.** Mean peak onset temperature (°C), peak temperature (°C) and enthalpy (ΔH, J/g) values
769 for each thermal transition associated with micronized and non-micronized DPV forms I and IV.
770 Endothermic transitions 1 & 2 are observed during the 1st heat cycle, endothermic transitions 3 &
771 5 and exothermic transition 4 are observed during the 2nd heat cycle.
772

DPV material *	Transition No.	Onset (˚C)	Peak Maximum (˚C)	Enthalpy (ΔH, J/g)	Assignment
form I (m)	1	101.1	104.1	8.0	I→II
form I (nm)	1	97.8	99.3	10.4	I→II
form IV (nm)	1	205.8	209.3	10.9	IV→II
form IV (m)	1	189.4	199.0	8.0	IV→II
form I (m)	2	219.9	221.9	114.7	II melting
form I (nm)	2	219.9	221.9	119.2	
form IV (nm)	2	220.0	221.8	121.9	
form IV (m)	2	220.1	221.8	104.2	
form I (m)	3	80.9	85.6	1.6	T_g with amorphous relaxation
form I (nm)	3	80.9	85.5	1.8	
form IV (nm)	3	81.2	85.6	1.4	
form IV (m)	3	81.2	85.7	1.6	
form I (m)	4	163.0	167.9	-82.9	Recrystallization to form II
form I (nm)	4	159.8	167.4	-87.4	
form IV (nm)	4	154.4	163.0	-87.4	
form IV (m)	4	153.8	164.8	-68.4	
form I (m)	5	219.6	221.9	112.8	II melting
form I (nm)	5	219.5	221.7	117.0	
form IV (nm)	5	219.7	221.9	118.1	
form IV (m)	5	219.8	221.9	100.2	

773 * nm – non-micronized, m – micronized

774 **Table 2**. Experimentally determined d_{90}, d_{50} and d_{10} values for both non-micronized and
775 micronized DPV form I and form IV materials with comparative certificate of analysis values
776 where available.
777

DPV Batch *	Experimentally Determined Particle Size (µm)			CoA# Specified Particle Size (µm)		
	d_{90}	d_{50}	d_{10}	d_{90}	d_{50}	d_{10}
form I $_{(nm)}$	324	111	22.1	302	101	19
form I $_{(m)}$	14.7	6.00	2.20	14.0	5.9	2.0
form IV $_{(nm)}$	250	74.4	17.8	N/A	N/A	N/A
form IV $_{(m)}$	14.5	5.00	1.58	14.6	4.82	0.557

778
779
780
781
782
783 * nm – non-micronized, m – micronized; # CoA – certificate of analysis

784 **Table 3.** Mean ring weight, external diameter and cross-sectional diameter for five rings assessed
785 from each micronized DPV manufacturing batch.
786

DPV polymorph (Batch No.)	Ring Weight (Mean ± SD; g)	C.S.D. (Mean ± SD; mm)	Mean Ex.D. ± SD (mm)
form I (B1)	7.93 ± 0.24	7.58 ± 0.10	56.41 ± 0.04
form I (B2)	7.99 ± 0.01	7.62 ± 0.01	56.41 ± 0.03
form IV (B1)	7.99 ± 0.06	7.62 ± 0.01	56.39 ± 0.02
form IV (B2)	8.05 ± 0.01	7.62 ± 0.02	56.39 ± 0.02

787 B1 – batch 1, B2 – batch 2; acceptable limits for weight (7.2 – 8.8 g), external diameter (Ex.D.; 54.9 – 57.1 mm) and
788 cross sectional diameter (C.S.D.; 7.3 – 8.1 mm).
789

Table 4. Mean Shore A hardness measurement for five rings assessed from each micronized DPV manufacturing batch.

Batch Details	Shore A Hardness ± SD (arbitrary units)
DPV form I (B1)	64.9 ± 1.0
DPV form I (B2)	65.1 ± 0.5
DPV form IV (B1)	65.1 ± 0.3
DPV form IV (B2)	65.7 ± 0.2

B1 – batch 1, B2 – batch 2

795 **Table 5.** Release rates and coefficients of correlation (r^2) obtained from linear regression analysis
796 of the cumulative DPV release vs. root time plots for matrix-type vaginal rings containing different
797 forms of micronized DPV released into IPA+H_2O or SVF+Tween.

798

DPV type	Release medium	Release rate (µg/day $^{0.5}$)	r^2 value
form I	IPA+H_2O	2330	0.9983
form IV	IPA+H_2O	2323	0.9980
form I	SVF+Tween	959.9	0.9823
form IV	SVF+Tween	887.8	0.9880
form I	SVF+Tween (day 8-30)	1146.4	0.9993
form IV	SVF+Tween (day 8-30)	1027.5	0.9995

799

Table 6. Thermodynamic solubility values for DPV forms I and IV, micronized and non-micronized, into SVF + 0.2%Tween 80 and 1:1 v/v IPA/water. Both release media have been used routinely throughout the development process for the DPV-releasing vaginal ring. Solubility values are reported as mean ± SD of n=4 replicates.

DPV polymorph	Solvent system	DPV solubility at 37 °C (Mean ± SD; µg/mL)	PXRD analysis of residual solid
form I $_{(nm)}$	SVF + 0.2% (w/v) Tween 80	16.78 ± 0.66	form I
form I $_{(m)}$	SVF + 0.2% (w/v) Tween 80	16.12 ± 0.29	form I
form I $_{(nm)}$	IPA/water (1:1 v/v)	1171 ± 53	form I
form I $_{(m)}$	IPA/water (1:1 v/v)	1249 ± 46	form I
form IV $_{(nm)}$	SVF + 0.2% (w/v) Tween 80	14.74 ± 0.99	form IV
form IV $_{(m)}$	SVF + 0.2% (w/v) Tween 80	15.83 ± 0.14	form IV
form IV $_{(nm)}$	IPA/water (1:1 v/v)	1193 ± 36	form IV
form IV $_{(m)}$	IPA/water (1:1 v/v)	1214 ± 34	form IV

Table 7. Amount of DPV released, residual DPV content and calculated initial content values for 25 mg (nominally) DPV polymorph rings.

DPV polymorph	Release medium	DPV released (mg)	Residual DPV (mg)	Calculated initial DPV content (mg)
form I	IPA+H_2O	13.1 ± 0.2	12.0 ± 0.3	25.1 ± 0.4
form IV	IPA+H_2O	13.1 ± 0.5	12.6 ± 0.3	25.7 ± 0.3
form I	SVF+Tween	4.6 ± 0.1	20.3 ± 0.4	24.9 ± 0.3
form IV	SVF+Tween	4.5 ± 0.4	21.3 ± 0.4	25.9 ± 0.5