



## Research paper

## Sustained release of proteins from a modified vaginal ring device

Ryan J. Morrow<sup>a</sup>, A. David Woolfson<sup>a</sup>, Louise Donnelly<sup>a</sup>, Rhonda Curran<sup>a</sup>, Gavin Andrews<sup>a</sup>, Dietmar Katinger<sup>b</sup>, R. Karl Malcolm<sup>a,\*</sup>

<sup>a</sup> School of Pharmacy, Queen's University Belfast, Belfast, Northern Ireland, UK

<sup>b</sup> Polymun Scientific GmbH, Vienna, Austria

## ARTICLE INFO

## Article history:

Received 30 June 2010

Accepted in revised form 25 October 2010

Available online 3 November 2010

## Keywords:

Vaginal ring

Protein drug delivery

Vaccine

Microbicide

2F5

Lyophilisation

## ABSTRACT

A new vaginal ring technology, the insert vaginal ring (InVR), is presented. The InVR overcomes the current shortfall of conventional vaginal rings (VRs) that are generally ineffectual for the delivery of hydrophilic and/or macromolecular actives, including peptides, proteins and antibodies, due to their poor permeation characteristics in the hydrophobic polymeric elastomers from which VRs are usually fabricated. Release of the model protein BSA from a variety of insert matrices for the InVR is demonstrated, including modified silicone rods, directly compressed tablets and lyophilised gels, which collectively provided controlled release profiles from several hours to beyond 4 weeks. Furthermore, the InVR was shown to deliver over 1 mg of the monoclonal antibody 2F5 from a single device, offering a potential means of protecting women against the transmission of HIV.

Crown Copyright © 2010 Published by Elsevier B.V. All rights reserved.

## 1. Introduction

There is a strong consensus within the HIV microbicide field that a greater diversity of delivery technologies is needed to both effectively formulate the increasing number of microbicide candidates progressing through the development pipeline and to meet the needs and preferences of women in different cultural settings [1,2]. The inherent disadvantages associated with many vaginal delivery systems, such as poor user acceptability, leakage, short duration of vaginal retention, coital dependence (in the case of spermicides and HIV microbicides) and the inability to be used covertly without the knowledge of the male sexual partner, are widely recognised [3,4]. Considerable advances have been made toward the use of vaginally retentive semi-solid polymeric systems, which aim to reduce leakage and improve vaginal retention through mucoadhesion thereby prolonging the delivery of actives [5]. Vaginal ring (VR) delivery systems, however, eliminate all of the above listed disadvantages associated with vaginal delivery systems and consequently are attracting interest for the delivery of HIV microbicides and vaginally administered vaccines [6–10]. Conventional vaginal ring technologies, comprising hydrophobic elastomeric polymers such as silicone or ethylene–vinyl acetate copolymers (PEVA) (extensively reviewed elsewhere [11,12]), generally contain the active agent(s) homogeneously dispersed

throughout the polymeric carrier (matrix-type VR) or located in a drug-loaded central core overmoulded by rate-controlling metering sheath (reservoir-type VR). Matrix VRs provide higher but non-linear release rates, whereas lower but zero-order drug release can be achieved with reservoir systems [13,14].

Active agents are released from hydrophobic, non-biodegradable, elastomeric polymers via a permeation-controlled mechanism that depends upon the solubility and diffusivity of the active within the rate-controlling polymer [7,15–17]. Consequently, the format of current VRs devices are particularly well-suited to the sustained administration of therapeutically potent, small molecular weight and lipophilic actives, such as steroid molecules, which typically require release rates in the order of low micrograms per day [13,14]. Hydrophilic and/or high molecular weight actives are generally poorly released from conventional VR devices owing to poor permeation characteristics. Although the inclusion of hydrophilic excipients has been reported to enhance release of hydrophilic/macromolecular actives from hydrophobic polymers [18,19], this approach is not useful for the fabrication of VRs, since the excipients also cause swelling of the device through imbibing of the aqueous fluids, particularly at the high excipient loadings required to achieve effective release rates.

A large number of hydrophilic and/or macromolecular actives are currently being developed as potential vaginal HIV microbicide and vaccine candidates, including several monoclonal antibodies such as 2F5, b12, 2G12, and recombinant proteins gp160, gp140 and gp41 [21–26]. Naturally occurring antibodies are of particular interest as potential microbicides since there is less concern about

\* Corresponding author. School of Pharmacy, Medical Biology Centre, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK. Tel.: +44 (0)28 9097 2319; fax: +44 (0)28 9024 7794.

E-mail address: [k.malcolm@qub.ac.uk](mailto:k.malcolm@qub.ac.uk) (R.K. Malcolm).

acquiring resistance to HIV as with antiretroviral microbicide therapies [27,28]. In addition, VR technologies offer the potential to sustain the delivery of both microbicide and vaccine candidates to the vaginal mucosa. However, the potential of VR delivery of such actives will continue to be constrained by their poor permeability characteristics in conventional VR designs.

Here, we describe a new vaginal ring device, the 'insert vaginal ring' (InVR), comprising a ring body into which various drug-loaded inserts can be placed. The device is effective for the sustained release of proteins, as exemplified by the model protein BSA and the HIV monoclonal antibody 2F5 [25,26]. The ring device comprises one or more protein-loaded rods, fabricated from either modified silicone elastomer, a compressed solid tablet, or a lyophilised gel, that are inserted into cavities contained within a non-medicated silicone elastomer ring carrier.

## 2. Materials and methods

### 2.1. Materials

The monoclonal antibody 2F5 was supplied by Polymun Scientific (Vienna, Austria). Silicone elastomers MED-6382 and LSR9-9508-30, and tetrapropoxysilane were supplied by Nusil Technology LLC (Carpinteria, USA). BSA, hydroxypropylmethylcellulose (HPMC, 10 kDa, 86 kDa and 120 kDa), hydroxyethylcellulose (HEC, 250 K), glycine and sucrose were purchased from Sigma–Aldrich (Gillingham, UK). Ultra-pure water was obtained using an Elga Purelab Maxima system. Medical grade PVC tubing (3.00 mm *id*, 4.00 mm *od*) was purchased from BDH (Belfast, UK).

### 2.2. Preparation of BSA-loaded silicone rod insert formulations

Dispersed mixtures (2.0 g, Table 1) comprising Parts A and B of LSR9-9508-30 addition-cure silicone elastomer mix, BSA (2% w/w), and glycine, sucrose or HPMC (10, 30, 50% w/w) were prepared using a DAC 150 FVZ-K Speedmixer (30 s, 3000 rpm). The mixtures were injected into PVC tubing (100 mm length, 3.0 mm *id*) via disposable plastic syringe and allowed to cure 24 h at room temperature. The outer PVC tubing was removed and the elastomeric cord cut into 7.6-mm length sections (rod inserts).

### 2.3. Preparation of BSA-loaded directly compressed HPMC tablets

Directly compressed tablet formulations, comprising BSA (2% w/w) and HPMC (98% w/w, molecular weight grades 10 kDa, 86 kDa and 120 kDa) (Table 2), were prepared according to the following general method. BSA and HPMC were mixed in a DAC 150 FVZ-K Speedmixer (60 s, 3000 rpm) followed by direct compression of the powder mixture on a Riva MK II Minipress using a punch and die set specifically designed for production of tablets

suitable for ring insertion. The final tablet inserts had the following dimensions: 7.6 mm length, 3.0 mm width and 3.2 mm depth. The mean mass per tablet insert was 73.30, 70.71, 74.65 mg of BSA for 10 kDa, 86 kDa and 120 kDa HPMC, respectively.

### 2.4. Preparation of HPMC lyophilised gel insert formulations containing BSA or 2F5

Lyophilised inserts, comprising 10% w/w dry-weight BSA or 2F5, were prepared by lyophilisation of aqueous 9% w/w HPMC gels (various molecular weight grades of HPMC – 10 kDa, 86 kDa and 120 kDa) containing 1.0% w/w BSA or 2F5 (Table 2). Briefly, the required quantities of water, BSA powder or 2F5 solution (14.59 mg/mL), and HPMC were mixed in a DAC 150 FVZ-K Speedmixer (30 s, 3000 rpm) and allowed to hydrate overnight at 5 °C. The gels were mixed again before injection via syringe into PVC tubing (3.0 mm *id*). The tubing was cut into approximately 40 mm length sections which were subsequently lyophilised using a laboratory freeze-dryer (VirTis adVantage). Tube sections were placed inside the freeze-dryer at room temperature, the shelf temperature ramped to –60 °C and held for 2 h, followed by primary drying at –30 °C for 15 h. The shelf temperature was then increased to +20 °C over 60 min and held at this temperature for 10 h. Chamber pressure was maintained at 100 millitorr throughout the drying cycle. After lyophilisation, the silicone tubing was removed, the inner lyophilised cord cut into rods of 7.6 mm length, and the rods stored in a desiccator until further use.

### 2.5. Preparation of silicone elastomer vaginal ring devices

Silicone elastomer base MED-6382 and tetrapropoxysilane (ratio 25:1) were blended to produce the silicone elastomer mix. Silicone elastomer mix (30.0 g) was mixed with stannous octoate (0.5% w/w) for 30 s before injection into a laboratory-scale, electrically heated, reaction injection moulding machine fitted with specially designed stainless steel injection moulds for manufacture of vaginal rings containing cavities for placement of the drug-loaded inserts. The injection mixes were cured at 80 °C for 2 min producing VRs with dimensions 7.6 mm *csd*, 43.0 mm *id*, 58.0 mm *od* and comprising three equidistant holes (3.0 mm *id*) traversing through the body of the ring (Fig. 1).

### 2.6. In vitro release testing of BSA and 2F5 from Silicone rod InVRs

VR devices containing a single BSA-loaded silicone rod, a single BSA-loaded directly compressed tablet, or three BSA or 2F5-loaded lyophilised gel inserts were individually placed in 100-mL polypropylene jars with ammonium acetate buffer (30 mL, 0.1 M, pH 5.5) and stored in an orbital shaking incubator (37 °C, 60 rpm). VRs containing directly compressed tablets and lyophilised gel inserts

**Table 1**

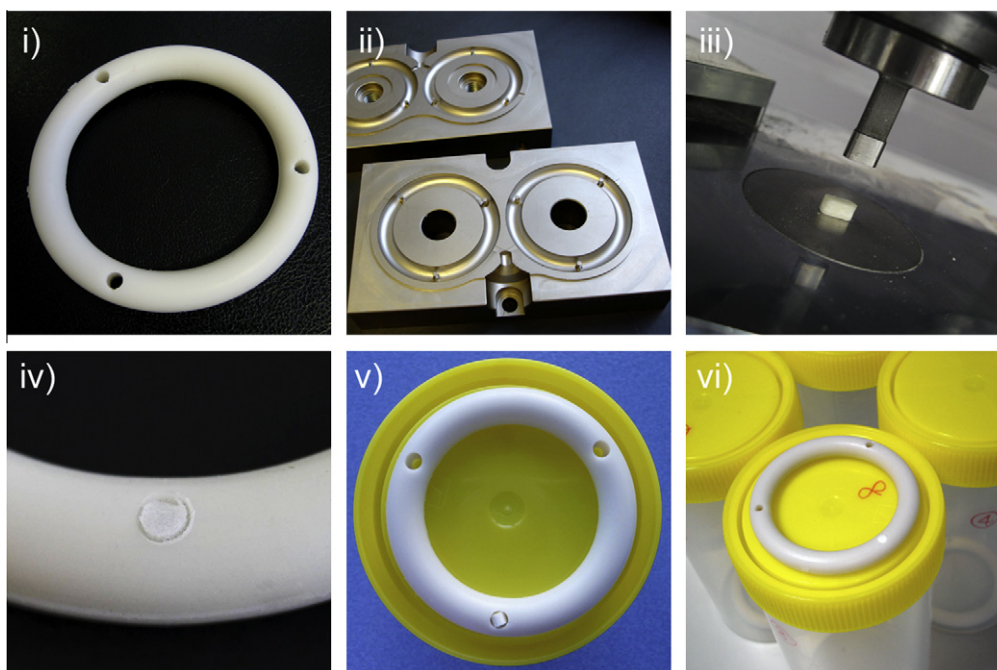
Composition and release characteristics of 2% BSA-loaded silicone rod insert formulations and their in vitro release characteristics.

Formulation	Excipient (% w/w)			Day 28 cumulative release amount (µg)/%	Release exponent	Mean BSA release rate over 28 days (µg day <sup>-0.5</sup> )
	Glycine	Sucrose	HPMC			
Si-0	–	–	–	124.8 ± 17.4/11.4 ± 1.6	0.44	21.0
Si-G10	10	–	–	295.1 ± 93.6/27.0 ± 8.6	0.46	51.3
Si-G30	30	–	–	516.1 ± 37.1/47.2 ± 3.4	0.56	101.6
Si-G50	50	–	–	712.5 ± 68.3/65.1 ± 6.2	0.54	130.2
Si-S10	–	10	–	144.6 ± 17.2/13.2 ± 1.6	0.35	19.6
Si-S30	–	30	–	265.3 ± 45.6/24.3 ± 4.2	0.56	51.3
Si-S50	–	50	–	840.8 ± 74.1/76.9 ± 6.8	0.62	162.7
Si-HP10	–	–	10	150.8 ± 26.2/13.8 ± 2.4	0.39	21.6
Si-HP30	–	–	30	198.9 ± 43.6/18.2 ± 4.0	0.38	28.9
Si-HP50	–	–	50	463.2 ± 54.5/42.3 ± 5.0	0.50	89.0

**Table 2**

Composition and release characteristics of directly compressed tablets and lyophilised BSA and 2F5 gels.

Formulation	Formulation components (%)			Cumulative release amount (mg)/%	Release exponent	Mean BSA release rate ( $\mu\text{g h}^{-0.5}$ )
	Protein	HPMC	Water			
Tab-BSA-10K	BSA/2%	98	–	$59.0 \pm 5.7/0.87 \pm 0.08$ @48 h	0.67	186.6
Tab-BSA-86K	BSA/2%	98	–	$50.2 \pm 2.9/0.71 \pm 0.05$ @168 h	0.65	75.8
Tab-BSA-120K	BSA/2%	98	–	$48.0 \pm 3.5/0.72 \pm 0.05$ @158 h	0.72	76.2
Lyo-BSA-10K	BSA/1%	9	90	$94.0 \pm 5.7/2.19 \pm 0.13$ @3 h	–	–
Lyo-BSA-86K	BSA/1%	9	90	$105.0 \pm 6.5/1.78 \pm 0.11$ @48 h	0.59	303.3
Lyo-BSA-120K	BSA/1%	9	90	$108.0 \pm 7.5/2.20 \pm 0.14$ @120 h	0.58	204.8
Lyo-2F5-10K	2F5/1%	9	90	$60.9 \pm 0.5/1.0 \pm 0.01$ @6 h	–	–
Lyo-2F5-86K	2F5/1%	9	90	$65.9 \pm 4.1/1.07 \pm 0.07$ @48 h	0.55	236.7
Lyo-2F5-120K	2F5/1%	9	90	$80.7 \pm 19.8/1.38 \pm 0.34$ @168 h	0.66	118.7



**Fig. 1.** (i) Silicone insert vaginal ring, (ii) Injection moulds for In VR manufacture, (iii) directly compressed insert manufacture, (iv) silicone insert, (v) directly compressed tablet insert, and (vi) lyophilised insert.

were sampled (2 mL) with media replacement at 1, 3, 6, 24, 48, 72, 96, 120, 144, 168 h. VRs containing excipient-modified silicone elastomer rod inserts were sampled with complete media replacement on days 1, 2, 3, 4, 7, 11, 14, 18, 21, 24 and 28. BSA was quantified by SEC-HPLC, and 2F5 by ELISA.

### 2.7. Quantification of BSA by high performance liquid chromatography

BSA was quantified using SEC-HPLC with ultraviolet detection (Waters Breeze HPLC system); Biosep SEC S3000 column (5  $\mu\text{m}$ , 25 cm  $\times$  4.6 mm *id*, Phenomenex, Ireland); mobile phase ammonium acetate (0.1 M, pH 5.5) flow rate 1.0 mL/min; detection wavelength 220 nm; injection volume 100  $\mu\text{L}$ ; BSA retention time 8 min). A linear calibration plot for BSA was obtained over the range 0.5–100  $\mu\text{g/mL}$  ( $R^2 = 0.99$ ;  $y = 2E-05x + 1.3971$ ).

### 2.8. Quantification of 2F5 by ELISA assay

Antibody levels of 2F5 were quantified by a double-sandwich ELISA using the 2F5 antibody and the peptide aa:GGGLELDKQWASL as the capture antigen. Bound 2F5 was detected with goat anti-human (GAH) IgG ( $\gamma$ -chain specific) antibody conjugated with

horseradish peroxidase. TMBE was added as substrate, and optical density values of the formed blue coloured product were obtained at 450 nm.

### 2.9. Determination of fluid uptake into silicone rod InVRs

In parallel with release studies, VRs containing silicone rod inserts were immersed in deionised water (30 mL). The rings were removed at the same sampling time points, blotted dry and the mass of each ring measured to quantify fluid uptake.

### 2.10. Qualitative assessment of water ingress into silicone rod, lyophilised rod and directly compressed tablet inserts

Sections of PVC tubing ( $n = 4$ , 3.0 mm *id* and 7.6 mm length; to mimic the cavities in the vaginal ring holder) containing the various solid dosage inserts (rods, tablets, lyophilised gels) were prepared and immersed in a methylene blue aqueous solution (20  $\mu\text{g/mL}$ ). The samples were removed after 1, 2, 4, 6, 24, 48 and 72 h, blotted dry and the ingress/uptake of dye assessed visually. The silicone elastomer rod samples were also assessed at extended timepoints (7, 12, 21 and 28 days).

### 3. Results

#### 3.1. In vitro BSA release from InVRs containing excipient-modified silicone elastomer inserts

BSA was released continuously over 28 days from InVR devices containing silicone elastomer rod inserts (Fig. 2). The rate of BSA release was observed to depend significantly upon both the type of excipient (sucrose > glycine > HPMC) and its initial loading (50% > 30% > 10% > 0%) in the rod insert. With no excipient included, only 11% BSA was released (and most within the first four days), compared to 76% (day 28) for the 50% sucrose insert. Summary release data are presented in Table 1 for each silicone rod insert VR formulation.

#### 3.2. Water uptake into InVRs loaded with excipient-modified silicone elastomer inserts

InVRs containing a single excipient-modified silicone elastomer BSA rod insert showed an increase in weight of between 2.0% and 3.5% (total ring weight) due to water uptake upon immersion (Fig. 3), compared to 1.5% for the control InVR containing a silicone elastomer BSA rod insert without excipient. Compared to sucrose and glycine, HPMC displayed the lowest percentage weight change over the 28-day dissolution. For glycine InVRs, the 10% loaded inserts displayed the highest percentage weight change, whereas with sucrose and HPMC InVRs with 50% loadings produced the highest increase in mass after 28 days. In general, the percentage

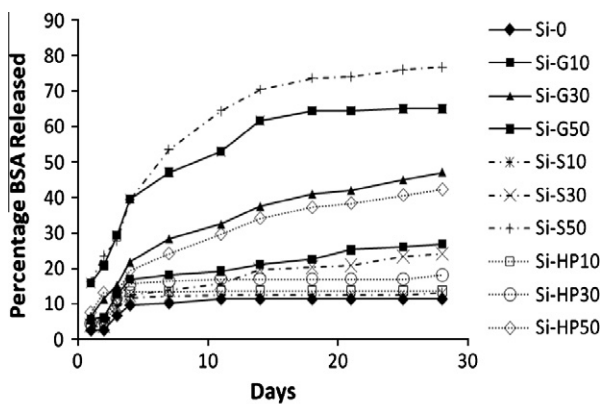


Fig. 2. In vitro percentage release profile for BSA from vaginal rings containing a single excipient-modified silicone elastomer rod insert (mean BSA loading per rod insert 1.09 mg).

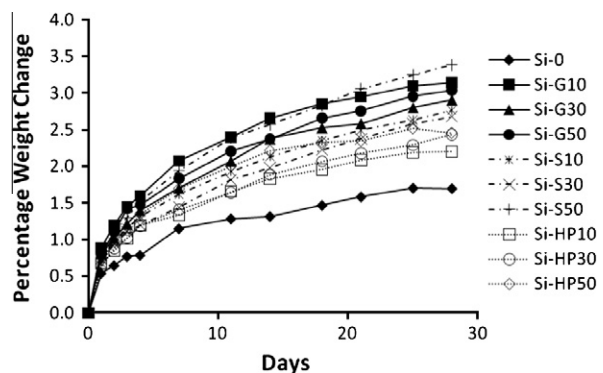


Fig. 3. Percentage weight change for vaginal rings containing a single excipient-modified silicone elastomer rod insert immersed in deionised water.

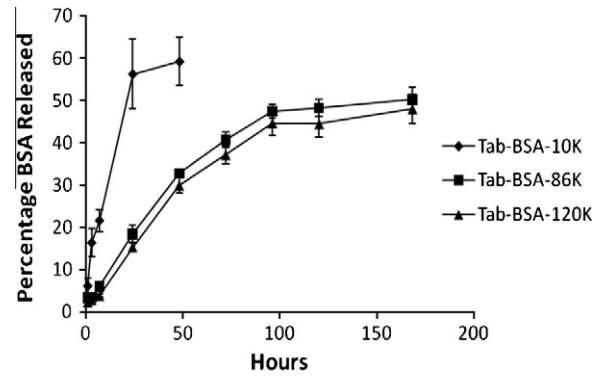


Fig. 4. In vitro percentage release profile for BSA from vaginal rings containing a single directly compressed tablet insert (mean BSA loading per tablet insert 1.47, 1.41, 1.49 mg of BSA for 10 kDa, 86 kDa and 120 kDa HPMC, respectively).

weight changes for the various rod insert formulations correlated in vitro release (Fig. 2).

#### 3.3. In vitro BSA release from InVRs loaded with directly compressed HPMC tablet inserts

Percentage BSA release versus time profiles for VRs containing HPMC tablet inserts are presented in Fig. 4 and the release data summarised in Table 2. It is evident as molecular weight of the HPMC tablet inserts increased so the rates of BSA release decreased. For the 10 kDa molecular weight HPMC insert, BSA is released over two days, compared with four days for the higher molecular weight grades of HPMC.

#### 3.4. In vitro BSA release from InVRs loaded with lyophilised HPMC gel rod inserts

Release of BSA from InVRs containing three lyophilised HPMC gel inserts showed a marked dependency on HPMC molecular weight (Fig. 5, Table 2), where an increase in molecular weight provides a reduction in the release rate. Release was sustained over approximately 3, 50, and 100 h for low (10 kDa), medium (86 kDa) and high (120 kDa) MW HPMC, respectively.

#### 3.5. Qualitative assessment of methylene blue dye uptake by the various ring insert formulations

Representative photographs indicating the extent of methylene blue ingress into the ring inserts via their exposed ends are

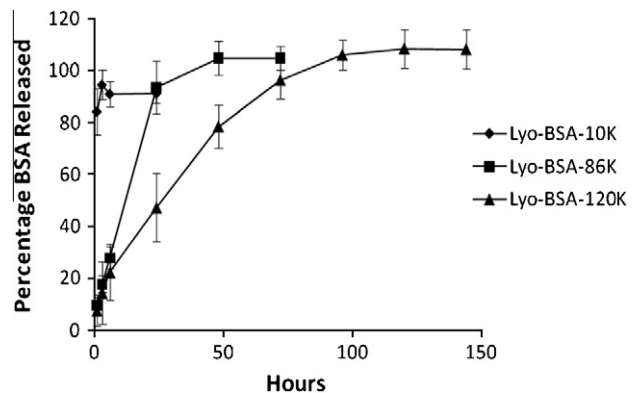


Fig. 5. In vitro percentage release profile for BSA from vaginal rings containing three lyophilised gel inserts (mean BSA loading per three inserts 2.31, 1.70, 1.86 mg of BSA for 10 kDa, 86 kDa and 120 kDa HPMC, respectively).

presented in Table 3. The low MW HPMC lyophilised gel insert allowed rapid ingress of dye within 1 h and complete dissolution of the dosage form within 2 h. The higher MW HPMC systems (86 kDa and 120 kDa) showed slower ingress of the dye solution and were still intact at 48 h; complete dissolution occurred within 72 h.

All of the directly compressed HPMC tablets showed dye uptake at their exposed ends after 1 h. After 24 h, dye had ingressed into all but a 2-mm section in the middle of the low MW HPMC tablet, and by 48 h dissolution was complete. Both medium and high MW directly compressed HPMC tablets showed considerable swelling at their exposed ends after 24 h. By 72 h (not shown), most of the medium MW HPMC tablet had dissolved while the high MW HPMC still had 2 mm of gel extending out each end of the tube. After 7 days, the medium MW HPMC tablet had completely dissolved and only a small portion remained for the high MW tablet.

For the silicone elastomer rod inserts, only the glycine-loaded formulations are shown; the other excipients showed similar characteristics. Silicone rods with no excipient displayed a very light blue colouration over the length of the rod by day 28, indicating limited ingress of the dye solution. The rate and extent of ingress

of dye solution increased with increasing glycine loading, and some swelling at the rod ends was observed with prolonged immersion.

### 3.6. Release of 2F5 from InVRs containing lyophilised HPMC gel inserts

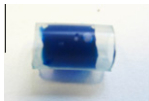
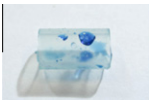
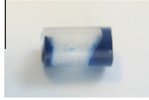






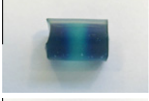




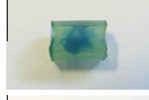









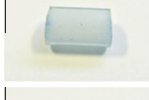






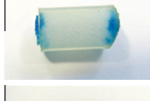
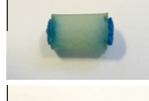


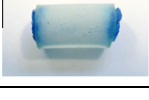

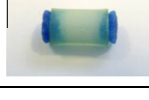

In vitro release of monoclonal antibody 2F5 from InVRs containing three lyophilised HPMC gel inserts depended significantly on the HPMC molecular weight (Fig. 6); time to complete dissolution was approximately 2, 24 and 100 h for the 10 kDa, 86 kDa and the 120 kDa MW inserts, respectively. With the current insert formulations, a InVR device containing three lyophilised HPMC inserts was capable of delivering over 1 mg 2F5 in total, representing approximately 70% of the total 2F5 loading.

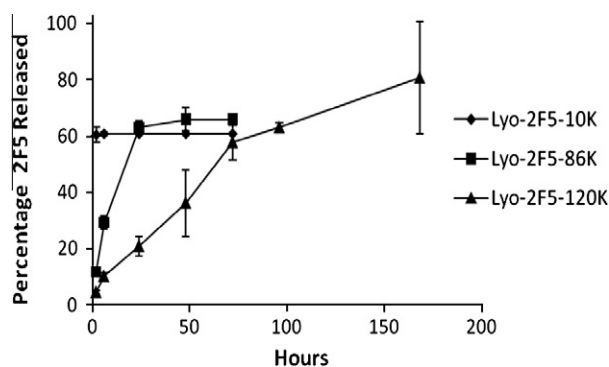
## 4. Discussion

The concept of an insert vaginal ring device (InVR), in which the active pharmaceutical agent is loaded into one or more discrete solid dosage units which are subsequently inserted into the ring body

**Table 3**

Photographic images showing the ingress of aqueous methylene blue dye into covered silicone rods, lyophilised gels and directly compressed tablets. (For interpretation to colours in this table, the reader is referred to the web version of this paper.)

Formulation	1 h	24 h	48 h	Day 7	Day 28
Lyo-BSA-10k					
Lyo-BSA-86k					
Lyo-BSA-120k					
Tab-BSA-10k					
Tab-BSA-86k					
Tab-BSA-120k					
Si-0					
Si-G10					
Si-G30					
Si-G50					



**Fig. 6.** In vitro percentage release profile for 2F5 from vaginal rings containing three lyophilised gel inserts (mean 2F5 loading per three inserts 1.64, 1.62, 1.70 mg for 10 kDa, 86 kDa and 120 kDa HPMC, respectively).

to aid its vaginal retention, is novel, and offers a number of distinct advantages over more traditional vaginal drug formulations. Conventional vaginal dosage forms, such as tablets, pessaries and semi-solid gels, generally display poor mucosal retention [3,4], require frequent dosing, and employ manufacturing methods that are generally not conducive to the stability of complex biological macromolecules, such as peptides, proteins and DNA [29–31]. By comparison, conventional vaginal ring designs can provide effective, controlled or sustained release of actives over many months, at least for those molecules that have the requisite physicochemical characteristics (relatively lipophilic and small molecular volume) to permit both significant permeation in the polymer materials used to fabricate ring devices and some degree of solubility in cervicovaginal fluid [7]. Release of hydrophilic and/or macromolecular actives from non-biodegradable elastomers is generally not feasible unless relatively high concentrations of hydrophilic excipients are incorporated [18,33]. When placed in an aqueous environment, the excipients absorb fluid to form a network of interconnected aqueous pores throughout the polymeric device that serve as conduits for subsequent drug permeation [18,19]. However, this approach has limited use in the fabrication of vaginal rings, since the absorption of aqueous fluid is accompanied by swelling of the polymeric device [20,34]. Also, commercial production of both thermoplastic and silicone elastomer vaginal rings requires high-temperature processing techniques (hot melt extrusion and reaction injection moulding, respectively) which are detrimental to protein structure. With the InVR design, the elastomeric ring body acts primarily as a holder for various solid dosage inserts, potentially providing increased vaginal retention and sustained release compared to the insert alone.

Three different types of ring inserts were selected for evaluation. The excipient-modified silicone elastomer rods were based on implantable matrix and covered-rod formulations described previously in the literature [34–38]. The directly compressed tablets represent the most common and inexpensive drug dosage form, with widespread use for oral administration and occasionally used vaginally. The lyophilised gel inserts, described recently as vaginal dosage forms for the HIV microbicide dapivirine [39], were selected as a means of administering an aqueous gel dose in a format likely to maintain protein stability. Previous studies involving the HIV envelope protein gp140 as a mucosal vaccine candidate formulated in an aqueous gel demonstrated poor protein stability [40].

The in vitro release data for the silicone elastomer rod insert rings (Fig. 2, Table 1) underlines the difficulties in achieving sustained delivery of proteins from silicone elastomers except with the incorporation of high concentrations of hydrophilic excipients. For all formulations, the release profiles display a biphasic pattern, i.e. rapid surface release from the exposed ends of the ring inserts

during the first four days followed by slower sustained release out to 28 days. The amount of BSA released during both phases was dependent on the type and loading of the excipient, with higher loadings of the glycine and sucrose showing the greatest release enhancement. The control silicone elastomer insert (no excipient) and those containing 10% excipient loadings generally showed no release after four days, illustrating the inability of these systems to form an interconnected pathway of aqueous channels into the bulk of the insert. The dye ingress studies (Table 3) and the weight change data (Fig. 3) with the same insert ring formulations confirm the mechanism by which protein is released from the rod inserts; slow and minimal penetration of the aqueous medium into the rod inserts is observed at lower excipient loadings, while the 30% and 50% loadings show very significant ingress of the aqueous dye solution. The increase in the weights of the rings, ranging from 1.5% to 3.5% of total ring weight, is a function of excipient type and loading attributed to diffusion of the aqueous medium into the excipient-modified rod insert.

For certain clinical applications (such as vaginal vaccination), it may be desirable to have protein release sustained over shorter time periods, as exemplified by BSA release from the directly compressed tablet insert rings (Fig. 4) and the lyophilised gel insert rings (Fig. 5). HPMC was selected as a hydrophilic mucoadhesive polymeric commonly used as an excipient in pharmaceutical gels and solid dosage forms. In these systems, release of BSA was sustained for up to 4 days depending on the molecular grade of the HPMC used to prepare the inserts. The 10K HPMC lyophilised inserts provided fastest release, a consequence of their highly porous structure permitting rapid fluid uptake and dissolution of the short polymer chains. Increasing the HPMC molecular weight in the lyophilised systems served to significantly prolong BSA release, consistent with increased polymer dissolution time. Unlike the silicone elastomer rod inserts, practically all the original BSA loading was quantifiably released from the lyophilised HPMC gel inserts, a pertinent issue when dealing with highly expensive recombinant protein actives. By comparison, total release of BSA from the directly compressed tablet inserts was less than 60%. It is postulated that the high compression and shear forces generated in direct compression tablet manufacture are responsible for protein degradation [29,30,32].

Vaginal administration of HIV monoclonal antibodies such as b12, 2F5 and 2G12 to inhibit intravenous, intravaginal or oral challenge of SHIV (a genetically engineered hybrid virus having an HIV envelope and a simian immunodeficiency virus core) has been established, and thus if present in the vaginal cavity in high concentrations could offer protection against HIV during intercourse [25,26]. Vaginal administration of a buffer solution containing the broadly neutralizing human monoclonal antibody b12 has been shown to protect macaques against infection following vaginal challenge with simian-human immunodeficiency virus (SHIV) [37]. Combinations of the monoclonal antibodies 4E10, 2F5 and 2G12, each having broad cross-clade neutralizing properties and relatively conserved binding epitopes, are also being investigated as vaginal HIV microbicides in gel formulations [26]. However, both solution and gel-based formulation strategies for HIV microbicides will require vaginal administration immediately before intercourse in order to be effective; both adherence and effectiveness might be increased if release can be sustained over multiple days from a ring device. In vitro 2F5 release from vaginal rings comprising three lyophilised gel inserts (Fig. 6) demonstrates that between 60% and 80% of the original 2F5 loading was effectively released from the inserts, with approximately 10–15% of 2F5 activity being lost during the lyophilisation stage as measured by ELISA. As with release of BSA from the directly compressed tablet and lyophilised gel insert rings, the HPMC molecular weight of the HPMC employed significantly influenced the 2F5 release rates. The 120 kDa HPMC lyophilised gel inserts sustained 2F5 release

over at least five days with daily release rates of between 250 and 350 µg/day for the first three days followed by approximately 100 µg/day for subsequent days (Fig. 6). Assuming upper limits for the volumes of cervicovaginal fluid (8 mL) and semen (8 mL), and assuming that in vivo release rate from the insert vaginal ring is similar to that observed in vitro, then vaginal concentrations in the combined fluids greater than 10 µg/mL might be established within hours of ring placement, similar to the in vitro IC<sub>50</sub> value for 2F5. Increased 2F5 release could be readily achieved by (i) increasing the rod diameter (up to 4 mm diameter inserts can be included in the current design), (ii) increasing the number of holes in the ring body to accommodate up to 12 inserts, and (iii) increasing the 2F5 loading in each insert.

## 5. Conclusion

The insert vaginal rings offer the potential to extend the remit of vaginal ring technology to actives that do not possess the permeation characteristics to achieve clinically effective release rates from conventional matrix and reservoir ring designs. The study has demonstrated that both the rate and duration of model protein release can be readily tailored through choice of insert type, number of inserts, and the molecular weight grade of the polymeric excipients. In particular, the lyophilised gel inserts offer mild manufacturing conditions conducive to protein stability. The multi-compartment nature of the insert vaginal ring may also ultimately prove useful in the administration of combination actives. For example, the ring body might be used to provide release of a small-molecule antiretroviral microbicide, such as dapivirine [7,8], while inserts within the device simultaneously release a protein or DNA-based HIV microbicide or antigen. From a mucosal vaccination perspective, it might also be possible to mimic traditional prime-boost schedules by developing separate inserts that provide immediate and delayed release behaviour.

## References

- [1] C.A. Varga, Sexual decision-making and negotiation in the midst of AIDS: Youth in KwaZulu/Natal, South Africa, *Health Trans. Rev.* 7 (3) (1997) 45–67.
- [2] T.T. Langen, Gender power imbalance on women's capacity to negotiate self-protection against HIV/AIDS in Botswana and South Africa, *Afr. Health Sci.* 5 (September) (2005) 188–197.
- [3] R.A. Ayton, G.M. Darling, A.L. Murkies, E.A. Farrell, E. Weisberg, I. Selinus, I.S. Fraser, A comparative study of safety and efficacy of continuous low dose estradiol released from a vaginal ring compared with conjugated equine oestrogen vaginal cream in the treatment of postmenopausal urogenital atrophy, *Br. J. Obstet. Gynecol.* 103 (1996) 351–358.
- [4] R. Barentsen, P. van de Weijer, J. Schram, Continuous low dose estradiol released from a vaginal ring versus estriol vaginal cream for urogenital atrophy, *Eur. J. Obstet. Gynecol. Reprod. Biol.* 71 (1997) 73–80.
- [5] R.M. Curran, L. Donnelly, R.J. Morrow, C. Fraser, G. Andrews, M. Cranage, R.K. Malcolm, R.J. Shattock, A.D. Woolfson, Vaginal delivery of the recombinant HIV-1 clade-C trimeric gp140 envelope protein CN54gp140 within novel rheologically structured vehicles elicits specific immune responses, *Vaccine* 27 (2009) 6791–6798.
- [6] K. Gupta, S. Pearce, A. Poursaid, H. Aliyar, P. Tresco, M. Mitchnick, P. Kiser, Polyurethane intravaginal ring for controlled delivery of dapivirine, a nonnucleoside reverse transcriptase inhibitor of HIV-1, *J. Pharm. Sci.* 97 (10) (2008) 4228–4239.
- [7] R.K. Malcolm, A.D. Woolfson, R.J. Morrow, C.F. Toner, S.D. McCullagh, Long-term, controlled release of the HIV microbicide TMC120 from silicone elastomer vaginal rings, *J. Antimicrob. Chemother.* 56 (5) (2005) 954–956.
- [8] A.D. Woolfson, R.K. Malcolm, R.J. Morrow, C.F. Toner, S.D. McCullagh, Intravaginal ring delivery of the reverse transcriptase inhibitor TMC 120 as an HIV microbicide, *Int. J. Pharm.* 325 (1–2) (2006) 82–89.
- [9] H. Shen, E. Goldberg, W. M. Saltzman, Gene expression and mucosal immune responses after vagina I DNA immunization in mice using a controlled delivery matrix, *J. Control. Release* 86 (2003) 339–348.
- [10] P.F. Zhang, F. Cham, M. Dong, A. Choudhary, P. Bouma, Z. Zhang, Y. Shao, Y. Feng, L. Wang, N. Mathy, G. Voss, C. C Broder, G. V Quinnan, Extensively cross-reactive anti-HIV-1 neutralizing antibodies induced by gp140 immunization, *PNAS* 104 (24) (2007) 10193–10198.
- [11] R.K. Malcolm, S. D McCullagh, R.J. Morrow, D. Woolfson, Vagina and uterus as drug-absorbing organs, in: E. Touitou, B. Barry (Eds.), *Enhancement in Drug Delivery*, CRC Press, Boca Raton, FL, 2007, pp. 395–440.
- [12] G. Dezarnaulds, I.S. Fraser, Vaginal ring delivery of hormone replacement therapy – a review, *Expert. Opin. Pharmacol.* 4 (2) (2003) 201–212.
- [13] A.D. Woolfson, G.R.E. Elliott, C.A. Gilligan, C.M. Passmore, Design of an intravaginal ring for the controlled delivery of 17β-estradiol as its 3-acetate ester, *J. Control. Release* 61 (3) (1999) 319–328.
- [14] R.K. Malcolm, The intravaginal ring, in: M.J. Rathbone, J. Hadgraft, M.S. Roberts (Eds.), *Modified-release Drug Delivery Technology*, Marcel Dekker Inc., New York, 2003, pp. 775–790.
- [15] D.R. Mishell, Vaginal contraceptive rings, *Ann. Med.* 25 (1993) 191–197.
- [16] Y.W. Chien, *Novel Drug Delivery Systems, Drugs and the Pharmaceutical Sciences*, second ed., vol. 50, Marcel Dekker Inc., New York, 1992, pp. 565–576.
- [17] A.D. Woolfson, R.K. Malcolm, R. Gallagher, Drug delivery by the intravaginal route, *Crit. Rev. Ther. Drug Carr. Syst.* 17 (2000) 509–555.
- [18] G. Di Colo, V. Careli, E. Nannipieri, M.F. Serafini, D. Vitale, F. Bottari, Effect of different water-soluble additives on the sustained release of sulphanylamide from silicone rubber matrices, *II Farmaco* 37 (1982) 377–389.
- [19] G. Di Colo, V. Campigli, V. Careli, E. Nannipieri, M.F. Serafini, D. Vitale, Release of osmotically active drugs from silicone rubbers, *II Farmaco* 37 (1984) 310–319.
- [20] D.S.T. Hsieh, Y.W. Chien, Enhanced release of drugs from silicone elastomers (II): induction of swelling and changes in microstructure, *Drug Dev. Ind. Pharm.* 11 (1982) 1411–1432.
- [21] J.R. Mascola, M.G. Lewis, G. Stiegler, H. Katinger, D. Harris, D. Hayes, M. Louder, T. Vancott, S. Frankel, C. Brown, C. Sapan, M. Robb, D. Birx, Protection of macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies, *J. Virol.* 73 (1999) 4009–4018.
- [22] J.R. Mascola, G. Stiegler, T.C. VanCott, H. Katinger, C. B Carpenter, C.E. Hanson, H. Beary, D. Hayes, S.S. Frankel, D. L. Birx, M.G. Lewis, Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies, *Nature Med.* 6 (2000) 207–210.
- [23] T.W. Baba, V. Liska, R. Hofmann-Lehmann, J. Vlasak, W. Xu, S. Ayehunie, L.A. Cavacini, M.R. Posner, H. Katinger, G. Stiegler, B.J. Bernacki, T.A. Rizvi, R. Schmidt, L.R. Hill, M.E. Keeling, Y. Lu, J.E. Wright, T.C. Chou, R.M. Ruprecht, Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection, *Nature Med.* 6 (2000) 200–206.
- [24] C. Armbruster, G.M. Stiegler, B.A. Vcelarb, W.J. Ègerc, N.L. Michael, N. Vetter, H.W.D. Katinger, A phase I trial with two human monoclonal antibodies (hMAb 2F5, 2G12) against HIV-1, *AIDS* 16 (2002) 227–233.
- [25] J.M. Binley, T. Wrin, B. Korber, M.B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, C.J. Petropoulos, D.R. Burton, Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies, *J. Virol.* (2004) 13232–13252.
- [26] S. Mehandru, T. Wrin, J. Galovich, G. Stiegler, B. Vcelar, A. Hurlley, C. Hogan, S. Vasan, H. Katinger, C. Petropoulos, M. Markowitz, Neutralization profiles of newly transmitted human immunodeficiency virus type 1 by monoclonal antibodies 2G12, 2F5, and 4E10, *J. Virol.* (2004) 14039–14042.
- [27] V. Tozzi, M. Zarcarelli, S. Bonfigli, P. Lorenzini, G. Liuzzi, M.P. Trotta, F. Forbici, C. Gori, A. Bertoli, R. Bellagamba, P. Narciso, C.F. Perno, A. Antinorini, Drug-class-wide resistance to antiretrovirals in HIV-infected patients failing therapy: prevalence, risk factors and virological outcome, *Antivir. Ther.* 11 (2006) 553–560.
- [28] P.J. Weidle, Kityo, C.M. Cissy, P. Mugenyi, R. Downing, A. Kebba, D. Pieniazek, R. Respass, K. Hertogs, V. De Vroey, P. Dehertogh, S. Bloor, B. Larder, E. Lackritz, Resistance to antiretroviral therapy among patients in Uganda, *J. Acq. Immun. Def. Synd.* 26 (5) (2001) 495–500.
- [29] S. Jacob, A.A. Shirwaikar, K.K. Srinivasan, J. Alex, S.L. Prabu, R. Mahalaxmi, R. Kumar, Stability of proteins in aqueous solution and solid state, *Indian J. Pharm. Sci.* 68 (2) (2006) 154–163.
- [30] J. Japse, S.J. Hagen, Do protein molecules unfold in a simple shear flow?, *Biophys J.* 91 (2006) 3415–3424.
- [31] S.T. Zannis, W.J.M. Hrushesky, P.A. Wood, T.M. Przybycien, Adsorption of a formulated protein on a drug delivery device surface, *J. Colloid Interface Sci.* 189 (1997) 216–228.
- [32] P.M. Bummer, S. Koppenol, Chemical and physical considerations in protein and peptide stability, in: Eugene McNally (Ed.), *Protein Formulation and Delivery*, Marcel Dekker, New York, 2000.
- [33] R. Siegel, R. Langer, Controlled release of polypeptides and other macromolecules, *Pharm. Res.* 1 (1984) 2–10.
- [34] T.J. Johnson, K.M. Gupta, J. Fabian, T.H. Albright, P.F. Kiser, Segmented polyurethane intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine and tenofovir, *Eur. J. Pharm. Sci.* 39 (2010) 203–212.
- [35] M. Kajihara, T. Sugie, M. Mizuno, N. Tamura, A. Sano, K. Fujioka, Y. Kashiwazaki, T. Yamaoka, S. Sugawara, Y.E. Otto, Z. Long, Y. Chiang, G.J. McGarrity, L.M. Muul, Development of new drug delivery system for protein drugs using silicone (I), *J. Control. Release* 66 (2000) 49–61.
- [36] M. Kajihara, T. Sugie, T. Hojo, H. Maeda, A. Sano, K. Fujioka, Y.S. Sugawara, Y. Urabe, Development of a new drug delivery system for protein drugs using silicone (II), *J. Control. Release* 73 (2001) 279–291.

- [37] H. Maeda, E. Ohashi, A. Sano, H. Kawasakib, Y. Kurosaki, Investigation of the release behaviour of a covered-rod-type formulation using silicone, *J. Control. Release* 90 (2003) 59–70.
- [38] H. Maeda, M. Brandon, A. Sano, Design of controlled-release formulation for ivermectin using silicone, *Int. J. Pharm.* 261 (2003) 9–19.
- [39] A.D. Woolfson, M.L. Umrethia, V.L. Kett, R.K. Malcolm, Freeze-dried, mucoadhesive system for vaginal delivery of the HIV microbicide, dapivirine: optimisation by an artificial neural network, *Int. J. Pharm.* 30 (388:1-2) (2010) 136–143.
- [40] R.S. Veazey, R.J. Shattock, M. Pope, J.C. Kirijan, J. Jones, Q.X. Hu, T. Ketas, P.A. Marx, P.J. Klasse, D.R. Burton, J.P. Moore, Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120, *Nat. Med.* 9 (3) (2003) 343–346.