Physicochemical considerations in the formulation development of silicone elastomer vaginal rings releasing 5-nitroimidazole drugs for the treatment of bacterial vaginosis

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Physicochemical considerations in the formulation development of silicone elastomer vaginal rings releasing 5-nitroimidazole drugs for the treatment of bacterial vaginosis

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ABSTRACT

Bacterial vaginosis (BV) is a common dysbiosis of the human vaginal microbiota characterized by depletion of hydrogen peroxide and lactic acid-producing Lactobacillus bacteria and an overgrowth of certain facultative anaerobic bacteria. Although short-term cure rates following treatment with frontline antibiotics (most notably oral metronidazole (MNZ), clindamycin vaginal cream, and MNZ vaginal gel) are generally high, longer-term recurrence rates are an issue. The development of vaginal formulations offering continuous/sustained administration of antibiotic drugs over one or more weeks might prove useful in reducing recurrence. Here, we report the manufacture and preclinical testing of matrix-type vaginal rings offering sustained release of four 5-nitroimidazole antimicrobial drugs either being used clinically or having potential in treatment of BV – MNZ, tinidazole (TNZ), secnidazole (SNZ) and ornidazole (ONZ). All four drugs showed good compatibility with a medical-grade addition-cure silicone elastomer based upon thermal analysis experiments, and matrix-type rings containing 250 mg (3.125 %w/w) of each drug were successfully manufactured by reaction injection molding. 28-day in vitro drug release studies demonstrated root-time kinetics, with daily release rates of 25, 22, 9 and 6 mg/day for SNZ, ONZ, MNZ and TNZ, respectively. The rank order of drug release from rings correlated with the simple molecular permeability parameter $S/V$, where $S$ is the measured drug solubility in silicone fluid and $V$ is the drug molecular volume. The relative merits of SNZ and ONZ over MNZ (the current reference treatment) are discussed. The data support development of vaginal rings for sustained release of 5-nitroimidazole compounds for treatment of BV.

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Intravaginal ring
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1. Introduction

Bacterial vaginosis (BV) is a common dysbiosis of the human vaginal microbiota, characterized by a shift from *Lactobacillus* dominance (which helps maintain the normal healthy acidic vaginal pH by metabolizing glycogen to lactic acid) to a high diversity microbiome having increased *Gardnerella vaginalis*, *Atopobium vaginae* and other anaerobic microorganisms and elevated vaginal pH (Shipitsyna et al., 2013). In the United States, the Centers for Disease Control and Prevention (CDC) recommend various first-line treatments for BV in nonpregnant women, including oral metronidazole (MNZ; 500 mg tablet twice daily for seven days), clindamycin vaginal cream (2% once daily for seven days), and MNZ vaginal gel (0.75% twice daily for five days) (Walensky et al., 2021) (Table 1). In the UK, according to the National Institute for Health and Care Excellence (NICE), oral MNZ is the treatment of choice for the treatment of BV in both non-pregnant and pregnant women, although MNZ vaginal gel and clindamycin vaginal cream have been shown to achieve similar clinical cure rates (Ferris et al., 1995; Li et al., 2020). The 5-nitroimidazole compounds MNZ, tinidazole (TNZ) and secnidazole (SNZ) have limited or no activity against *lactobacilli* recovered from the vagina (Armstrong and Wilson, 2009; Austin et al., 2006) – a preferred characteristic for an antibiotic used in topical vaginal therapy – while clindamycin does show *lactobacillus* activity (Aroucheva et al., 2001; Austin et al., 2005; Petrina et al., 2017).

TNZ, SNZ and ornidazole (ONZ) are second-generation 5-nitroimidazole drugs either being used or considered as new therapeutic strategies to treat BV, and particularly recurrent BV which is associated with clinical resistance to MNZ (Bradshaw et al., 2006). These 5-nitroimidazole drugs have similar chemical structures, each containing a nitro group at position 5 on the imidazole ring and different substituents at position 2 (Table 2). The drugs share a common spectrum of activity...
against anaerobic microorganisms associated with BV and have similar efficacy in their treatment of BV (Abd El Aziz et al., 2019; Petrina et al., 2017). In fact, these 5-nitroimidazole drugs are prodrugs, activated by reduction of the nitro group in the target microbe followed by covalent attachment of the corresponding nucleic acid (Edwards, 1980). Marketed pharmaceutical products containing 5-nitroimidazoles for the treatment of BV and other pharmaceutical formulations in development are summarized in Table 1.

Five-day treatment with MNZ vaginal gel (0.75%) is a common treatment regimen for BV (Sexually Transmitted Infections Treatment Guidelines, 2021). In a clinical study comparing the efficacy, safety and patient acceptability of four MNZ regimens – MNZ gel 1.3% (75 mg) administered once daily for 5 days/weeks have the potential to further enhance patient acceptability and decrease BV recurrence rates. However, the silicone elastomers commonly used to fabricate vaginal rings are very different from the water-based vaginal gel formulations currently marketed, and the relatively water-soluble 5-nitroimidazole drugs used to treat BV have distinctly different physicochemical properties to the poorly water-soluble steroid drugs found in most marketed silicone elastomer vaginal ring products. Both hydrophilic thermoplastic polyurethane vaginal rings containing and releasing MNZ for up to seven days and poly(ε-caprolactone) matrices loaded with MNZ have been reported previously (Pathak et al., 2014; Verstraete et al., 2017).

In this study, we manufactured matrix-type silicone elastomer vaginal rings containing four different 5-nitroimidazole drugs suitable for the treatment of BV – TNZ, ONZ, SNZ, MNZ. Given the different physicochemical properties of these drugs, we assessed how their properties influenced the formulation performance and in vitro drug release characteristics. Specifically, (i) compatibility between the drugs and silicone elastomers was assessed using DSC analysis; (ii) UPLC-UV methods were developed and validated for the quantification of the four drugs; (iii) matrix-type silicone elastomer vaginal rings containing 200 mg (3.125 %w/w) of each drug were manufactured and tested for 28-day in vitro release; and (iv) a simple permeability model was developed to explain the rank order of in vitro release.

Therefore, higher dosing (75 mg vs. 37.5 mg) and reduced frequency (1 day vs. 5 days) correlated with increased patient acceptability without compromising efficacy or incidence of recurrence.

Vaginal MNZ gels typically contain 37.5 mg or 75 mg per application. By comparison, much larger vaginal dosages are often required for other 5-nitroimidazole drugs when used to treat BV, e.g., 500 mg ONZ vaginal tablet and 300 mg TNZ vaginal ovule (Table 1). A study comparing the efficacy of 500 mg ONZ vaginal ovules versus 500 mg ONZ vaginal tablets showed that these two formulations had similar cure rates (94% vs. 92%) according to Nugent scores and no safety concerns (Baloglu et al., 2003). A multi-center, open-label study by Regidor and Sailer explored the efficacy and safety of a vaginal suppository (vaginal ovulum, Gynomax XL®) containing 300 mg TNZ, 200 mg tinocazole and 100 mg lidocaine in the treatment of BV, vulvovaginal candidiasis, and co-infections. 67 patients diagnosed with a vaginal infection were treated once daily for three consecutive days with the Gynomax XL® (Regidor and Sailer, 2019). Patients achieved complete clinical recovery at day 10 (80.6%) and day 30 (86.6%), respectively. This 300 mg TNZ formulation showed high efficacy and safety in the therapy of BV and vulvovaginal candidiasis.

MNZ-releasing vaginal rings in which MNZ is incorporated into the ring device during manufacture and, upon vaginal insertion, is released in a sustained and continuous fashion over an extended period, e.g., days/weeks have the potential to further enhance patient acceptability and decrease BV recurrence rates. However, the silicone elastomers commonly used to fabricate vaginal rings are very different from the water-based vaginal gel formulations currently marketed, and the relatively water-soluble 5-nitroimidazole drugs used to treat BV have distinctly different physicochemical properties to the poorly water-soluble steroid drugs found in most marketed silicone elastomer vaginal ring products. Both hydrophilic thermoplastic polyurethane vaginal rings containing and releasing MNZ for up to seven days and poly(ε-caprolactone) matrices loaded with MNZ have been reported previously (Pathak et al., 2014; Verstraete et al., 2017).

Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>MNZ</th>
<th>ONZ</th>
<th>SNZ</th>
<th>TNZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical structure</td>
<td><img src="image1.png" alt="Chemical structure" /></td>
<td><img src="image2.png" alt="Chemical structure" /></td>
<td><img src="image3.png" alt="Chemical structure" /></td>
<td><img src="image4.png" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Relative molecular weight (g/mol)</td>
<td>171.2</td>
<td>219.6</td>
<td>185.2</td>
<td>247.3</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>160</td>
<td>82</td>
<td>76</td>
<td>127</td>
</tr>
<tr>
<td>Experimental log P</td>
<td>-0.10</td>
<td>-0.60</td>
<td>-0.37</td>
<td>-0.36</td>
</tr>
<tr>
<td>Predicted log P **</td>
<td>-0.46, -0.15</td>
<td>0.23, 0.37</td>
<td>-0.043, -0.25</td>
<td>0.58, -0.41</td>
</tr>
</tbody>
</table>

* Literature values: [Comptox.epa.gov/dashboard/](https://comptox.epa.gov/dashboard/).
** Log P is log octanol/water partition coefficient. ChemAxon and ALOGPS predicted values are presented, obtained from DrugBank ([https://go.drugbank.com](https://go.drugbank.com)).
2. Materials and methods

2.1. Materials

Addition-cure liquid silicone rubber (LSR) DDU-4320 was supplied by NuSil® (Carpinteria, CA, USA). ONZ, SNZ (hemihydrate), and TNZ (>98.0% purity) were purchased from Tokyo Chemical Industry UK Ltd. (Oxford, UK) and used as supplied. MNZ was supplied by Farchemia Srl (Treviglio, Italy) and used as supplied. Trimethylsiloxy-terminated polydimethylsiloxane (viscosity 5 cSt) was purchased from Fluorochem (Hadfield, UK). HPLC-grade water was obtained using a Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK). Tween 80, HPLC-grade acetonitrile and acetonitrile were purchased from Sigma-Aldrich (Gillingham, UK). Potassium dihydrogen orthophosphate was purchased from Fisher Scientific (Leicestershire, UK) and phosphoric acid from Honeywell (Fluka, Germany).

2.2. Vaginal ring manufacture

DDU-4320 matrix-type vaginal rings loaded with 3.125% w/w (~250 mg) of either SNZ, ONZ, TNZ or MNZ were manufactured using a manual injection molding machine fitted with custom stainless steel vaginal ring molds (cross-sectional diameter 7.6 mm, outer diameter 56.7 mm). Different ring manufacturing parameters (Table 3) were selected for each drug to avoid drug melting during ring manufacturing; drug melting temperatures are presented in Table 2. Part A and B silicone premixes were prepared by weighing appropriate quantities of active ingredients and Parts A or B silicone elastomer into individual screw-cap polypropylene mixing containers (100 g capacity) and mixing at 3000 rpm for 10 s in a dual asymmetric centrifugal (DAC) mixer, (SpeedMixer, DAC-150 FVZ-K, Hauschild, Germany). Premixes A and B were combined (1:1 ratio), hand-mixed for 30 s using a stainless steel spatula, and then mixed at 3000 rpm for 30 s using the DAC. This active mix was transferred to a SEMCO cartridge and the mixture injected manually into the heated ring mold assembly using an adhesive dispensing gun. After a specified cure time (Table 3), the fully formed rings were removed from the molds, allowed to cool to room temperature, and then sealed in labelled plastic pouches.

2.3. Thermogravimetric analysis (TGA)

TGA analysis of MNZ, TNZ, SNZ and ONZ powders (1–3 mg) was performed using a Thermal Advantage Model TGA Q500 (TA instruments, New Castle, PA, US) in heat ramp mode (25–180°C at a rate of 10°C/min) in sealed aluminium pans under a nitrogen atmosphere (50 mL/min). SNZ powder and drug-loaded silicone elastomer were also analysed using an alternative protocol to further assess its more complex melting behaviour. This alternative protocol comprised ramp heating from 25 to 90°C at 10°C/min, ramp cooling to 0°C and ramp heating to 90°C at 10°C/min. An empty sealed aluminium pan with lid was used as a reference in all DSC runs, and triplicate measurements were made for all samples. Onset temperature (C), peak temperature (C) and enthalpy (ΔH, J/g) values were determined for all thermal transitions. DSC traces were plotted using GraphPad Prism (version 8.4.2).

2.4. Differential scanning calorimetry (DSC) analysis

Powder samples of MNZ, TNZ, SNZ and ONZ (1–5 mg) and cured drug-loaded silicone elastomer samples (10% w/w; 5–10 mg) were analysed using a Thermal Advantage Model DSC Q100 (TA instruments, New Castle, PA, US) in heat ramp mode (25–180°C at a rate of 10°C/min) in sealed aluminium pans under a nitrogen atmosphere (50 mL/min). SNZ powder and drug-loaded silicone elastomer were also analysed using an alternative protocol to further assess its more complex melting behaviour. This alternative protocol comprised ramp heating from 25 to 90°C at 10°C/min, ramp cooling to 0°C and ramp heating to 90°C at 10°C/min. An empty sealed aluminium pan with lid was used as a reference in all DSC runs, and triplicate measurements were made for all samples. Onset temperature (C), peak temperature (C) and enthalpy (ΔH, J/g) values were determined for all thermal transitions. DSC traces were plotted using GraphPad Prism (version 8.4.2).

2.5. UPLC method development

Working solutions (0.5 200 µg/mL) were prepared by dissolving each of MNZ, SNZ, ONZ and TNZ in ultrapure water for the production of calibration curves. Samples (5 µL) were injected onto a Waters Acquity UPLC® system (Waters Corporation, Dublin, Ireland) fitted with an Acquity UPLC BEK C18 column (2.1 mm × 50 mm, 1.7 µm particle size) and an in-line filter (0.2 µm). The column temperature, mobile phase flow rate and run time were 25°C, 0.25 mL/min and 3 min, respectively. The mobile phase compositions, wavelengths of detection, and retention times for MNZ, SNZ, ONZ and TNZ are presented in Table 4. Chromatograms were analysed using Empower™ 3.0 software.

A series of solutions having different concentrations of MNZ, ONZ, SNZ and TNZ were prepared for validation of the UPLC methods. Linearity across the range 25–1000 µg/mL was assessed on three different days (n = 3). Three injections were performed for each sample. The average peak areas of these injections were plotted against concentrations to produce a linear plot. Three different concentrations with nine determinations (n = 3) were used to assess the accuracy of recovery (theoretical concentrations/concentrations calculated from the linear equation × 100%) in 90–110%. Repeatability was assessed by calculating RSD% of three injections of the same concentrations in accuracy determination (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2005). Limit of detection (LOD) was calculated with a signal to noise (S/N) of 3. Limit of quantification (LOQ) was determined with S/N of 10. Linear ranges and values for R², inter-day precision (% RSD), accuracy (% recovery), LOD and LOQ for the MNZ, SNZ, ONZ and TNZ are presented in Table 5.

2.6. In vitro release testing of vaginal rings

Four rings were randomly selected from each ring manufacturing batch for in vitro release testing over 28 days. On Day 0, each ring was placed into a 250 mL DURAN flask containing 200 mL of an aqueous solution comprising 0.2% w/w Tween 80 (adjusted to pH 4.2) and the flask placed into a shaking incubator (37°C, 60 rpm, 25 mm orbital temperature. All TGA analyses were conducted in triplicate.

Table 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug loading in ring (mg, %w/w)</th>
<th>Cure temperature (°C)</th>
<th>Cure time (min)</th>
<th>Ring appearance</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNZ</td>
<td>250, 3.125</td>
<td>50</td>
<td>10</td>
<td>white</td>
<td>sticky ring surface</td>
</tr>
<tr>
<td>ONZ</td>
<td>250, 3.125</td>
<td>60</td>
<td>10</td>
<td>white</td>
<td>no manufacturing issues</td>
</tr>
<tr>
<td>TNZ</td>
<td>250, 3.125</td>
<td>90</td>
<td>2</td>
<td>white</td>
<td>no manufacturing issues</td>
</tr>
<tr>
<td>MNZ</td>
<td>250, 3.125</td>
<td>100</td>
<td>1.5</td>
<td>white</td>
<td>no manufacturing issues</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mobile phase composition</th>
<th>Detection wavelength (nm)</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNZ</td>
<td>70% phosphate buffer (7.7 mM), 30% ACN</td>
<td>310</td>
<td>0.56</td>
</tr>
<tr>
<td>SNZ</td>
<td>85% TFA (1% v/v), 15% ACN</td>
<td>320</td>
<td>0.97</td>
</tr>
<tr>
<td>ONZ</td>
<td>80% TFA (1% v/v), 20% ACN</td>
<td>320</td>
<td>1.22</td>
</tr>
<tr>
<td>TNZ</td>
<td>70% phosphate buffer (7.7 mM), 30% ACN</td>
<td>310</td>
<td>1.25</td>
</tr>
</tbody>
</table>
throw; Unitron HT Infors, Switzerland). The release medium was sampled daily and replaced with 100 mL fresh medium Monday through Thursday, with 200 mL replacement on Fridays and no weekend sampling. Quantities of MNZ, SNZ, TNZ and ONZ in the release media were measured using UPLC-UV.

2.7. Determination of drug solubility in silicone oil

Each drug (~50 mg) was added to 5.0 mL of 5 cSt polydimethylsiloxane fluid ($n = 4$) and stored in a shaking incubator (37°C, 60 rpm, 25 mm orbital throw; Unitron HT Infors, Switzerland) for 72 h. The resulting saturated silicone oil solutions were passed through 0.2 µm filters to remove excess drug, and then ~2.0 g samples of the filtrates were added to 50 mL acetonitrile at 37°C. The biphasic mixture of silicone oil and acetonitrile was stored at 37°C and 60 rpm for a further 72 h before sonicating the flasks (Branson Bransonics® CPHX Digital Bath 5800; Danbury, USA) at 37°C for 15 min. 1 mL samples of the acetonitrile layers were transferred into 1.5 mL centrifuge tubes and centrifuged at 4°C, 12000 rpm for 10 min (Hettich Mikro 200R; DHLab, Belfast, UK). Samples of the acetonitrile medium (0.8 mL) were transferred into UPLC vials and values for drug solubility in silicone oil determined by UPLC-UV analysis.

2.8. Statistical analysis

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 (v 8.4.2; GraphPad Software, LLC.).

3. Results and discussion

3.1. Vaginal ring manufacture

Injection molding is a well established, highly scalable, and efficient method for commercial manufacture of polymeric medical and drug delivery devices, including vaginal rings (Malcolm et al., 2016; Rothen-Weinhold et al., 1999; Zema et al., 2012). Of the eight marketed drug-releasing vaginal rings, six – Estring, Femring, Fertiring, Progering, Annovera and the recently approved dapivirine ring – are manufactured from silicone elastomers by elevated temperature reaction injection molding. The other two rings (NuvaRing and Ornibel) are manufactured from thermoplastic polymers by hot-melt extrusion. Advantages of injection molding include: (i) well-established manufacturing processes for medical and drug delivery devices, (ii) useful for fabrication of silicone elastomer devices using different cure chemistries, (iii) can be used to manufacture both simple and complex designs, (iv) relatively inexpensive to manufacture devices once the mold(s) are fabricated, (v) a

![Fig. 1. Representative photographs of matrix-type silicone elastomer (DDU-4320) vaginal rings: (A) 250 mg SNZ ring, (B) 250 mg ONZ ring, (C) 250 mg TNZ ring, (D) 250 mg MNZ ring.](image-url)
broad selection of medical and drug delivery grades of silicone elastomers are available allowing optimisation of manufacturing temperature and cycle time, and (vi) by comparison, hot melt extrusion processes for manufacture of rings require the extrudate to be cut and thermally welded to form the final ring format.

All manufactured rings were uniform white and slightly translucent due to the relatively low drug loading (3.125% w/w) (Fig. 1). Mean weights of the SNZ, ONZ, TNZ and MNZ rings were $7.43 \pm 0.03$, $7.42 \pm 0.01$, $7.34 \pm 0.04$ and $7.32 \pm 0.04$ g, respectively; any differences in ring weight reflect the different powder densities of the drug substances. The SNZ and ONZ rings were shinier than the TNZ and MNZ rings, due to the physical characteristics of the SNZ and ONZ crystalline powders. The surface of the SNZ rings were slightly sticky to the touch due to incomplete cure of the silicone elastomer; the curing issue was attributed to at the relatively low temperature ($50^\circ C$) used to manufacture the SNZ rings due to the drug’s relatively low melting point ($74^\circ C$) and the preference to manufacture rings without drug melting occurring (to avoid issues around subsequent drug recrystallisation). Manufacturing at a slightly higher temperature (e.g., $65^\circ C$) or post-curing the rings resolved this issue. The ONZ, TNZ and MNZ rings cured fully in the mold, were easy to demold, and had a smooth ring surface.

### 3.2. Thermogravimetric analysis (TGA)

TGA thermograms of the supplied MNZ, ONZ, TNZ and SNZ drug powders are presented in Fig. 2; the onset temperatures for thermal degradation for these drugs were 150, 145, 160 and 120 C, respectively. The small weight loss observed at $\sim 70^\circ C$ in the TGA trace for SNZ (Fig. 2D; measured 4.4%; 4.6% theoretical) is due to loss of water of crystallization from its hemihydrate form, which is known to be stable at room temperature (Bezerra et al., 2016). The preparation of drug-loaded silicone elastomer formulations by injection molding requires elevated temperatures to ensure rapid cure rates. To avoid changes to the drug substance during manufacture, it is preferable to select manufacturing (curing) temperatures below any thermal transitions (Beyer et al., 2019).

### 3.3. Differential scanning calorimetry (DSC) analysis

Representative DSC thermograms of the supplied drug powders and silicone elastomer samples loaded with 10% w/w of each drug are presented in Fig. 3. Thermal transitions are summarized in Table 6. ONZ and TNZ displayed melting peaks at 87 and 126 C, respectively (Fig. 3B and 3C), corresponding with literature values (85–90 C and 127–128 C, respectively) (World of Chemicals, 2023). Similar thermal transitions were measured by DSC for the corresponding silicone elastomer samples containing 10% w/w of either ONZ or TNZ (Fig. 3B and 3C), indicating no significant drug-elastomer interactions and that the drugs exist, at least in part, in the crystalline form within the silicone elastomer. For the 10% w/w ONZ-loaded silicone elastomer sample (Fig. 3B), the enthalpy value of 10.2 J/g was less than 10% of that for the ONZ drug powder (107 J/g) (Table 6), indicating that a fraction of the 250 mg ONZ loading was dissolved in the silicone elastomer matrix (Malcolm et al., 2002; Woolfson et al., 2003). For the 10% w/w TNZ-loaded silicone elastomer (Fig. 3C), the enthalpy value (12.6 J/g) was proportionately similar to that of the pure TNZ powder (126.3 J/g), indicating that TNZ had not dissolved in the silicone elastomer to any appreciable extent and the drug was present in the crystalline state within the silicone elastomer (Table 6). The higher silicone elastomer solubility of ONZ compared with TNZ correlates with the lipophilicity values of the drugs, as indicated by their log partition coefficient (log P) values (ONZ 0.60; TNZ –0.36; Table 2).

SNZ displayed two endothermic peaks in the DSC trace at 69 C and 77 C (Fig. 3D), attributed to dehydration of the SNZ hemihydrate and subsequent melting of the anhydrous SNZ, respectively (Bezerra et al., 2016). The enthalpy of melting is significantly reduced for the anhydrous SNZ in the silicone elastomer sample compared to the DSC trace of the drug alone (Table 6), which is attributed at least in part to dissolution of the anhydrous SNZ in the silicone elastomer. A heat-cool-heat DSC experiment was conducted on both the SNZ powder and a SNZ-

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**Fig. 2.** Representative TGA thermograms of (A) MNZ, (B) ONZ, (C) TNZ and (D) SNZ drug powders.
Fig. 3. Representative DSC traces showing the thermal behavior of (A) MNZ powders and MNZ (10% w/w) silicone elastomer samples, (B) ONZ powders and ONZ (10% w/w) silicone elastomer samples, (C) TNZ powders and TNZ (10% w/w) silicone elastomer samples, (D) SNZ powders and SNZ (10% w/w) silicone elastomer samples in a ramp heating mode; (E) SNZ powders in a heat-cool-heat mode; (F) SNZ (10% w/w) silicone elastomer samples in a heat-cool-heat mode. Downward direction is endothermic.
silicone elastomer (SE) samples (10% w/w). Note that SNZ showed two thermal transitions, hence the inclusion of Peak II values. That SNZ had formed an amorphous phase upon cooling which recrystallised at 30 °C, close to the 77 °C melting point in the first heating and suggesting the formation of the original polymorphic form (Table 6). The recrystallisation at 30 °C resulted in formation of the anhydrous form (not the hemihydrate), as evidenced by absence of an endothermic transition at 69 °C.

Given the thermal characteristics of SNZ, rings containing SNZ were manufactured at 50 °C for 10 min, significantly below the dehydration and melting temperatures. As a result, the SNZ rings were only partly cured upon removal from the injection mold; addition-cure silicone elastomer materials are usually cured at much higher temperatures – typically 80–170 °C, depending upon the type – since the high temperature is required to drive off the cure inhibitor (Malcolm et al., 2016). Based on TGA information reported by Bezerra et al., SNZ hemihydrate starts losing water close to 45 °C and is complete by 75 °C (Bezerra et al., 2016). Therefore, SNZ likely exists in a variety of forms within these silicone elastomer rings, including (i) dissolved SNZ hemihydrate, (ii) crystalline SNZ hemihydrate, (iii) dissolved anhydrous SNZ, (iv) crystalline anhydrous SNZ. (Had the SNZ rings been manufactured at a higher temperature to obtain better cure and mechanical properties, the SNZ hemihydrate would have lost its water of crystallisation and melted). Upon cooling of the rings, SNZ was retained in the amorphous state. Interestingly, SNZ rings placed in vivo or in vitro at 37 °C would be above the 30 °C recrystallisation temperature, such that amorphous SNZ might be expected to recrystallise to some extent within the ring.

### 3.4. In vitro release testing

Mean daily release vs. time plots and cumulative release vs. root time plots for matrix-type silicone elastomer DDU-4320 vaginal rings containing 250 mg of SNZ, ONZ, TNZ or MNZ incubated in 0.2% w/w Tween aqueous solution (pH 4.2) are presented in Fig. 4. Cumulative release was modelled based on the Higuchi equation, and correlation coefficients (R²) and release rates (mg/t²) for each formulation are reported in Table 7.

For all ring formulations, a burst release was observed on Day 1 followed by steadily decreasing daily release values with time (Fig. 4). Linear (R² = 0.997–0.999) cumulative release vs. square root time profiles were also obtained (Fig. 4), consistent with a permeation-controlled drug release mechanism from a polymeric matrix device containing dispersed solid drug (Higuchi, 1961; Malcolm et al., 2003). The Day 1 burst was highest for SNZ (23.7 mg), followed by ONZ (16.5 mg), MNZ (8.6 mg) and TNZ (4.0 mg) (Fig. 4A). Thereafter, the quantities of drug release declined daily, typical of matrix-type silicone elastomer vaginal rings (Boyd et al., 2019; Malcolm et al., 2003, 2016).

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**Table 6** Thermal parameters (mean ± SD, n = 3) for each thermal transition in the DSC thermograms for MNZ, ONZ, TNZ, and SNZ drug powders and the corresponding silicone elastomer (SE) samples (10% w/w). Note that SNZ showed two thermal transitions, hence the inclusion of Peak II values.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak I Onset Temp. (°C)</th>
<th>Peak Temp. (°C)</th>
<th>Enthalpy (ΔH, J/g)</th>
<th>Peak II Onset Temp. (°C)</th>
<th>Peak Temp. (°C)</th>
<th>Enthalpy (ΔH, J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNZ</td>
<td>160.02 ± 0.19</td>
<td>160.95 ± 0.07</td>
<td>196 ± 28</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MNZ SE</td>
<td>159.76 ± 0.11</td>
<td>160.96 ± 0.19</td>
<td>15.9 ± 3.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ONZ</td>
<td>86.92 ± 0.04</td>
<td>89.33 ± 0.13</td>
<td>107.3 ± 6.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ONZ SE</td>
<td>87.03 ± 0.15</td>
<td>89.48 ± 0.11</td>
<td>10.20 ± 0.43</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TNZ</td>
<td>125.76 ± 0.36</td>
<td>127.03 ± 0.39</td>
<td>126.3 ± 5.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TNZ SE</td>
<td>125.34 ± 0.05</td>
<td>126.53 ± 0.18</td>
<td>12.6 ± 1.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SNZ</td>
<td>68.82 ± 0.12</td>
<td>70.19 ± 0.14</td>
<td>8.8 ± 3.8</td>
<td>76.70 ± 0.31</td>
<td>78.03 ± 0.14</td>
<td>99.9 ± 8.1</td>
</tr>
<tr>
<td>SNZ SE</td>
<td>68.59 ± 0.16</td>
<td>69.94 ± 0.32</td>
<td>5.9 ± 1.5</td>
<td>76.31 ± 0.20</td>
<td>77.91 ± 0.19</td>
<td>3.60 ± 0.65</td>
</tr>
<tr>
<td>SNZ 1st heating</td>
<td>68.54 ± 0.35</td>
<td>69.95 ± 0.79</td>
<td>16.0 ± 2.2</td>
<td>76.59 ± 0.11</td>
<td>78.11 ± 0.36</td>
<td>86 ± 16</td>
</tr>
<tr>
<td>SNZ 2nd heating</td>
<td>30.15 ± 0.48</td>
<td>36.64 ± 0.66</td>
<td>51 ± 16</td>
<td>75.57 ± 0.15</td>
<td>77.32 ± 0.24</td>
<td>94 ± 20</td>
</tr>
<tr>
<td>SNZ SE 1st heating</td>
<td>68.30 ± 0.11</td>
<td>69.67 ± 0.12</td>
<td>5.57 ± 1.04</td>
<td>76.10 ± 0.17</td>
<td>77.78 ± 0.17</td>
<td>2.2 ± 0.78</td>
</tr>
<tr>
<td>SNZ SE 2nd heating</td>
<td>33.12 ± 1.46</td>
<td>39.70 ± 1.64</td>
<td>3.34 ± 0.97</td>
<td>75.79 ± 0.12</td>
<td>77.33 ± 0.17</td>
<td>10 ± 1</td>
</tr>
</tbody>
</table>

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**Fig. 4.** Mean daily release vs. time (A) and cumulative release vs. root time (B) profiles for release into 0.2% w/w Tween 80 (pH 4.2) for 250 mg MNZ, SNZ, ONZ and TNZ silicone elastomer DDU-4320 matrix-type vaginal rings (MNZ, ONZ, TNZ n = 4; SNZ n = 2).

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Release values for each sampling day followed the rank order SNZ > ONZ > MNZ > TNZ. After 28 days, cumulative release values were SNZ (132 mg) > ONZ (109 mg) > MNZ (46 mg) > TNZ (31 mg) (Fig. 4B, Table 7). Release rates – obtained from the line gradients of the cumulative release vs. time plots – followed the rank order SNZ (25.3 mg/t<sup>1/2</sup>) > ONZ (21.8 mg/t<sup>1/2</sup>) > MNZ (9.4 mg/t<sup>1/2</sup>) > TNZ (6.3 mg/t<sup>1/2</sup>) (Table 7).

When the drug loading in a silicone elastomer vaginal ring exceeds the drug’s solubility in the silicone elastomer, the drug then exists within the ring in two distinct forms – a small quantity of drug is dissolved within the silicone matrix and the remainder is present in the solid (usually crystalline) state. Upon placement into the release medium, drug at the surface of the ring dissolves rapidly and diffuses into the release medium, contributing to the observed ‘burst release’ (Malcolm et al., 2003; Woolfson et al., 2003). Following release of drug particles at the surface of the ring and assuming no ingress of medium into the ring matrix (as is true for rings having relatively low initial drug loadings), drug release thereafter occurs entirely via a permeation-controlled mechanism with the drug molecules dissolved in the silicone elastomer matrix diffusing to the ring surface and then dissolving in the release medium. This diffusional process, accompanied by replenishment of the dissolved drug component in the matrix via further dissolution of the embedded drug particles, and the increasing thickness of the drug depletion zone as drug release continues (Fetherston et al., 2013; Murphy et al., 2019), gives rise to the typical root-time release kinetics observed with matrix-type rings (Boyd et al., 2019; Malcolm et al., 2016). Thus, the release rate of drug molecules from silicone elastomer matrix-type vaginal rings is dependent upon the extent of drug solubility in the silicone elastomer, the diffusivity of the drug through the silicone elastomer, the initial drug loading, and the surface area of the ring device (Malcolm et al., 2016). In this study, the 5-nitroimidazole rings were manufactured to have the same dimensions, surface areas, and initial drug loading. The ~0.1 g differences in ring weights (reported earlier) were deemed insignificant and likely due to variability in injection pressures associated with the manual injection process used to inject the active silicone mix into the ring molds.

Therefore, silicone solubility and silicone diffusivity are the major parameters influencing the permeation of drugs through silicone elastomers (Malcolm et al., 2003).

Measurement of drug solubility in silicone elastomers may also be obtained using the ‘thin film’ method (Kunst and Lee, 2015; van Laarhoven et al., 2002a; van Laarhoven et al., 2002b; Woolfson et al., 2003). However, the method is time consuming and difficult to perform with accuracy and precision. Here, we opted to measure solubility in a low molecular polydimethylsiloxane oil as a proxy for solubility in silicone elastomer, similar to that described previously (Malcolm et al., 2003, 2002; Woolfson et al., 2003). The rank order of measured solubility in silicone oil was SNZ > ONZ > MNZ = TNZ (Table 7). Further, the diffusivity of the solvated drug molecules in silicone elastomer is assumed to inversely correlate with the drug molecular weight (Table 2), or better, the molecular volume (Table 7). Thus, plots of log (S/V) – where S is the drug mean solubility value measured in silicone oil (mg/cm<sup>3</sup>) and V is the molecular volume of the drug substance (nm<sup>3</sup>) – versus in vitro drug release rate (mg/day<sup>1/2</sup>) produced a strong positive correlation (Fig. 5, Table 7). Here, the parameter S/V can be considered a form of permeability coefficient, functioning in a similar fashion to the well established understanding that the permeability (P) is the product of solubility (S) multiplied by diffusivity (D), i.e., P = S*D. In other words, 1/V is being used as a proxy for D. According to this analysis, the drug release rates for the rings and the values of the permeability parameter S/V follow the rank order SNZ > ONZ > MNZ > TNZ (Table 7), reflecting the dependence of both upon drug molecule solubility in silicone (S) and drug molecular volume (V).

Significantly greater quantities of SNZ and ONZ were released from rings compared to MNZ or TNZ (Fig. 4), reflecting their relatively low molecular volume and relatively high silicone solubility values (Table 7). Given that SNZ shows similar in vitro activity against BV-associated bacteria to MNZ and TNZ and no detriment to lactobacilli (Petrina et al., 2017), SNZ or ONZ may be the preferred choice of 5-nitroimidazole compound for sustained release from a vaginal ring.

Data obtained from in vitro drug release experiments can be plotted as log (M<sub>1</sub>/M<sub>n</sub>) versus log t (M<sub>1</sub>/M<sub>n</sub> < 0.6) (Korsmeyer-Peppas model) to determine n, the slope of linear regression (Korsmeyer et al., 1983; Peppas, 1985). Log fractional release vs log time plots are presented in Fig. 6 for each 250 mg matrix ring. A summary of the parameters derived from Korsmeyer-Peppas mathematical modelling of the data is presented in Table 8. High correlation coefficients (R<sup>2</sup> = 1.00) were obtained for all four 5-nitroimidazoles. The diffusional exponents (n) of the drugs ranged from 0.51 to 0.61, values higher than the 0.45 commonly
development of gel formulations for vaginal administration of SNZ, vaginal MNZ. However, regimens involving vaginal administration SNZ although none have yet progressed to the clinic (Argenta et al., 2021; et al., 1978) that make it eminently suitable as a single dose oral MNZ, TNZ and ONZ, respectively) (Schwartz and Jeunet, 1976; Videau Arun Karthick et al., 2018).

72% were reported across all treatment regimens, and data since rings are toruses and not cylinders, such that release from the Fickian) transport. However, it is important not to over-interpret these -

3.5. Clinical efficacy of the 5-nitroimidazoles

A comprehensive review describing use of SNZ for treatment of BV, and particularly the 2 g single-dose regimen, has been published (Nyirjesy and Schwebke, 2018). SNZ has been shown to be as effective as oral MNZ for treatment of BV, both in single and multiple-dose regimens (Bohbot et al., 2010). A previous study evaluated the clinical efficacy of oral and vaginal ONZ, SNZ and MNZ – both alone and in various combinations – for treatment of BV (Saraçoğlu et al., 1998). Cure rates of ≥ 72% were reported across all treatment regimens, and > 90% for oral ONZ, oral ONZ + vaginal ONZ, oral SNZ + vaginal ONZ, oral SNZ + vaginal MNZ. However, regimens involving vaginal administration SNZ were not considered. More recently, studies have reported preclinical development of gel formulations for vaginal administration of SNZ, although none have yet progressed to the clinic (Argenta et al., 2021; Arun Karthick et al., 2018).

It is SNZ’s relatively long terminal elimination half-life following oral administration (~14 h in women; compare with ~8, 12 and 14 h for MNZ, TNZ and ONZ, respectively) (Schwartz and Jeunet, 1976; Videau et al., 1978) that make it eminently suitable as a single dose oral regimen, since plasma concentrations are maintained for ~72 h above the minimum inhibitory concentrations for BV-associated pathogens (Gillis and Wiseman, 1996; Nyirjesy and Schwebke, 2018). This long half-life, coupled with its significantly greater solubility and in vitro release characteristics in silicone elastomer compared to other drugs in the class (Table 7), make SNZ a particularly good candidate for sustained administration using a vaginal ring device.

Of course, one issue that will need to be addressed in future studies is the potential for the development of bacterial resistance resulting from prolonged administration of SNZ from a ring device. Although semidazole is currently administered as a single 2 g oral dose (Table 1), extended and sustained release regimens/formulations have previously been reported (Arun Karthick et al., 2018; McNeil et al., 2023). However, there is no data describing emergence of resistance strains for semidazole treatment of BV; bacterial resistance appears to develop only rarely to 5-nitroimidazole drugs (Gillis and Wiseman, 1996).

Finally, given recent advances and success in the use of vaginal ring technology for administering antiretroviral drugs for prevention of sexually acquired infection with human immunodeficiency virus (HIV) (Baeten et al., 2016; Malcolm et al., 2016; Nel et al., 2016) and recognizing that BV is implicated in increased risk of HIV acquisition (Armstrong and Kaul, 2021; Atashili et al., 2008; Cohen et al., 2012; McKinnon et al., 2019; Sewankambo et al., 1997), it might prove fruitful to develop combination drug vaginal rings targeted at both HIV prevention and treatment/prevention of BV. For example, a vaginal ring device offering sustained release of both dapivirine and one of the 5-nitroimidazole compounds described in this paper should be feasible and could further reduce rates of sexually acquired HIV infection. While BV is not a sexually transmitted infection, it does lead to increased risk of acquiring such infections. As such, the strategies and technologies being pursued currently for multipurpose prevention technology vaginal rings could be adopted and adapted to include treatment of BV (Benhabbour, 2019; Boyd et al., 2016; Dallal Bashi et al., 2021, 2019; Moss et al., 2013; Smith et al., 2017; Thurman et al., 2013).

4. Conclusions

All four 5-nitroimidazole drugs – MNZ, SNZ, TNZ and ONZ – showed good compatibility with the addition-cure silicone elastomer. The curing temperatures used for ring manufacture were lower than the melting points of the drugs, such that the drugs existed in both the dissolved and solid (crystalline) state, similar to marketed silicone elastomer ring products. The 50°C temperature used to manufacture SNZ rings was too low to obtain a fully cured vaginal ring with mechanical properties similar to the pure elastomer; therefore, the manufacturing method for SNZ rings would need to be further optimized, by increasing the cure temperature, for example to 60°C. The release rates of the four 5-nitroimidazole drugs followed the rank order SNZ > ONZ > MNZ > TNZ, and this order further correlated with a simple permeation parameter based upon drug solubility and drug molecular weight (the latter indicative of drug diffusivity in the silicone elastomer). The results presented here will be useful in supporting development of sustained release vaginal ring products as new treatment options for bacterial vaginoses and may help reduce the high recurrence rates reported with current treatment options. The potential for combining these 5-nitroimidazole drugs with antiretroviral drugs (such as dapivirine) in next-generation multipurpose vaginal rings as a means of bolstering ring-based HIV prevention strategies is particularly intriguing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 8

Korsmeyer-Peppas mathematical modelling of the cumulative release of SNZ, ONZ, MNZ and TNZ from matrix-type silicone elastomer vaginal rings (drug loading: 250 mg, 3.125 % w/w).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Release exponent (n)</th>
<th>k (constant)</th>
<th>Drug transport mechanism</th>
<th>R² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNZ</td>
<td>0.51</td>
<td>0.10</td>
<td>Fickian diffusion</td>
<td>1.00</td>
</tr>
<tr>
<td>ONZ</td>
<td>0.56</td>
<td>0.07</td>
<td>Anomalous transport</td>
<td>1.00</td>
</tr>
<tr>
<td>MNZ</td>
<td>0.53</td>
<td>0.03</td>
<td>Fickian diffusion</td>
<td>1.00</td>
</tr>
<tr>
<td>TNZ</td>
<td>0.61</td>
<td>0.02</td>
<td>Anomalous transport</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Fig. 6. Log fractional drug release (M/M∞) versus log t (days) (Korsmeyer-Peppas mathematical modelling) of the cumulative release of SNZ, ONZ, MNZ and TNZ from matrix-type silicone elastomer vaginal rings. M is the amount of drug released at time t; M∞ is the amount of drug released after an infinite time (equivalent to the total drug loading).


