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***In vitro* release testing methods for drug-releasing vaginal rings**

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Abstract

Drug-releasing vaginal rings are torus-shaped devices, generally fabricated from thermoplastics or silicone elastomers, used to administer pharmaceutical drugs to the human vagina for periods typically ranging from three weeks to twelve months. One of the most important product performance tests for vaginal rings is the *in vitro* release test. Although it has been fifty years since the a vaginal ring device was first described in the scientific literature, and despite seven drug-releasing vaginal rings having been approved for market, there is no universally accepted method for testing *in vitro* drug release, and only one non-compendial shaking incubator method (for the estradiol-releasing ring Estring®) is described in the US Food and Drug Administration's *Dissolution Methods Database*. Here, for the first time, we critically review the diverse range of test methods that have been described in the scientific literature for testing *in vitro* release of drug-releasing vaginal rings. Issues around *in vitro-in vivo* correlation and modelling of *in vitro* release data are also discussed.

Keywords

Dissolution testing; *In vitro* release testing; Intravaginal rings; Sustained release; Controlled release; Sink conditions; Novel dosage form; IVIVC; Release models

1. Introduction

Drug-releasing vaginal rings are flexible annular-shaped devices – mostly fabricated from medical grade silicone elastomers or thermoplastic polymers – that offer sustained or controlled delivery of therapeutic agents to the vagina for clinical benefit [1–4]. Since the concept of a polymeric vaginal ring for continuous drug administration was first described in a 1968 patent application [5], seven vaginal ring products have been approved for marketing (Estring[®], Nuvaring[®], Femring[®], Progering[®], Fertiring[®], Ornibel[®] and Annovera[®]; Table 1). One other ring device – a vaginal ring providing sustained release of the antiretroviral compound dapivirine for HIV prevention – is currently under review by the European Medicines Agency (EMA), and many others are in preclinical and clinical development (Table 1) [1].

In accordance with compendial requirements, two types of *in vitro* test are generally performed for drug-releasing vaginal rings: product quality tests and product performance tests [6]. Product quality tests are used to assess drug product quality attributes of vaginal rings, and include items such as assay value (drug content), drug substance and drug product identification, content uniformity, related substances, degradation products, microbial limits, etc. [7,8]. Mechanical testing is also considered an important product quality test for drug-releasing vaginal rings [9,10]. By comparison, performance tests for drug products invariably involve assessment of *in vitro* drug release. For oral immediate release dosage forms, these tests are commonly referred to as 'dissolution tests', reflecting the fact that drug release is generally achieved by disintegration and/or dissolution of the dosage form. However, for many non-oral dosage forms, including vaginal rings, the drug product itself does not generally disintegrate or dissolve. Instead, the incorporated drug substance(s) is commonly released relatively

slowly from the device via a permeation mechanism (usually involving passive diffusion of solubilised drug molecules through a polymeric material), and often over an extended period of time. Here, it is preferable to use the term '*in vitro* drug release testing', or simply '*in vitro* release testing', commonly abbreviated to IVRT. The aim of *in vitro* release testing is to characterise the drug product to ensure consistent product batch quality within a defined set of specification criteria, and to act, where possible, as a surrogate for assessment or prediction of *in vivo* performance [11,12].

Interest in vaginal ring technology for drug delivery applications has piqued in recent years, driven primarily by a surge of innovation around the use of new ring designs and new polymeric materials for fabrication of rings. In fact, much of this innovation has stemmed directly from efforts to develop antiretroviral-releasing rings for prevention of HIV infection and multi-purpose prevention technologies the combine HIV prevention with contraception and prevention of other sexually transmitted infections [1–3,10,13–29]. In particular, great advances have been made in extending ring technology beyond conventional low molecular weight and relatively hydrophobic drugs to the formulation and release of hydrophilic drugs, biomolecular drugs and drug combinations. Fig. 1 illustrates the extended range of drug molecules for which new approaches to vaginal ring formulation are now being considered.

Table 1. Descriptions of vaginal rings (both marketed products and products currently undergoing clinical testing) and details of *in vitro* testing methods.

Vaginal ring (Status) [#]	Developer	Device type / duration of release	Active agent(s) (loading / release rate)	Polymer	Indication	Apparatus	Medium, volume, speed [†]	Sampling timepoints	References
Estring® (A)	Pharmacia & Upjohn	reservoir 3 months	17β-estradiol (2 mg / 7.5 µg/day)	silicone elastomer core and sheath	ERT	linear shaking water bath or shaking incubator / dissolution medium changed periodically	<ul style="list-style-type: none"> • 0.9% saline • 250 mL • 60 or 130 rpm 	Medium sampled and replaced days 1, 9, 16, 17, 18, 19, 45 days	[30,31]
Nuvaring® (A)	Merck	reservoir 21 days	etonogestrel (11.7 mg / 120 µg/day) ethinyl estradiol (2.7 mg / 15 µg/day)	28% EVA copolymer core and 9% EVA sheath	contraception	incubator with magnetic bar stirring / ring suspended in flask with nylon string	<ul style="list-style-type: none"> • water • 200 mL • 750 rpm 	NA	[32–35]
Femring® (A)	Actavis / Warner Chilcott	reservoir 3 months	17β-estradiol-3-acetate (12.4, 24.8 mg / 50, 100 µg/day)	silicone elastomer core and sheath	ERT	shaking orbital incubator / ring suspended by thread / dissolution medium replaced daily	<ul style="list-style-type: none"> • 0.9% w/w saline • 0.133 or 1.0% w/v benzalkonium chloride • 500 mL • rpm not specified 	NA	[36]
Progering® (A)	Population Council / / Silesia SA / Grupo Grünenthal Chile	matrix 3 months	progesterone (2074 mg / ~10 mg/day)	silicone elastomer	post-partum contraception in breastfeeding women	constant-flow release system comprising peristaltic pump and ring suspended in flask with nylon string	<ul style="list-style-type: none"> • isotonic saline • 250 mL • 4 L/day 	NA	[37–39]
Fertiring® (A)	Population Council/Silesia SA/ Grupo Grünenthal Chile	matrix 7 days	Progesterone (1000 mg / ~10 mg/day)	silicone elastomer	IVF / hormone supplementation	same as Progering®	same as Progering®	NA	
Ornibel® / Myring™ (A)	INSUD PHARMA / Exeltis	reservoir 21 days	etonogestrel (11.0 mg / 120 µg/day) ethinyl estradiol (3.47 mg / 15 µg/day)	polyurethane sheath and 28% EVA copolymer core	contraception	shaking incubator	<ul style="list-style-type: none"> • sodium acetate solution (25 mM, pH 4.2) + 0.05% Solutol HS 15 	NA	[40]

Annovera® (A)	Population Council	reservoir 1 year	Nestorone® (103 mg / 150 µg/day) ethinyl estradiol (17.4 mg / 13 µg/day)	silicone elastomer cores (x2) and sheath	contraception	linear shaking water bath (1 inch) / ring suspended in flask with nylon string	<ul style="list-style-type: none"> • 100 mL • 60 rpm • water • 400 mL • 100 opm • 1-inch stroke length 	Medium sampled and replaced every 24 h on weekdays	
Dapivirine ring (Ring-004) (R)	IPM	matrix 28 days	dapivirine (25 mg / 2600–180 µg/day in IPA+water / 350–100 µg/day in SVT+Tween)	silicone elastomer	HIV prevention	orbital shaking incubator (25 mm) / screw-cap glass flasks	<ul style="list-style-type: none"> • IPA+water (1:1 v/v) or SVF+0.2% Tween 80 • 100 mL weekdays, 200 mL weekend • 60 rpm • 25 mm orbital diameter 	Medium sampled and replaced every 24 h on weekdays	[7,8,41]
tenofovir and tenofovir + levonorgestrel rings	CONRAD	Segmented dual-reservoir 90 days	tenofovir (1.2 – 1.6 g / 10 mg/day) levonorgestrel (6 mg / 20 µg/day)	polyurethane	HIV prevention, contraception	shaking incubator / 250 or 500 mL glass flasks	<ul style="list-style-type: none"> • 25 mM sodium acetate buffer, pH 4.2 • Volumes adjusted throughout the experiment • 80 rpm 	Medium replaced every 24 h on weekdays	[15,42,43]
Combination MZCL ring (D) ¹	Population Council	core-matrix 90 days	MIV-150 (3 mg / > 4 µg/day) zinc acetate (30 mg / > 50 µg/day) carrageenan (70 mg / > 100 µg/day) levonorgestrel (0.6 mg / > 2 µg/day)	ethylene vinyl acetate (28) core with a 500 or 800 µm pore	HIV prevention, HSV-2 prevention, HPV prevention and contraception	shaking incubator	<ul style="list-style-type: none"> • 25 mM acetate buffer, pH 4.2 • 10 mL daily • 100 rpm 	Medium replaced every 24 h on weekdays and sampled periodically	[14,27]
Multipurpose pod-intravaginal ring (D) ²	Oak Crest Institute of Science	pod ring 35 days	tenofovir alafenamide hemifumarate (45 mg / 400 µg/day) acyclovir (4 mg / 700 µg/day) etonogestrel (11 or 22 mg / 600 µg/day) ethinyl estradiol (11 mg / 55 µg/day)	silicone elastomer	HIV prevention, HSV prevention and contraception	orbital shaking incubator	<ul style="list-style-type: none"> • 25 mM acetate buffer, pH 4.2 + NaCl to 220 mOs • 100 mL • 60 rpm 	Medium replaced daily or samples removed and replaced periodically	[22,44–46]

5 Abbreviations (in alphabetical order): ERT – estrogen replacement therapy; EVA – ethylene vinyl acetate copolymer; HIV – human immunodeficiency virus; IPA –
6 isopropanol; IPM – International Partnership for Microbicides; IVF – *in vitro* fertilization; NA – data not available; opm – linear oscillations per minute; rpm – revolutions per
7 minutes; SVF – simulated vaginal fluid.
8 # (A) – approved; (R) – under review; (D) – in clinical development
9 † All *in vitro* tests are performed at 37 °C.
10 ¹ Macaque-sized ring and target release rates reported.
11 ² Macaque and human-sized rings reported. Also, several drug combinations were investigated, including tenofovir disoproxil fumarate with emtricitabine and the
12 monoclonal antibody VRC01-N.

14
15

16 **Fig. 1.** Plot of log P (experimental or calculated log partition coefficients; protein
17 molecules arbitrarily assigned a value of 1) vs. molecular weight for drug compounds
18 investigated in vaginal rings. Plot symbols inside the dashed box include estradiol,
19 ethinyl estradiol, etonogestrel, estradiol-3- acetate, dapivirine, progesterone,
20 levonorgestrel, maraviroc, MIV-150, oxybutynin, segesterone acetate (Nestorone®),
21 norethindrone acetate, ulipristal acetate, medroxyprogesterone acetate, UC781, danazol,
22 MC1220, CMPD-167, drospirinone, norgestol acetate, and vicriviroc. Labelled
23 molecules outside the dashed box are currently being considered for vaginal ring
24 formulations and that, due to physiochemical constraints, generally require novel
25 formulation approaches. Figure and caption used with permission (McBride et al.,
26 2019a).

2. General principles of *in vitro* release testing for vaginal rings

2.1. Background

Currently, there is no established compendial apparatus or method for *in vitro* release testing of vaginal rings. Moreover, existing apparatus and methods used for dissolution testing of more conventional dosage forms are often unsuitable for vaginal rings. For example, based on unpublished data from within our own research group, release testing of rings using USP Apparatus 2 (rotating paddle) leads to very significant loss of release medium between daily sampling timepoints due to evaporation, both for aqueous-based media and particularly for solvent/water media. Theoretically, USP Apparatus 4 (flow-through cell) would seem a good option for *in vitro* release testing of vaginal rings, since the closed system minimises evaporation of medium and the rate of flow can be easily adjusted to mimic the dynamics of vaginal fluid production *in vivo* [47]. However, a major limitation of existing Apparatus 4 systems is the small size of the flow cells for housing the test device, such that full-sized ring devices cannot usually be accommodated. Cutting vaginal rings into smaller segments for testing in Apparatus 4 is also not recommended, since the drug release characteristics for the ring segments would be different from that of the full ring product. This is particularly true for reservoir-type rings, which comprise a drug-loaded core encapsulated by a non-medicated rate-controlling membrane; cutting a segment from the ring would directly expose the drug-loaded core to the release medium. Some researchers have sought to overcome this limitation by using ring segments having high-density polyethylene caps glued to the ends of the segments [25,48,49]. Even for ring devices that can be inserted into a flow-through cell, the ring often needs to be folded and squeezed beyond what would normally be encountered *in vivo*, potentially impacting both the extent of ring exposure to the release medium and fluid dynamics around the device.

52

53 2.2. *Key criteria for in vitro release testing of vaginal rings*

54 Selection of experimental apparatus, release media, and release methods for *in vitro*
55 release testing of vaginal rings is largely governed by the nature of both the drug and the
56 device. However, certain key criteria should be carefully considered, including: (i) the
57 method should be biorelevant, i.e. should mimic as closely as possible physiological
58 conditions in the vagina, (ii) the system should comprise well-defined components for
59 reproducibility of results, (iii) simplicity of design of experimental apparatus is
60 preferable, (iv) the test apparatus should be convenient in terms of handling, operation
61 and cleaning, (v) it should be easy to introduce the test product into the testing systems,
62 and to withdraw release medium samples for analysis, (vi) the method should allow
63 effective and controlled agitation, (vii) the method should be easily adapted for use in
64 accelerated drug release tests, and (viii) the method should be economical [50].

Table 2. Drug product factors and release test parameters that can affect the *in vitro* drug release performance of vaginal rings.

Drug product factors	Release test parameters
<ul style="list-style-type: none"> • ring type (e.g. matrix, reservoir, pod, insert, exposed core, etc.) • overall ring dimensions • core length (for reservoir rings) • membrane thickness (for reservoir rings) • drug type • drug solubility in the ring polymer • drug diffusivity in the ring polymer • initial drug loading (for matrix rings) • drug particle size distribution • salt form of drug • polymorphic form of drug • co-formulation / drug-drug interactions • polymer type and grade • cure temperature and time (for silicone elastomer) • molding/extrusion temperature (thermoplastics) • formulation excipients • drug photosensitivity • ring storage / storage conditions 	<ul style="list-style-type: none"> • type of test (shake-flask vs. flow-through) • type of agitation (orbital vs. linear shaking) • composition of release medium • pH of release medium • volume of release medium • sink vs non-sink conditions • sampling frequency and interval • frequency of medium replacement • rate of stirring • rate and diameter of orbital shaking • temperature of release medium / prewarming of medium • position of ring in flask (suspended or lying flat) • type of flask / shape of flask (affects fluid dynamics around the ring)

To date, a variety of non-compendial methods for *in vitro* release testing of vaginal rings have been reported in the literature (Table 1). Factors common to most of these methods include: (i) placement of the ring device in a sealed glass flask; (ii) shaking or stirring of the flask; and (iii) periodic sampling and replacement of the release medium. Typically, the ring device is either allowed to rest at the bottom of the flask or suspended in the medium by a string (usually nylon) (Fig. 2).

Fig. 2. Sealed glass flasks for in vitro release testing of vaginal rings. A – ring placed on bottom of flask containing 100 mL of release medium. B – ring suspended by a nylon thread in glass flask containing 200 mL release medium.

2.3. Stirring or agitation of the release medium

Reported agitation rates vary widely, from 60 rpm when using shaking incubators (which is most common) to 750 rpm for in-flask stirring with a magnetic bar (Nuvaring® testing protocol, Table 1). Just as for dissolution testing of oral dosage forms, agitation rates are known to significantly impact *in vitro* drug release from vaginal rings, although only very limited data on this topic has been reported in the literature [51,52]. The thickness of the *aqueous diffusion layer* (also known as the *hydrostatic layer* or the *boundary layer*) immediately adjacent to the surface of the ring device when placed into a liquid medium (*in vivo* or *in vitro*) can be readily modulated by adjusting the stirring rate, which in turn influences the drug release rate. According to long-established theories of drug dissolution, faster stirring rates will lead to increased drug release rates [53,54].

Further evaluation of the impact of stirring rates on drug release from vaginal rings could prove useful in developing methods that more closely mimic *in vivo* release.

3. Dissolution media for *in vitro* release testing of vaginal ring

3.1. Types of release media

A wide range of different media have been reported for *in vitro* release testing of marketed vaginal ring products and rings currently undergoing clinical testing (Table 1), including: water [10], saline [15], buffer solutions [14,15,55–57], aqueous surfactant solutions [23,48,55,58], polyethylene glycol/water mixtures [59–61], simulated vaginal fluid [7,26,62,63], and organic solvent/water mixtures [7,62–68]. Although polyethylene glycol/water mixtures were used in many early studies, these media see limited use today. Invariably, the release medium is judiciously selected to provide sink conditions for the drug(s) under investigation, and therefore factors such as solubility of the drug, the volume of medium used, the rate of release of drug(s) from the device, and the sampling interval are critical in guiding selection.

3.2. Types of drugs released from vaginal rings

All of the marketed vaginal rings and many of the rings currently undergoing development contain relatively potent (μg –low mg daily dosing) and hydrophobic (log P typically > 3) drug molecules (Table 1; Figure 1). Hydrophobic drugs are particularly well-suited to incorporation into and release from the hydrophobic and non-biodegradable polymers commonly used to fabricate vaginal rings (i.e. silicone elastomer, ethylene vinyl acetate copolymer, thermoplastic polyurethanes), since it is necessary for the drug to have some degree of solubility within the polymer in order to permit molecular permeation and release. Consequently, *in vitro* release testing of rings

often requires (i) use of relatively large volumes of aqueous release media, (ii) addition of surfactants to water/buffer mixtures, or (iii) use of water-miscible organic solvents in order to maintain sink conditions between sampling timepoints (Table 1).

3.3. Sink conditions

The principle of sink conditions is often poorly understood outside of those with specialist knowledge of pharmaceuticals and drug delivery, and yet it is one of the most important considerations when developing *in vitro* dissolution/release methods. Here, in the context of *in vitro* release testing of vaginal rings, we define sink conditions as selection and maintenance of a release medium with sufficient solvating power such that the concentration of the drug in the release medium (following drug release from the ring) does not exceed 10% of its saturation concentration during the release experiment. For example, dapivirine is soluble to the extent of ~1200 µg/mL in 1:1 isopropanol/water mixture at 37°C. Day 1 *in vitro* release of dapivirine from a 25 mg dapivirine matrix-type vaginal ring into 200 mL is ~2500 µg, which corresponds to ~1% of saturation concentration [7], significantly below the 10% sink condition threshold.

Sink conditions are commonly assumed for vaginal rings *in vivo*, even for highly water-insoluble drugs and despite the very low volumes (< 2mL) of vaginal fluid normally present [47]. From a theoretical perspective, sink conditions are more likely to be operating *in vivo* for rings offering relatively low microgram per day release rates, such as the reservoir rings Estring[®], Nuvaring[®] and Ornibel[®]. By comparison, the marketed progesterone rings Fertiring[®] and Progering[®] (Table 1), which provide release of approx. 10 mg/day, may saturate the vaginal fluid. Surprisingly, for many vaginal ring products, it is not known (or at least it has not been reported) to what extent initial drug loading

within the ring impacts systemic pharmacokinetics. For highly water-insoluble drugs delivered from rings, it is entirely possible that drug saturation is maintained in vaginal fluid, such that drug solubility – rather than the device itself – is rate-controlling. However, the relatively large sink offered by the vaginal mucosal tissue will likely compensate for poor vaginal fluid solubility of poorly water-soluble drugs. In fact, it is entirely possible that drug at the ring surface could permeate directly from the ring into the surrounding mucosal tissue via a ring-to-tissue partitioning process. By comparison, release of relatively water-soluble molecules (having relatively low log P values) from vaginal rings may quickly saturate the limited volume of vaginal fluid available, and yet be effectively trapped there due to an unfavourable partition/distribution coefficient with the mucosal tissue. In this scenario, sink conditions may not apply, and release could theoretically be solubility controlled. Unfortunately, more fundamental studies to address such issues relating to vaginal ring performance *in vivo* are lacking, highlighting the need for further careful research.

3.4. Biorelevant release media

Although it is strongly encouraged, drug regulatory authorities do not strictly require the selected release medium to reflect *in vivo* physiological conditions. Non-physiologically relevant *in vitro* release test methods can be approved if they are demonstrated to be sufficiently robust and discriminatory, i.e. capable of reliably detecting changes in the dosage form that *could* potentially lead to changes of the drug product performance *in vivo*.

Driven by efforts to provide better *in vitro-in vivo* correlations (IVIVCs; see later section) and physiological relevance for vaginally-administered products, there has been

a move in recent years towards use of release media that more closely mimic the chemical composition, pH and volume of vaginal fluid [47,69]. Although its composition is affected by contributions from cervical mucus (pH ~8.0) [70], semen (pH ~7.5) [71] and menstrual material, normal vaginal fluid in pre-menopausal and post-menopausal women is generally within the pH range 4–5 and >5, respectively [47]. Daily production of vaginal fluid is estimated at around 6 g/day, with approximately 0.5–0.75 g present in the vagina at any time [47]. Taking the lower value within this range, the 10% solubility value commonly defined for sink conditions translates to approximately 50 mg drug in 0.5 mL of vaginal fluid. Fortunately, such large drug doses are not possible (nor clinically warranted) with marketed low-dose steroid-releasing vaginal ring products. However, vaginal drug doses close to or in excess of 50 mg are not unusual for other vaginally-administered products. For example, vaginal application of metronidazole gel for treatment of bacterial vaginosis requires relatively high daily dosing in the range 37.5–70 mg (one or two doses daily). This level of dosing could also potentially be achieved with a ring device (assuming sufficiently high release characteristics), and for which sink conditions may not hold.

Aqueous-based media adjusted to pH 4–5, and most notably simulated vaginal fluid (SVF), are now commonly used in the *in vitro* dissolution/release testing of vaginal dosage forms, including vaginal rings [7,28,46,47,62,66,69,72–90]. SVF is prepared either following the published recipe [47], or additionally containing small quantities of surfactants to enhance its otherwise limited solvating power (Table 1) [7,69]. SVF was primarily developed to simulate the chemical composition of vaginal fluid, with a particular emphasis on modelling pH and osmolarity; it is not intended to mimic the viscosity of vaginal fluid [47]. Several surfactants have been reported for addition to

SVF media, including Tween 80 (also known as Polysorbate 80), Solutol HS15 (also known as Kolliphor HS15) and benzalkonium chloride (Table 1) [7,36,48,69,91–94].

3.5. Solvent/water release media

For ring devices containing very poorly water-soluble drug molecules, *in vitro* release testing under sink conditions has been widely reported using solvent/water mixtures. In fact, much of the early research into steroid-releasing vaginal rings made use of highly concentrated aqueous solutions of polyethylene glycol [59–61]. More recently, for vaginal rings releasing highly water-insoluble antiretroviral drugs, alcohol/water mixtures have become common (Boyd et al., 2016; Fetherston et al., 2013b, 2013a; Malcolm et al., 2012b, 2005; McCoy et al., 2017; Murphy et al., 2016). For example, vaginal rings containing the antiretroviral drug dapivirine (water solubility $\ll 1$ $\mu\text{g/mL}$) [67,96] are routinely tested using 100 mL of a 1:1 v/v mixture of isopropanol/water (Table 1). In fact, similar solvent-based mixtures are used for USP compendial dissolution testing of oral tablets containing the anti-malarial drug atovaquone (water solubility <0.2 $\mu\text{g/mL}$) and a 5% methanol solution is used to test an injectable suspension of triptorelin pamoate [31]. In general, the solvent-water ratio can be readily adjusted to achieve the desired solvating power [97], and potentially to match *in vivo* release rates.

4. *In vitro* release test methods for vaginal rings

Here, we describe in more detail the various *in vitro* test methods for marketed vaginal rings and rings that are currently in late-stage development. For other drug-releasing vaginal ring devices, the interested reader is directed to the scientific literature, particularly journal articles and information provided in patents (both applications and

granted). Invariably, these rings are also tested for *in vitro* release using similar test methods to those described here. The following review articles will provide a useful entry into the journal literature [1,98–100].

4.1. Nuvaring®

Despite the lack of published information on the *in vitro* release testing method used for the contraceptive vaginal ring product Nuvaring®, much can be gleaned from the patent literature and a supporting PhD thesis [33–35,101,102]. According to these sources, individual Nuvaring® devices are immersed in 200 mL water at 37 °C under continuous stirring at 750 rpm. In order to maintain sink conditions, water is refreshed daily by an auto-sampler. Based on the relatively high stirring speed reported, and the reference to 'stirring' in the source documents, it is assumed that a magnetic stirring bar method is being used, which also explains the need to suspend the ring in the release medium using a nylon string. Experimental water solubility for etonogestrel is not available. However, ethinyl estradiol, has a reported pH-dependent water solubility of between 9.1 and 10.8 mg/L at 25 °C (>1.8 mg/200 mL) [103]. With a mean ethinyl estradiol release rate of 15 µg/day, the 200 mL release volume easily provides sink conditions over the 1-day sampling interval.

Externbrink *et al.* have reported the use of a USP Apparatus 7 (reciprocating holder, 40 dips/min) to assess real-time and temperature-accelerated etonogestrel release (but not ethinyl estradiol) from Nuvaring® [104]. Ring segments of length 1–1.5 cm, having their ends sealed with an acrylate glue to prevent release from the segments ends, were placed into 50-mesh basket holders within individual dissolution cells containing 10 mL of either simulated vaginal fluid with 0.1% sodium azide or water. Release experiments

were conducted at 37, 44, 50, and 55 °C with automated sampling and replacement of media performed every 12 h. Etonogestrel daily release vs. time profiles measured at 37 °C for ring segments standardised to release per ring showed slightly decreased release for the USP 7 apparatus compared with the FDA-approved standard test method [33,35,101,102]. Also, etonogestrel release increased with temperature, with zero-order release constants consistent with the Arrhenius equation. The closed system USP 7 apparatus was also useful in minimising media loss by evaporation at higher temperatures, an issue that is known to impact release when using USP Apparatus 2 (rotating paddle) for testing of vaginal rings [Karl Malcolm; unpublished data].

4.2. *Estring*[®]

There are two reports of an *in vitro* release test method for *Estring*[®], a silicone elastomer reservoir-type ring offering 90-day controlled release of 17 β -estradiol for estrogen replacement therapy (Table 1). The first, a 1994 journal article by Schmidt et al., describes placement of each ring in a 500 mL conical flask containing 250 mL of 0.9% saline, the flasks placed in linear shaking water bath at 37 °C operating at 60 strokes/min (Table 1) [30]. Based on literature values for estradiol water solubility of (1.5–3.6 mg/L at various temperatures and pH; i.e. >375 μ g/250 mL) and the relatively low 17 β -estradiol release rate from the ring (7.5 μ g/day), the 250 mL volume of saline might easily maintain sink conditions over cumulative release periods of up to 5 days [103]. A modified incubator method for *in vitro* release testing of *Estring*[®] is also reported in the FDA's Dissolution Methods Database, for which a shaking speed of 130 rpm is used [31].

4.3. *Femring*[®]

No method describing the *in vitro* release testing of Femring[®] is publicly available. However, as part of preclinical development of Femring[®], Woolfson *et al.* reported 90-day *in vitro* release testing of core-type silicone elastomer vaginal rings containing 17 β -estradiol or one of its esters (Woolfson et al., 1999). The method involving suspending each ring by a thread in an individual closed flask containing 500 mL of the dissolution medium (either 0.9% w/v saline, 0.133% w/v or 1.0% w/v aqueous benzalkonium chloride, BKC) maintained at a constant temperature of 37 °C in a shaking orbital incubator, with the dissolution medium renewed every 24 h. Based on solubility measurements for each drug, it was determined that only the 1.0% w/v BKC solution provided sink conditions.

4.4. Annovera[®]

A reservoir-type, silicone elastomer, contraceptive vaginal ring offering controlled release of Nestorone[®] (segesterone acetate) and ethinyl estradiol for one year has been developed by the Population Council and recently been approved by the FDA (Table 1). As with other marketed vaginal rings, a relatively large volume (400 mL) of water is used for *in vitro* release testing, with daily replacement of the medium to maintain sink conditions. In this instance, a linear shaking water bath set to operate at 100 oscillations (strokes) per minute is used to house the ring flasks during the test.

4.5. Dapivirine ring

A matrix-type silicone elastomer vaginal ring releasing dapivirine, an experimental non-nucleoside reverse transcriptase inhibitor, has been in development since 2002 and has recently completed Phase III clinical testing as a female-controlled method for prevention of HIV-1 infection [68,105–107]. Numerous journal articles have reported *in*

vitro release testing of vaginal rings containing either dapivirine alone or dapivirine in combination with another drug, most using a shaking incubator or shaking water-bath method [7,16,23,62,64,66,68,95,107]. Since dapivirine has very poor solubility in aqueous media ($<<1\text{ }\mu\text{g/mL}$) [67,96], much of the reported *in vitro* release data for the vaginal ring describes use of a 1:1 isopropanol/water mixture in which dapivirine has greatly enhanced solubility [7,64,68,97,107]. In general, compendia (USP, Ph. Eur., et al.) strongly discourage the use of organic solvents for dissolution methods, allowing such use only when no appropriate alternatives can be identified. However, other products containing very poorly water-soluble drugs (e.g. atovaquone tablets) are tested using a medium containing 40% isopropanol [31]. For the dapivirine ring, the 1:1 isopropanol/water medium was found to be most suitable, although recent publications have explored the use of aqueous solutions containing surfactants [7], concluding that the aqueous-based medium is complementary, but not superior, to the isopropanol/water medium.

5. Apparatus for *in vitro* release testing of vaginal rings

5.1. Shaking incubator apparatus

Most methods described in the literature for *in vitro* release testing of vaginal rings make use of a temperature-controlled shaking incubator (Table 1). Examples of different types of shaking incubators are presented in Fig. 3. There are three important operational variables for these incubators that are known to affect drug release characteristics: (i) the type of shaking, e.g. linear vs. orbital; (ii) the speed of shaking (e.g. oscillations or revolutions per min); and (iii) the distance through which shaking occurs, referred to as the stroke length for linear shaking incubators and orbital diameter for orbital shaking incubators. Most shaking incubators will allow adjustment of the shaking speed. With

some incubators, the shaking distance can also be adjusted. Changing any of these three variables will modify both the dynamics of fluid flow around the ring device and, in turn, the thickness of the diffusion boundary layer (the relatively static layer of fluid immediately adjacent to the ring surface) [59,108–110]. In general, increasing the shaking speed or distance leads to increased rate of drug release, although there is very limited data reported in the literature on this topic [111].

Fig. 3. Different type of shaking incubators commonly used for in vitro release testing of vaginal rings. A – a benchtop shaking incubator; B – a floor-standing top-opening orbital shaking incubator; C – stackable orbital shaking incubators; D – flasks containing suspended rings and stored in an orbital shaking incubator.

5.2. Flow-through apparatus

A simple continuous flow-through apparatus has previously been reported for *in vitro* release testing of matrix-type, core-type and sandwich-type silicone elastomer vaginal rings containing the progestogens D(–)-norgestrel, norethindrone or progesterone [37,38,112]. In general, the apparatus comprised (i) an isotonic saline solution, (ii) a peristaltic pump, (iii) a glass flow cell containing the sample ring device and maintained at 37 °C by immersion in water bath, and (iv) a vessel to collect the saline solution effluent. Flow rates in excess of 1000 mL/day were needed to maintain sink conditions, and the experiments were run continually for up to 60 days. The *in vitro* release rates were subsequently calculated based on the amount of progestogen present in a known aliquot of the effluent and the total volume of effluent collected over a defined period of time. Extraction and concentration of progestogen from the flow cell effluent was performed by extracting the aliquot of effluent (usually 100 mL) with methylene chloride (10 mL) containing a suitable internal standard, before quantitation of the progestogen using gas chromatography. Using this method, similarity of progestogen *in vitro* release rate was demonstrated for rings tested (i) immediately after manufacture, (ii) unused and after two years storage, (iii) on day 10 of *in vitro* release testing, (iv) at the end of *in vitro* release testing, and (v) after clinical testing of the ring.

5.3. Reciprocating holder apparatus

USP Apparatus 7 (reciprocating holder) is commonly used to test transdermal patch drug products. However, the apparatus can also be applied to extended release tablets, capsules, osmotic pumps, beads and arterial stents. There is only one report in the literature describing use of this apparatus for vaginal ring testing. Externbrink *et al.* described use of Apparatus 7 for accelerated *in vitro* release testing of Nuvaring® [104]. Ring segments, 1–1.5 cm in length and sealed with an acrylate glue that was

impermeable to the steroid molecules to eliminate end effects, were placed into 50-mesh basket holders within heated glass dissolution cells containing 10 mL vaginal fluid simulant or water and experiments performed at temperatures of 44, 50 and 55 °C.

5.4. Rotating paddle apparatus

Only one paper has reported use of a rotating paddle dissolution apparatus (Apparatus II) for *in vitro* release testing of a vaginal ring device [58]. Here, the authors tested a drospirinone-releasing silicone elastomer vaginal ring into 200mL of 0.3% sodium dodecyl sulfate (SDS) solution maintained at 37 °C and stirred at 50 rpm. The release medium was replaced every 24 hr over the 21-day study period. Drospirenone solubility was determined to be 13.0 µg/mL, 12.1 µg/mL, 8.9 µg/mL, 15.2 µg/mL, and 392.6 µg/mL in pure water, NaCl solution, PBS (pH 3.8), 0.03% SDS solution, and 0.3% SDS solution, respectively. 200 mL 0.3% SDS medium was selected to maintain sink conditions. Evaporative loss of the medium during release testing was not discussed in the paper.

6. Modelling of *in vitro* release data

It is not the purpose of this review to provide a detailed overview of the various mathematical models used to model the kinetics of *in vitro* drug release from vaginal rings. However, the most commonly reported kinetic models used to assess data obtained from the *in vitro* release testing of vaginal rings are briefly described.

All seven marketed vaginal rings are fabricated from thermoplastic polymers or silicone elastomers. Five rings - Estring[®], Nuvaring[®], Femring[®], Ornibel[®] and Annovera[®] - are reservoir-type rings, while Fertiring[®] and Progering[®] are matrix-type rings. The

379 dapivirine-releasing ring is also a matrix ring. The non-degradable and non-swelling
380 nature of these polymeric rings means that the incorporated drugs are released according
381 to a permeation-controlled mechanism (also commonly referred to as diffusion-
382 controlled mechanism). Thus, it is common to use the terms 'membrane-controlled
383 permeation' for reservoir/core rings, and 'matrix-controlled permeation' for matrix-type
384 rings. Regardless of the ring design, permeation-controlled drug release from a vaginal
385 ring requires at least three distinct mechanistic steps: (i) the incorporated drug must
386 dissolve to some extent in the polymer(s) used to fabricate the ring; (ii) the dissolved
387 drug molecules must then diffuse through the polymer(s); and (iii) upon reaching the
388 surface of the ring, the drug(s) must partition into the surrounding vaginal fluid/release
389 medium.

390

391 For matrix-type rings, in which the (usually) solid crystalline drug substance is dispersed
392 homogeneously throughout the ring body, drug release under sink conditions normally
393 follows so-called 'root-time kinetics'. This is confirmed by a linear plot when the
394 cumulative release data is plotted against the square root of time. This model was first
395 introduced by Higuchi back in 1961 for release of drug from a thin ointment film into the
396 skin [113]. Based upon certain key assumptions, the two classical forms of the 'Higuchi
397 equation' can be derived (Eqs. 1 & 2; Table 3) [114,115]. These equations have been
398 reported many times for modelling drug release from matrix-type vaginal rings
399 [41,49,55,62,64,65,89,92,95,116–122]. Only one matrix-type vaginal ring device has
400 been reported containing a liquid drug at room temperature, and for which release
401 kinetics are significantly modified compared to matrix-type ring containing solid drugs
402 due to the absence of a drug depletion zone [56].

Table 3. Equations that have been used to model *in vitro* release data for matrix-type and reservoir-type drug-releasing vaginal rings.

Type of ring	Equations	References / Comments
Matrix-type rings	Eq.1. $\frac{M_t}{A} = \sqrt{DC_s(2C_0 - C_s)t}$	[113,114,123,124]
	Eq.2. $\frac{M_t}{A} = \sqrt{DC_s(2C_0)t}$	Simplified version of Eq.1 where $C_0 \gg C_s$
	Eq.3. $M_t = k\sqrt{t}$	[125]
Reservoir-type rings	Eq.4. $M_t = \frac{(2\pi C_s DL)}{\ln b/a} t$	[33,36,126–128]
	Eq.5. $M_t = \frac{(C_s DA)}{h} t$	[55]

By comparison, reservoir-type rings, comprising a drug-loaded core surrounded by a rate-controlling polymeric membrane, generally offer controlled release of drug(s) at a near constant rate of release. Most often, the release rate is described in units of µg/day. For example, Femring® provides release of estradiol-3-acetate at rates equivalent to either 50 or 100 µg/day estradiol for 3 months (Table 1). An entirely similar permeation-controlled mechanism operates for reservoir rings, except that the drug-free membrane material and/or thickness is judiciously selected to differ from that of the drug-loaded core so as to control the drug release rate. For example, the drug-loaded core of Nuvaring® is fabricated using an ethylene vinyl acetate (EVA) copolymer containing 28% vinyl acetate residues, while the rate-controlling EVA sheath contains only 9% vinyl acetate residues (Table 1). A reduction in the vinyl acetate content leads to lower drug permeation rates, owing to increased proportion of impermeable crystalline domains within the semi-crystalline EVA polymer [129]. By comparison, most

reservoir-type silicone elastomer rings (e.g. Femring[®] and Estring[®]) use the same silicone elastomer material for fabrication of both the core and the membrane. Here, the thickness of the membrane and the length of the drug loaded core control the observed release rate.

Reservoir-type rings tested under sink conditions are usually characterised by linear daily release *vs.* time plots (neglecting any initial lag or burst effects) and linear cumulative release *vs.* time plots (Fig. 4), and follow 'zero-order' release kinetics. Equations 4 and 5 (Table 3), in which the cumulative amount of drug release increases proportionately with time, are commonly reported for the modelling of drug release from reservoir-type rings.

7. Risk of dose dumping

With monolithic matrix-type vaginal rings where the drug is evenly dispersed throughout the entire volume of the device, a natural drug burst is observed at the beginning of *in vitro* release testing due to rapid release of drug close to the ring surface (Fig. 4A and 4C). This is due to the fact that drug at or near the surface of a matrix ring has a relatively small diffusional pathway to overcome in order to be released. Even if a matrix-type ring were to rupture *in vivo*, the increase in drug release rate would not likely be clinically significant.

Fig. 4. Representative in vitro drug release vs. time profiles for matrix (A, C and E) and reservoir-type vaginal rings (B and D).

With reservoir or core-type vaginal rings, in which the drug(s) is located in one or more reservoirs or cores encapsulated with a drug-free membrane or sheath, lag or burst effects are often observed during the initial period of in vitro release testing (Fig. 4B and 4D). These effects are due to redistribution of the drug(s) within the ring caused by (i) enhanced polymer solubilisation of the drug during high-temperature manufacture of the rings, (ii) cooling and recrystallisation of the drug within the rings following manufacture, and (ii) subsequent long-term storage of the ring product.

In order to better capture data around initial lag and burst effects, it is becoming increasingly common to include additional early sampling timepoints (e.g. 4, 8, 12 hr) during *in vitro* release testing of rings. Thereafter, the release medium is typically sampled once daily (except weekends) for the first few weeks following initiation of *in vitro* release testing, and then less frequently (often once or twice weekly), ensuring sink conditions are maintained throughout the duration.

Over and beyond these burst and lag effects, there is also a potential risk of dose dumping with reservoir-type rings due to either defects introduced during manufacture or *in vivo* rupture of the rate-controlling membrane. Admittedly, the risk is very low for conventional reservoir-type rings containing polymeric cores (such as Estring[®], Femring[®] and Nuvaring[®]) since (i) the methods of manufacture (injection molding and extrusion) are highly reliable and robust, (ii) the rings are very unlikely to break under even extreme conditions of compressive and tensile stress, and (iii) even in the event of a break in the membrane, the drug in the exposed reservoir compartment would still be released according to a permeation-controlled mechanism, albeit no longer conforming to zero-order kinetics for at least a period of time. To date, there have been no reports or other evidence of dose dumping *in vivo* with use of vaginal rings. Nonetheless, regulatory agencies normally request *in vitro* release data for testing of vaginal rings in which the drug containing core(s) has been artificially exposed by rupturing the membrane. Introduction of flaws into the vaginal rings is also useful in demonstrating the discriminating ability of the *in vitro* release method.

Three-layer reservoir-type thermoplastic vaginal rings offering controlled release of the antidepressant drug mirtazapine or the antipsychotic drug risperidone have been tested

for the risk of dose dumping by making a single cut through the ring cross-section, thereby exposing two ring ends [130,131]. The *in vitro* drug release performances of the cut rings were entirely similar to those of control rings that had not been cut.

In more robust testing of the potential risk of dose dumping, the Population Council's NES+EE ring was cut to remove part of the rate-controlling membrane surrounding the drug-loaded cores. In one experiment, a certain thickness of the silicone elastomer membrane was removed without exposing the underlying drug-loaded cores, thereby significantly shortening the drug diffusion path and increasing the *in vitro* release rate. In another experiment, the membrane was removed to expose the cores within the ring body. These configurations are useful in assessing the discriminating ability of the *in vitro* release method (Bruce Variano; unpublished data).

8. Accelerated *in vitro* release testing of vaginal rings

A particular challenge with vaginal rings is the long time periods required for real-time drug release testing. Compared to immediate release oral dosage forms for which dissolution can often be completed in less than 1 h, drug release from rings usually extends over many weeks or months (Table 1), with major implications for batch release and shelf-life of the product. To overcome this time constraint, and in addition to real-time release testing, quality control testing for extended, sustained or controlled release drug products is often performed using accelerated *in vitro* release testing methods. This approach has been successfully applied to a wide range of dosage forms through modification of the test conditions, such as increasing temperature, adjusting the pH of the release medium, using solvent-based release media, adding surfactants to aqueous release media, or adjustment of the agitation rate [11,50,132–135]. However, to date, there have been only two reports

in the literature of accelerated release testing of a vaginal ring device using elevated temperature conditions, solvent+water mixtures (ethanol, isopropanol or acetonitrile), or both [49,104].

Accelerated testing methods (which must also be discriminating and, if possible, clinically relevant) should provide a distinct *in vitro* release profile in which at least 80% of the initial drug load has been released. Ideally, drug release in both real-time and accelerated tests should follow the same release mechanism with a 1:1 correlation between the release profiles. However, in practice, the release mechanism may change if the physical properties of the ring change upon exposure to the accelerated condition.

Conducting *in vitro* release at elevated temperature typically leads to an increased rate of release without change to the mechanism, due to an increase in the permeation coefficient of the drug in the polymer. Use of high concentrations of organic solvents in the release medium, however, may lead to changes in the underlying release mechanism, for example from a permeation control mechanism (where the rate-limiting step is diffusion of the polymer-dissolved drug through the polymer) to an extraction-type mechanism involving solvent influx into the ring, swelling of the ring polymer, and for which the rate limiting step is the solubility of the drug in the medium. Accelerated *in vitro* release testing of vaginal ring devices is only valuable in limited cases and with the understanding that a correlation to real-time drug release may not, and likely will not, be achievable.

9. *In vitro-in vivo* correlations and relationships

9.1. *In vitro-in vivo* correlations

In vitro-in vivo correlations (IVIVCs) are correlations between *in vitro* and *in vivo* data that support subsequent prediction of *in vivo* results based on measured *in vitro* data [136–139]. According to the FDA definition of IVIVC, the *in vitro* data usually takes the form of the rate or extent of drug dissolution or release, while the *in vivo* response is commonly plasma drug concentrations or the amount of drug absorbed [140]. The USP define IVIVC is similar but somewhat broader in its interpretation, extending the *in vivo* data to a "*biological property produced by a dosage form*", and the *in vitro* data as "*a physicochemical property or characteristic*" of the dosage form [141].

9.2. *In vitro-in vivo* relationships

By comparison, *in vitro-in vivo* relationships (IVIVRs) are semi-quantitative or rank-order relationships between *in vitro* release data and an *in vivo* outcome (e.g. blood levels of the drug or the amount of drug absorbed) [142–144]. As such, IVIVRs are not considered as robust as IVIVCs. However, if a qualitative relationship can be established between *in vitro* release rate and *in vivo* absorption, then the *in vitro* release rate method may be deemed clinically relevant and therefore a safe space can be built via bracketing approach. Subsequent major changes whose dissolution profiles fall within this safe space are considered bioequivalent [145].

9.3. IVIVCs and IVIVCs for vaginal rings

Given the focus of this review article on *in vitro* release testing methods for vaginal rings, it is worth taking time to consider the role of these methods in developing IVIVCs/IVIVRs for vaginal rings. IVIVCs are most commonly used by pharmaceutical companies to justify bioequivalence waivers for formulation changes (excipients, dosage strengths, etc.) without *in vivo* data, and are increasingly recommended by regulatory

authorities in order to demonstrate product understanding. Although *in vitro* release testing has been used extensively as a quality control tool for testing and comparing vaginal ring formulations, predictions of *in vivo* performance from *in vitro* release data are either rarely performed, or at least are rarely reported in the literature. Two contributing factors may help explain the scarcity of IVIVCs for ring products. First, IVIVCs require a validation step based on clinical studies (e.g. bioavailability /bioequivalence studies), which are expensive and typically require multiple developed formulations showing different release profiles in multiple media [146]. Second, other drug dosage forms (and particularly oral dosage forms) are typically more lucrative than vaginal rings, such that there is a financial component/incentive to develop IVIVCs, since line extensions (different strengths, more efficient manufacturing, etc.) will often be developed after initial product approval. The use of non-compendial apparatus and methods for *in vitro* release testing of vaginal rings, the often very poor water solubility of drugs formulated in vaginal rings, the relatively long duration of release offered by vaginal rings, and the use of non-biorelevant release media may also contribute to the lack of good predictive models.

However, for non-oral or locally acting (i.e. not systemically absorbed) drug products, including vaginal rings, establishing IVIVCs is often challenging due to the complexities of the formulations, the the lack of standardized compendial *in vitro* release testing methods, and significant inter-patient variability in pharmacokinetic data [72,147]. For example, the first IVIVC for a marketed transdermal drug delivery system (a drug-in-adhesive type estradiol-releasing transdermal patch offering multiple drug delivery rates) has only been reported within the past five years [148]. In the context of vaginal ring

research, the most anticipated use of IVIVC is to support biowaivers for manufacturing and non-compendial excipient changes.

9.4. Different levels of IVIVC

The FDA defines four main levels of IVIVC, depending upon the type of data used to establish the relationship and the ability of the correlation to predict the complete plasma profile of a dosage form [140,147,149]. A Level A correlation represents a point-to-point relationship (usually linear) between an *in vitro* release performance measure and an *in vivo* response measure over the entire time course of both studies. The FDA define Level A correlations as: "*A predictive mathematical model for the relationship between the entire in vitro dissolution/release time course and the entire in vivo response time course, e.g., the time course of plasma drug concentration or amount of drug absorbed*" [140]. For a vaginal ring device, the daily *in vitro* release vs. time and the plasma, vaginal fluid and vaginal tissue drug concentration vs. time data are likely to be most useful in establishing the correlation (Fig. 4), the latter two more so for drug products intended to act locally. For a matrix-type vaginal ring containing excess solid drug dispersed throughout the ring body and offering $t^{1/2}$ release kinetics (Fig. 4), plasma concentrations are anticipated to decline with time, in line with the declining daily *in vitro* release values [7,55,64,66]. It should be noted that for rings releasing very low quantities of drug such that plasma levels are extremely low and highly variable (e.g. the dapivirine-releasing matrix-type vaginal ring [150–153], any potential correlation may be lost in the noise. For reservoir-type rings, which offer near-constant daily *in vitro* release rates, plasma concentrations are observed to remain almost constant or to decline slowly with time [30,36,154]. Certain practical difficulties can arise with attempting Level A correlations for vaginal rings, including: (i) the requirement to support extended

601 measurement of *in vivo* drug concentrations over periods of many weeks or months; (ii)
602 variations in *in vivo* drug concentrations among ring users due to physiological factors,
603 such as vaginal pH, vaginal microbiome, body mass index, age, etc.

605

Fig. 5. Workflows describing methods for developing IVIVCs for matrix-type (A–D) and reservoir-type vaginal rings (E–H), where the *in vivo* parameter is the total dose administered at any time t . This dose is calculated by subtracting the value for the residual drug content in the ring after a specified period of clinical use (based on a solvent extraction method) from the value of the initial drug loading in the ring. Panels I and J illustrate other possible correlations based on use of either plasma AUC or measured drug concentration in a biological medium (usually plasma, vaginal tissue or vaginal fluid) as the readout for the *in vivo* parameter.

For the contraceptive vaginal ring product Nuvaring[®], an IVIVC with a level A correlation was successfully established, determined predictive for *in vivo* performance and thus accepted as a basis for obtaining *in vivo* bioequivalence waivers [155].

Level B correlations, like those of Level A, make use of all of the *in vitro* and *in vivo* data, but are not considered to be point-to-point correlations. Instead, a Level B IVIVC uses the principles of statistical moment analysis. The FDA define Level B correlations as: "*A predictive mathematical model for the relationship between summary parameters that characterize the in vitro and in vivo time courses, e.g., models that relate the mean in vitro dissolution time to the mean in vivo dissolution time, the mean in vitro dissolution time to the mean residence time in vivo, or the in vitro dissolution rate constant to the absorption rate constant*" [140]. Typically, the mean *in vitro* dissolution time is compared either to the mean residence time (MRT) or to the mean *in vivo* dissolution time. Level B correlations do not uniquely reflect the actual *in vivo* plasma level curve, because a number of different *in vivo* curves will produce similar mean residence time values [138]. Level B correlations are least useful for regulatory purposes, are rarely encountered even for new drug applications (NDAs) for oral dosage forms and have never been reported for vaginal rings.

A Level C correlation establishes a single point relationship between a dissolution/release parameter and a pharmacokinetic parameter. The FDA definition is as follows: "*A predictive mathematical model of the relationship between the amount dissolved in vitro at a particular time (or the time required for in vitro dissolution of a fixed percent of the dose, e.g., T50%) and a summary parameter that characterizes the in vivo time course (e.g., C_{max} or AUC)*" [140]. In the context of vaginal rings, the *in vitro*

parameter would typically be the daily release rate, while the *in vivo* parameter could be selected from plasma measurements over time – area under the curve (*AUC*), maximum plasma/serum concentration (*C_{max}*) or time to maximum concentration (*T_{max}*). Single point Level C correlations are not considered useful to IVIVC of vaginal ring products and are not acceptable to regulatory agencies, however, multiple point, Level C correlations may be used to justify a biowaiver provided the correlation is established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. Level C correlations are particularly useful in the early stages of formulation development when different formulations are being tested.

According to best practice, the development of IVIVC requires *in vivo* pharmacokinetic (PK) data with at least three different drug dissolution/release rates for the pharmaceutical product. In principle, this is relatively easy to achieve for many vaginal ring devices. For example, adjusting the drug loading in a matrix-type ring will effectively modulate the release rate [17,55,64,66,156]. For reservoir-type rings, adjusting either the length of the internal drug-loaded reservoir or the thickness of the rate-controlling membrane will effectively modulate the release rate [17,36,64,128]. For pod-type rings, the drug release rate can be adjusted either by changing the number of pods included in the ring body or by modifying the design characteristics of the delivery channel [21,25]. In practice, however, it is very unusual for a company to develop and market vaginal ring products offering different release rates. Femring[®] is the exception, with two different release rates available (50 µg/day and 100 µg/day estradiol, Table 1).

IVIVCs have not been attempted for antiretroviral-releasing vaginal rings for HIV prevention. In order to be clinically effective, the antiretroviral drug(s) needs to be

released from the ring device at sufficient rate to maintain the concentrations of drug in the vaginal fluid and/or vaginal tissue above a minimum protective level. For this reason, IVIVC studies are not considered, since there is no clinical rationale for commercialising anything but a single formulation type having an efficacious release rate. Moreover, in late-stage efficacy trials of antiretroviral-releasing rings, low dosing would increase the risk of HIV acquisition, and high dosing would likely increase the extent of systemic absorption and potentially drive the emergence of resistant strains of the virus in users who became infected while using the product.

The development and testing of different drug release rates for steroid-releasing vaginal rings for hormone replacement therapy or contraception is equally challenging from an IVIVC perspective. In general, contraceptive rings are developed to release drug(s) at a single clinically-relevant rate, as exemplified by Nuvaring and Annovera (Table 1). The fact that only one release rate may be required is not a limitation for developing an IVIVC. The situation is somewhat different for rings used for estrogen replacement therapy, where different rates of estrogen administration may be beneficial. For example, Femring (but not Estring) is supplied in two strengths (50 and 100 µg/day, Table 1). With this product, therapy is started at the lowest effective dose and the shortest duration consistent with treatment goals, and dosage adjustment is guided by the clinical response.

9.5. Literature examples of IVIVCs and IVIVRs for vaginal rings

One of the earliest reports of an IVIVC for an experimental vaginal ring was reported for ethynodiol diacetate-releasing silicone elastomer devices implanted in rabbits, in which both the plasma drug concentrations of norethindrone (a major metabolite of ethynodiol

diacetate) and the cumulative amount of drug released *in vivo* were correlated with the *in vitro* release rates obtained under three hydrodynamic conditions [59]. Due to the small size of the rabbit vagina relative to that of women, the test devices comprised 1 cm sections cut from rings designed for use in women. *In vitro* release experiments were conducted with similar devices into a 75% polyethylene glycol 400 aqueous solution (to provide sink conditions) at each of 0, 30 and 81 rpm rotational speeds. The cumulative amount of ethynodiol diacetate released *in vivo* was obtained by determining the residual drug content (by extraction with methanol) after different durations of implantation. The measured *in vivo* release rate of $2.10 \text{ mg/cm}^2/\text{day}^{0.5}$ was noted to lie between the *in vitro* release rates obtained for the rings tested under the 30 and 81 rpm conditions.

Timmer et al. have reported that *in vivo* release rates were in good agreement with *in vitro* release rates for silicone elastomer, reservoir-type, combination contraceptive vaginal rings offering controlled release of 3-keto-desogestrel (also known as etonogestrel) and ethinyl estradiol [157]. In fact, these rings were early prototypes for Nuvaring® (Table 1). Three different ring formulation were tested, offering mean daily release rates of 75, 100 or 150 $\mu\text{g/day}$ etonogestrel and 15 $\mu\text{g/day}$ ethinyl estradiol. The results from the study demonstrated (i) that *in vitro* release characteristics were independent of the conditions used, and (ii) a linear relationship between the *in vitro* release rates and the *in vivo* absorption rates for both drug molecules. Even though the results did not strictly meet the criteria outlined in the IVIVC guidance, the IVIVC was deemed acceptable due to the complex nature of the dosage form. The sponsor subsequently changed the batch size, the manufacturing process, and the amount of non-controlling excipients. Although these changes would normally require a bioequivalence study, the IVIVC was deemed acceptable and the changes were approved [137].

711

712 As part of study to develop a 90-day progesterone-releasing vaginal ring for use in
713 postpartum contraception, Landgren *et al.* have reported a direct correlation between
714 plasma progesterone levels (areas under curves) and *in vitro* release rates for rings
715 offering three different initial release rates (5, 8 and 20 mg/day) [158]. In a follow-on
716 clinical study, significantly higher concentrations of pregnanediol glucuronide – a major
717 progesterone metabolite excreted in the urine – were measured for the 20 mg/day ring
718 compared to the 5 mg/day ring [159].

719

720 Clark *et al.* makes reference to poor IVIVC in preliminary pharmacokinetic studies
721 testing a UC781-loaded silicone elastomer matrix-type ring in pig-tailed macaques [160].
722 However, the actual data was never published. UC781 is an experimental non-nucleoside
723 reverse transcriptase inhibitor that had previously been considered as a HIV microbicide
724 [119,161,162].

725

726 Johnson *et al.* have reported a polyurethane vaginal rings offering sustained release of
727 the experimental antiretroviral compounds IQP-0528 and IQP- 0532 [163]. The paper
728 describes *in vitro* release testing of the rings under both sink and non-sink conditions and
729 pharmacokinetic assessment in pig-tailed macaques. A simple *in vivo* correlation was
730 established between the drug levels measured in the vaginal fluid and the total amount of
731 the drugs administered *in vivo* during the 28-day pharmacokinetic study (as measured by
732 post-use solvent extraction). Although an IVIVC was not considered in the paper, the
733 authors noted that the cumulative percentages of the two drugs released under *in vitro*
734 non-sink conditions were similar to those observed *in vivo*, whereas *in vitro* testing using
735 a sink condition release medium overestimated release of the drugs.

736
737 McConville et al. reported the lack of an IVIVC in macaques for a matrix-type silicone
738 elastomer vaginal ring releasing the very poorly water-soluble experimental
739 antiretroviral drug UC781 [119]. However, once again, this was not an IVIVC. Different
740 sizes of rings, suitable for use in macaques and humans, were tested for *in vitro* release
741 using sink condition model (100 mL daily of 1:1v/v ethanol/water, 1:1v/v
742 isopropanol/water or 1 %w/v aqueous benzalkonium chloride solution). Residual drug
743 content values in rings initially loaded with 100mg UC781 were measured after both 28-
744 day vaginal placement in pig-tailed macaques and *in vitro* release testing. The results
745 showed 10-times less drug release *in vivo* compared to *in vitro*, which was attributed to
746 the limited solubility of UC781 in the aqueous vaginal fluid. Although the authors to not
747 comment on this issue, it is assumed that adjustment of the composition of the *in vitro*
748 release medium (to decrease its solvating power) would be useful in modulating the *in*
749 *vitro* release to more closely mimicking *in vivo* release.

750
751 Moss et al. have reported *in vivo* release rate divided by *in vitro* release rate as a measure
752 of IVIVR following pharmacokinetic testing of pod-type vaginal rings in macaques and
753 sheep [21,46]. The *in vitro* release rate was obtained from the slope of the *in vitro*
754 cumulative release vs. time profile. The *in vivo* release rates were based on the residual
755 drug mass remaining in the rings following *in vivo* use and the assumption, supported by
756 *in vitro* data, that drug release was linear over the test period. The calculated IVIVCs for
757 a range of antiretroviral drugs (including tenofovir disoproxil fumarate, maraviroc
758 and emtricitabine) ranged between 0.2–0.7. It should be noted that the release profile
759 does not have to be linear for a successful IVIVC/IVIVR, and the absorption profile in
760 the vaginal vault may not be the same for every woman.

761

762 Matrix-type rings containing dapivirine or progesterone [41,164] and reservoir-type and
763 sandwich-type silicone elastomer rings containing progesterone, d-norgestrel or
764 norethindrone [112] have been shown to provide estimated *in vivo* release rates (based
765 on measurement of post-use analysis of residual drug content and assuming a constant
766 release rate) that were not significantly different from measured *in vitro* release rates.

767

768 Finally, a physiologically-based pharmacokinetic model – the structural compartments of
769 which comprise the vaginal epithelium, vaginal stroma, lungs, liver and the rest of body
770 – has recently been reported for quantitative prediction of cervicovaginal tissue and
771 plasma concentrations for the 25 mg matrix-type dapivirine vaginal ring (Ring-004,
772 Table 1) [165]. (Fig. 5). Physiologically-based models may offer certain advantages over
773 more conventional approaches, including the potential to model the effect of
774 manufacturing changes, thereby allowing a potential better/easier prediction of *in vivo*
775 release rate.

Fig. 5. Structural compartments of a physiologically-based pharmacokinetic model for a drug-releasing vaginal ring. Figure adapted from (Kay et al., 2018).

10. Conclusions

Interest in vaginal ring technology for long-acting drug administration is growing, with seven products currently marketed and a raft of new products in preclinical and clinical development. In the continued absence of a compendial method for *in vitro* release testing of vaginal rings, there will likely remain significant variations in the apparatus and methods used for *in vitro* release testing of drug-releasing vaginal ring products. The methods described and referenced in this review, however, should prove useful for developers of new ring formulations. Further progress in the vaginal ring field and the

development of biopredictive *in vitro* drug release methods will help focus efforts towards the development of IVIVCs/IVIVRs.

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Disclosure of interests

PS is an employee of the International Partnership for Microbicides (IPM), owner and developer of various vaginal ring products, including the dapivirine ring. BV is an employee of the Population Council, developer of the Annovera[®] vaginal ring and with interests in other vaginal ring products. KM and PB have received research funding from both IPM and the Population Council.

Disclaimer

The views expressed here are the personal views of the authors and do not necessarily represent the views of their respective organisations.

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Table and figure captions

Fig. 1. Plot of log partition coefficient (log P; experimental or calculated values; protein molecules arbitrarily assigned a value of 1, due to lack of data) vs. molecular weight for drug molecules in marketed vaginal rings or having previously been considered for formulation in vaginal rings. Plot symbols inside the dashed box include estradiol, ethinyl estradiol, etonogestrel, estradiol-3- acetate, dapivirine, progesterone, levonorgestrel, maraviroc, MIV-150, oxybutynin, segesterone acetate (Nestorone[®]), norethindrone acetate, ulipristal acetate, medroxyprogesterone acetate, UC781, danazol, MC1220, CMPD-167, drospirinone, nomegestol acetate, and vicriviroc. Molecules outside the dashed box (see labels within the figure) are those currently being considered for vaginal ring formulations and that, due to physiochemical constraints, generally require novel formulation approaches. Figure used with permission [10].

Fig. 2. Different type of shaking incubators commonly used for *in vitro* release testing of vaginal rings. A – a benchtop shaking incubator; B – a floor-standing top-opening orbital shaking incubator; C – stackable orbital shaking incubators; D – flasks containing suspended rings *in vitro* undergoing release testing in an orbital shaking incubator.

Fig. 3. Sealed glass flasks for *in vitro* release testing of vaginal rings. A – ring placed on bottom of flask containing 100 mL of release medium. B – ring suspended by a nylon thread in glass flask containing 200 mL release medium.

Fig. 4. Workflows describing methods for developing IVIVCs for matrix-type (A–D) and reservoir-type vaginal rings (E–H), where the *in vivo* parameter is the total dose administered at any time t . This dose is calculated by subtracting the value for the residual drug content in the ring after a specified period of clinical use (based on a solvent extraction method) from the value of the initial drug loading in the ring. Panels I and J illustrate other possible correlations based on use of either plasma AUC or measured drug concentration in a biological medium (usually plasma, vaginal tissue or vaginal fluid) as the readout for the *in vivo* parameter.

Fig. 5. Biological compartments of a physiologically-based pharmacokinetic model for a drug-releasing vaginal ring. Figure adapted from [165].

1455

1456 **Fig. 6.** Representative *in vitro* drug release vs. time profiles for matrix (A, C and E) and
1457 reservoir-type vaginal rings (B and D).

1458

1459 **Table 1.**

1460 Descriptions of vaginal rings (both marketed products and products currently undergoing
1461 clinical testing) and details of *in vitro* testing methods.

1462

1463 **Table 2.** Factors that can potentially affect the *in vitro* drug release performance of
1464 vaginal rings.

1465

1466 **Table 3.** Equations that have been used to model *in vitro* release data for matrix-type and
1467 reservoir-type drug-releasing vaginal rings.

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