European Journal of Pharmaceutics and Biopharmaceutics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Development of disulfiram-loaded vaginal rings for the localised treatment of cervical cancer

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ARTICLE INFO

Article history:
Received 28 April 2014
Accepted in revised form 6 August 2014
Available online xxxx

Keywords:
Disulfiram
Cervical cancer
Vaginal ring
Localised delivery
Controlled release
Sustained release

ABSTRACT

Cervical cancer is the third most prevalent cancer in women and disproportionately affects those in low resource settings due to limited programs for screening and prevention. In the developed world treatment for the disease in the non-metastasised state usually takes the form of surgical intervention and/or radiotherapy. In the developing world such techniques are less widely available. This paper describes the development of an intravaginal ring for the localised delivery of a chemotherapeutic drug to the cervix that has the potential to reduce the need for surgical intervention and will also provide a novel anti-cancer therapy for women in low resource settings. Disulfiram has demonstrated antineoplastic action against prostate, breast and lung cancer. Both PEVA and silicone elastomer were investigated for suitability as materials in the manufacture of DSF eluting intravaginal rings. DSF inhibited the curing process of the silicone elastomer, therefore PEVA was chosen as the material to manufacture the DSF-loaded vaginal rings. The vaginal rings had an excellent content uniformity while the DSF remained stable throughout the manufacturing process. Furthermore, the rings provided diffusion controlled release of DSF at levels well in excess of the IC50 value for the HeLa cervical cancer cell line.

1. Introduction

Cervical cancer is the third most prevalent cancer in women, with 529,000 new cases diagnosed each year of which 275,000 result in death. 85% of new cases occur in developing countries due to a lack of cervical cancer prevention and screening programs. Where as in developed countries, where women have access to resources capable of detecting and treating precancerous lesions, the number of cases is reduced by approximately 80% [1]. Sexual transmission of the human papillomavirus (HPV) is the main cause of cervical cancer, with 15 types of HPV being classed as carcinogenic or high risk. Types 16 and 18 are the most carcinogenic and are the main contributors to cervical cancer [2] and persistent HPV infections have the potential of causing the development of precancerous lesions and cervical intraepithelial neoplasia (CIN) that may lead to cervical cancer [3].

How cervical cancer is treated will depend on the women's general health as well as the type, stage and grade of the cancer and usually involves a combination of surgery, chemotherapy and/or radiotherapy [4,5]. All of these treatment options are either very invasive and involve extended stays in or repeated visits to the hospital and in the case of chemo and radiotherapy can result in significant side-effects, reducing the patients overall quality of life during the treatment. The location of the cervix makes it easily accessible through the vagina and allows for non-invasive localised delivery of chemotherapeutic drugs offering a number of advantages over systemic administration such as direct delivery to the site of action resulting in a lower dose being required as well as a reduction in systemic side effects and increased drug stability as it remains in the delivery device until released [6].

The vagina has been used to deliver drugs for a range of clinical and research applications, including contraception, vaginal infections and HIV prevention, with many different vaginal formulations such as gels, creams, pessaries, suppositories rings, films

Abbreviations: CIN, cervical intraepithelial neoplasia; DDC, diethyldithiocarbamate; DSC, differential scanning calorimeter; DSF, disulfiram; HPV, human papillomavirus; IVRs, intravaginal rings; PEVA, poly ethylene vinyl acetate; ROS, reactive oxygen species; SDS, sodium dodecyl sulphate.

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http://dx.doi.org/10.1016/j.ejpb.2014.08.002
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and tablets available [7–15]. However, only a small number of these delivery systems have been investigated for the localised delivery of chemotherapeutic drugs to the cervix [16–20].

Intravaginal rings (IVRs) are torus-shaped drug delivery devices that are capable of providing controlled delivery of substances to the vagina for up to a period of 1–12 months where it slowly releases one or more drugs to provide either a local or systemic effect [21–24]. IVRs have already seen clinical and commercial success in contraception (Nuvaring®) [23,25,26] and oestrogen replacement therapy (Estring® and Femring®) [21,27]. Femring® and Estring® are both manufactured from silicone elastomer, whereas Nuvaring® is manufactured from ethylene–vinyl-acetate copolymer (EVA). The clinical and commercial success of these rings makes them an ideal candidate for the delivery of chemotherapeutic drugs to the cervix. The IVR overcomes many of the disadvantages associated with more traditional vaginal drug dosage forms, such as gels, tablets and pessaries, which are often messy, interfere with intercourse and are poorly retained within the vagina. However, the major advantage of the IVR is its ability and versatility in providing long-term, continuous release of drug(s) at constant pre-determined rates, thereby increasing cost-effectiveness, patient compliance and therapeutic efficacy. Furthermore the vaginal ring is user controlled and thus does not require minor surgery or a physician for it to be placed in the vagina.

Disulfiram (DSF), which is currently used for treating alcohol abuse, has shown potential anti-tumour activity by inducing apoptosis in some cell lines and reducing cell growth in certain tumours [28]. This anticancer effect has been demonstrated in prostate cancer, breast cancer, lung cancer, leukaemia and cervical adenocarcinoma [29–37] and has been shown to be copper (Cu) dependent [21,38,39] as Cu plays a crucial role in redox reactions and triggers the generation of reactive oxygen species (ROS) which induce apoptosis in human cells [40]. The transport of Cu into the cell is strictly mediated by the trans-membrane Cu transporter (Ctr115) and due to its strong divalent metal ion chelating properties, DSF can chelate Cu(II) forming a DSF/Cu complex which improves the transport of Cu into cancer cells. Furthermore, the DSF/Cu complex is a much stronger ROS inducer than Cu alone [41,42] and is also an inhibitor of ALDH [29], which is involved in detoxifying a wide range of aldehydes. Aldehyde accumulation induces lipid peroxidation and generation of highly reactive free radicals leading to protein and DNA cross-linking and cell death. ALDH also plays a critical role in scavenging ROS and reducing UV-induced oxidative stress [43]. Therefore, inhibition of ALDH by the DSF/Cu complex will result in ROS accumulation leading to apoptosis. Drug induced ROS accumulation is usually counterbalanced by the activation of NFκB, an anti-apoptotic factor inhibiting ROS and ROS-induced cytotoxicity [44]. However, DSF is also capable of inhibiting activity of NFκB [29]. The DSF/Cu complex has been shown to induce ROS and inhibit NFκB activity in brain, colon and breast cancer cell lines [29,45,46]. Furthermore, it has been demonstrated that DSF can potentiate the cytotoxic effect of other anticancer drugs and ionising radiation *in vitro* while protecting normal cells in the kidneys, gut and bone marrow *in vivo* thus having the potential to increase the therapeutic index of other anticancer treatments.

In this study we describe, for the first time, the development and characterisation of a DSF-loaded vaginal ring that has the potential to be used for the localised treatment of cervical cancer.

2. Materials and methods

2.1. Materials

The poly ethylene vinyl acetate (PEVA) copolymers Elvax 40 (40% vinyl acetate content) and Elvax 150 (32% vinyl acetate content) were purchased from Dupont (Delaware, USA). The tin catalysed silicone MED8-6382 was purchased from NuSil Technology (Carpinteria, CA). Stannous octoate, sodium dodecyl sulphate (SDS) and DSF were purchased from Sigma–Aldrich, (Dorset, UK). All were used as supplied.

2.2. Determination of the thermal stability of DSF

50 mg samples ($n = 4$) of DSF was weighed into separate vials. The vials were placed into an oven at various temperatures (60, 70, 80, 100, 120, 140 and 160 °C) where they remained for 5, 10, 15, 30, 45 or 60 min. After removal from the oven the contents of each vial was dissolved in 10 mL of ethanol and analysed using a DSF stability indicating HPLC method.

2.3. Shore-A hardness determination of post-cured DSF-loaded MED8-6382 silicone elastomers

10 g of MED8-6382 silicone elastomer containing various loadings (0, 1, 2, 3, 5 and 10% w/w) of DSF were manufactured by weighting the appropriate amounts of silicone and DSF into a sealed plastic container and speed mixing them for 30 s at 3500 rpm (SpeedMixer™ DAC 15FVZ-K, Synergy Devices). Varying amounts (0.5%, 1.0%, 1.5%, 2.5%, 5% and 10%) of stannous octoate catalyst was then added to the silicone elastomers, which were subsequently poured into 81 × 81 × 18 mm square moulds before being placed in an oven at 60 °C for 1 h. Upon removal from the oven the silicone elastomers were allowed to cool overnight when their shore-A hardness was measured at five different places along the surface using a HBA 100-0 Shore-A durometer hardness tester (SAUTER, Balingen, Germany).

2.4. Rheological evaluation of the curing rate of DSF-loaded MED8-6382 silicone elastomers

3 g of MED8-6382 silicone elastomer containing various loadings (1, 2 and 5% w/w) of DSF were manufactured by weighting the appropriate amounts of silicone and DSF into a sealed plastic container and speed mixing them for 30 s at 3500 rpm (SpeedMixerTM DAC 15FVZ-K, Synergy Devices). 2.5% w/w of stannous octoate catalyst was added to the silicone mix which was immediately placed onto the lower plate of a TA Instruments AR2000 Rheometer using a disposable plastic syringe. The upper 40 mm diameter crosshatch parallel plate was lowered to produce a gap between the plates of 1000 lm and the excess silicone mix removed before the oscillation experiment was begun. A stress of 15 Pa and a frequency of 1 Hz were selected based on previously published work [47] and used for the subsequent cure analysis. Samples were analysed at both 60 and 80 °C.

2.5. Manufacture of 5% w/w DSF-loaded PEVA vaginal rings

47.5 g of PEVA (either Elvax 40 or Elvax 150) was weighed into a sealed plastic container along with 2.5 g of DSF. The container was manually shaken for approximately 10 min to mix the PEVA and DSF. The Elvax 150/DSF active mix was compounded at 80 °C and 50 rpm screw speed using a Thermo Electron HAAKE minilab extruder, while the Elvax 40/DSF active mix was compounded at both 65 and 80 °C. The compounded PEVA/DSF active mixes were pelletised using a Thermo Scientific VARICUT pelletiser and compounded a second time. 5% w/w DSF-loaded PEVA vaginal rings were produced by feeding the pellets into a Babyplast 6/10P micro-moulding machine with a barrel temperature of either 65 or 80 °C (depending on the active mix) and a mould temperature of 40 °C. The mould temperature was used as a processing aid to ensure the polymer melt filled the mould cavity before freezing off.

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2.6. Content uniformity and drug stability of 5% w/w DSF-loaded PEVA vaginal rings

Each 5% w/w DSF-loaded PEVA vaginal ring ($n = 4$) was cut into smaller segments and placed into a round bottom flask containing 100 mL dichloromethane. The dichloromethane containing the ring segments was refluxed at 40 °C for 2 h to extract the DSF before allowing the flask to cool for a further 1 h. Ten millilitres of the dichloromethane solution was removed to a sample vial and the dichloromethane evaporated to leave the residual drug, which was subsequently reconstituted in 10 mL of ethanol and analysed using a DSF stability indicating HPLC method.

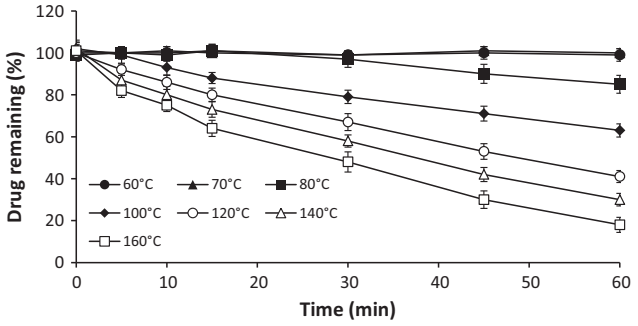

Fig. 1. The thermal stability of DSF at varying temperatures and exposure times.

2.7. Determination of the physical state of DSF in 5% w/w DSF-loaded PEVA vaginal rings

Thermal analysis of DSF and DSF-loaded ring segments was conducted using a Q200 TA Instruments differential scanning calorimeter (DSC). Approximately 10 mg of each sample was added to a DSC pan and placed in the thermal chamber of the DSC. The DSC analysis was performed between 30 and 90 °C at a heating rate of 10 °C/min. The control sample consisted of 9.5 mg of polymer and 0.5 mg of DSF, weighed into a DSC pan separately, which is the equivalent to a 5% w/w loading of DSF. The analysis was performed immediately after manufacture (time 0), as well as 1 month and 3 months after manufacture to determine if the drug crystallises out over time.

2.8. In vitro release of 5% w/w DSF-loaded PEVA vaginal rings

Each DSF-loaded PEVA vaginal ring ($n = 4$) was placed into a sealed flask containing either 100 mL of 2% SDS solution or 20 mL of water, and the flasks were placed into an orbital shaking incubator (Unitron HT infors) at 37 °C and 60 rpm. The release medium was sampled daily for 14 days, with complete replacement of

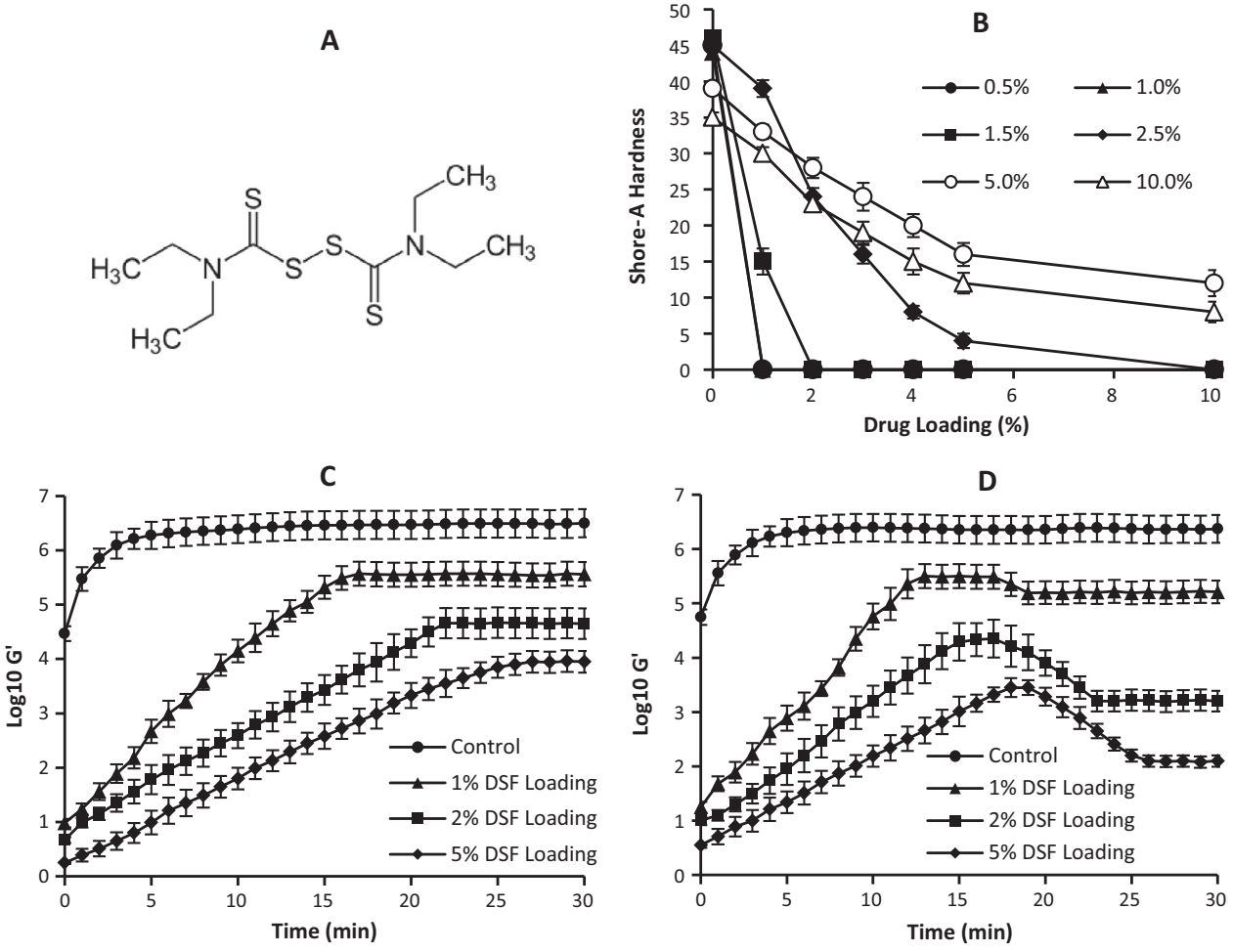

Fig. 2. The structure of DSF (A). The influence of drug and stannous octoate catalyst loading on the shore-A hardness of the MED8-6382 silicone elastomer cured at 60 °C (B). The effect of DSF loading on the cure rate and final storage modulus of the MED8-6382 silicone elastomer, containing 2.5% stannous octoate catalyst, cure at 60 (C) and 80 °C (D).

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release medium (except weekends), and the samples were analysed using the DSF stability indicating HPLC method.

2.9. In vitro release of DSF-loaded vaginal rings using a dual chambered release method

To mimic the dynamic nature of the vagina, where you have an aqueous environment (vaginal vault) surrounded by hydrophobic tissue, each DSF-loaded vaginal ring ($n = 4$) was placed into a sealed latex balloon, with a 5 mm thick wall (to simulate vaginal tissue), containing 20 mL of water to simulate vaginal fluid. The balloons were then submerged in 100 mL of 2% SDS solution (to simulate tissue) and placed into an orbital shaking incubator (Innova 43) at 37 °C and 60 rpm. 2 mL of sample was taken from the water and 5 mL from the 2% SDS solution each day for 14 days and replaced with fresh media. The samples were analysed using the DSF stability indicating HPLC method.

2.10. DSF stability indicating HPLC methodology

HPLC analysis was performed on an Agilent 1200 series HPLC with a Phenomenex Luna C18 4.6 × 150 mm column with a 5 μM particle size. The mobile phase was comprised of 80% HPLC grade methanol and 20% HPLC grade water. The flow rate was 1.00 mL/min, while UV detection was performed at a wavelength of 275 nm with an injection volume of 10 μL.

2.11. Statistical analysis

Statistical analysis was performed using a one way analysis of variance (ANOVA) (GraphPad Prism version 5.02 for Windows, GraphPad Software, San Diego, CA). Post-hoc comparisons of the means were performed using Tukey's Honestly Significance Difference test. A significance level of $p < 0.05$ was accepted to denote significance in all cases.

3. Results and discussion

3.1. Thermal stability of DSF

Fig. 1 demonstrates that at 60 and 70 °C DSF is completely stable for up to 1 h while at 80 °C it degrades by 3% after 30 min, 10% after 45 min and 15% after 1 h. At 100 °C it remains stable for 5 min, losing 7% after 10 min and by 1 h it had degraded by 37%. For temperatures of between 120 °C and 160 °C the DSF started to degrade with in the first 5 min and by 1 h had degraded between 59% and 82% ($p < 0.05$). This data tells us that in order to manufacture a stable DSF-loaded vaginal ring we need to manufacture the rings at temperatures below 80 °C and keep the residence time in both the extruder at injection moulder as short as possible. Therefore, we selected the silicone elastomer MED8-6382 to manufacture silicone vaginal rings as it cures within minutes at 80 °C. The thermoplastic rings will be manufactured from Elvax 40 and

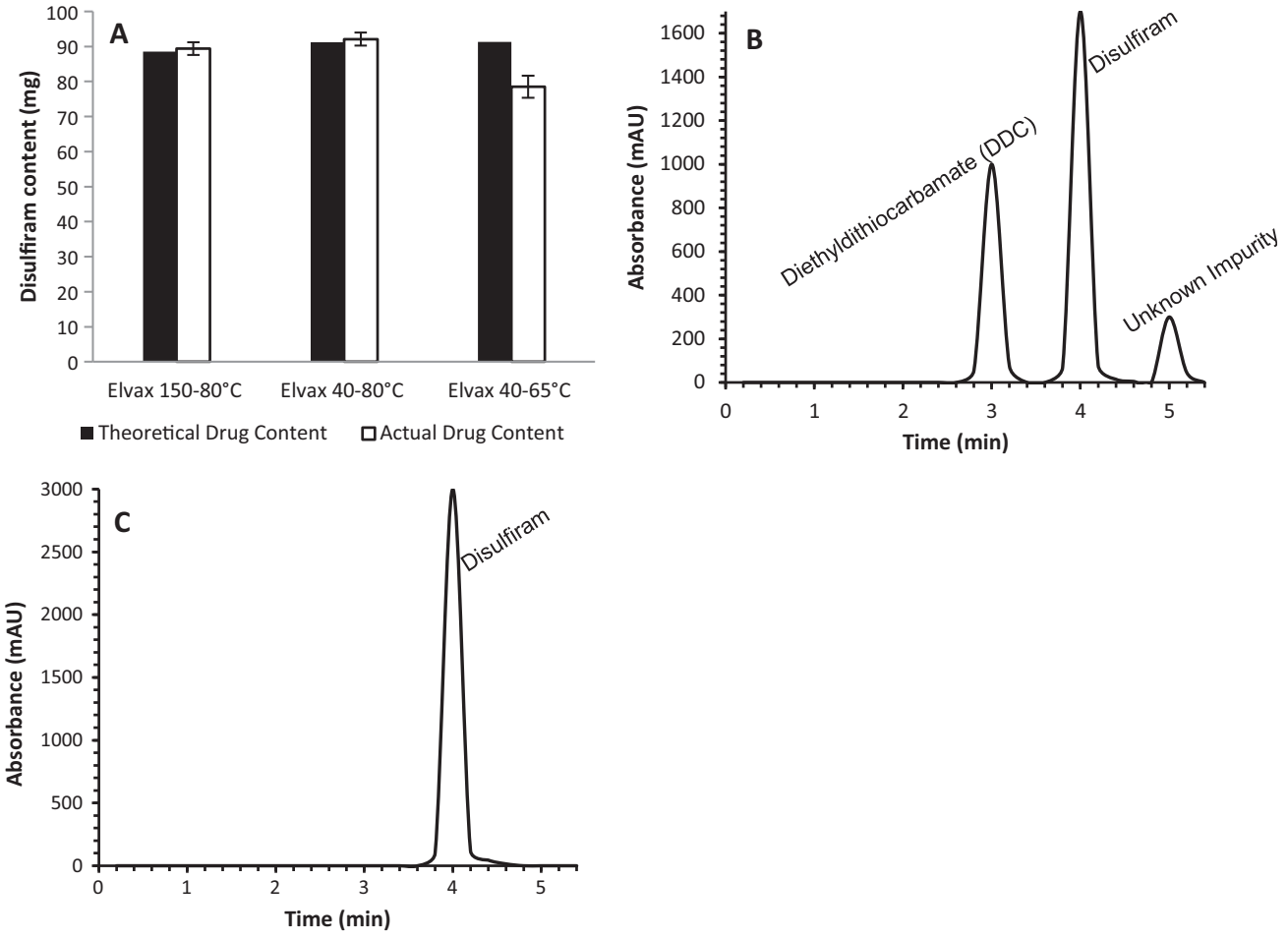

Fig. 3. DSF content of 5% w/w DSF-loaded thermoplastic vaginal rings (A). Representative HPLC chromatograms for heat degraded DSF (B) and DSF extracted from the thermoplastic vaginal rings (C).

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Elvax 150, which have melting temperatures of 45 and 50 °C respectively.

3.2. Determination of the shore-A hardness of post cured DSF-loaded MED8-6382 silicone elastomer

The curing process of tin catalysed silicone elastomers is known to be inhibited by sulphur. DSF contains four sulphur atoms (Fig. 2A), two of which are thio-ketones and have been shown to be reactive with silica [48]. Therefore, it was decided to perform a cure test to determine if it was possible to manufacture DSF-loaded silicone elastomer vaginal rings at 65 °C (to ensure drug stability). Fig. 2B demonstrates that the control sample (which contained no DSF) had a shore-A hardness of approximately 45 with differing amounts of catalyst. However, upon the addition of DSF the shore-A hardness of the cured silicone elastomer was lowered significantly ($p < 0.05$) with the decrease dependant on both the DSF and catalyst loading (Fig. 2B). With a catalyst loading of 0.5% and 1.0% the shore hardness was reduced to 0 across all DSF loadings, which suggests that the system did not cure. However, by adding 2.5% catalyst the shore-A hardness was reduced initially to 39 with a 1% DSF loading, but continued to decrease with an increase in DSF loading. The addition of 5.0% and 10.0% catalyst resulted in a shore-A hardness of 33 and 30 with a 1% DSF loading respectively and as with the 2.5% catalyst the shore-A hardness continued to decrease with an increase in the DSF loading. Fig. 2B demonstrates that once the catalyst loading went above 2.5% the shore-A hardness across all drug loadings was significantly reduced ($p < 0.05$). The reason for this is that the catalyst is a liquid and at 5.0% and 10.0% loadings the overall liquid content of the silicone elastomer is high, thus reducing the final shore-A hardness. This is demonstrated by a decrease in the shore-A hardness of the control at 5.0% and 10.0% catalyst (Fig. 2B).

3.3. Rheological evaluation of the curing rate of DSF-loaded MED8-6382 silicone elastomers

Rheological evaluation of the DSF-loaded silicone elastomers was used to determine their curing rate. Based on the shore-A hardness data a 2.5% loading of catalyst was chosen along with 1%, 2% and 5% DSF loadings. Furthermore, to determine if increasing the temperature would improve the curing process, the DSF-loaded silicone elastomers were evaluated at 65 and 80 °C. Fig. 2C and D demonstrates that the addition of DSF significantly ($p < 0.05$) reduces the rate of cure and the final log10 G' of the silicone elastomer. Furthermore, at 80 °C there is a further reduction in the final log10 G' at around 18–20 min. The reason for this is that the melt temperature of DSF is approximately 70 °C (Fig. 4). Therefore after 18 min at 80 °C the DSF begins to melt, thus reducing the final log10 G' of the silicone, which is proportionate to the DSF loading in the silicone (Fig. 2D). Based on the shore-A hardness data and the rheological evaluation it was decided to no longer consider the use of the silicone elastomer MED8-6382 for the manufacture of DSF-loaded vaginal rings.

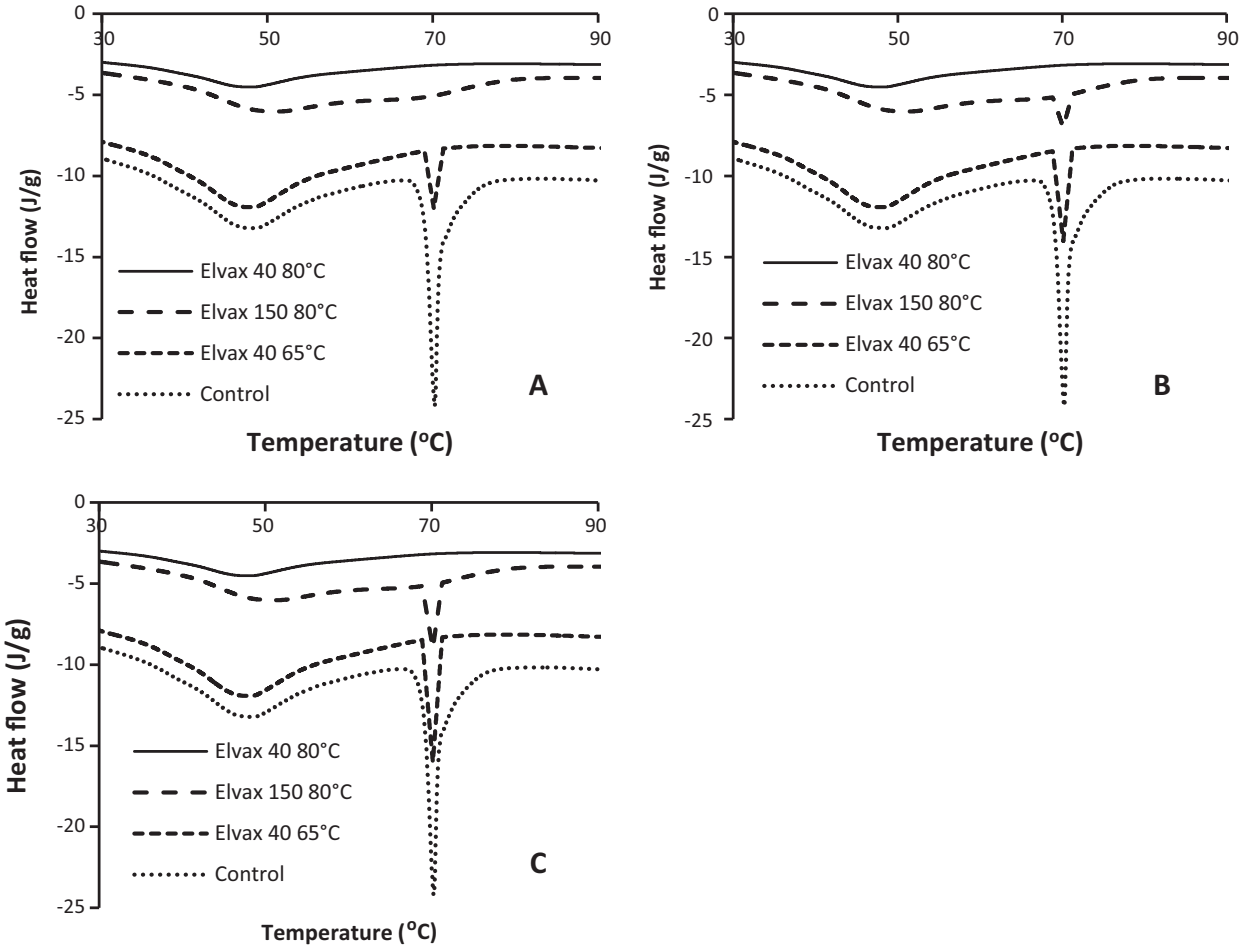

Fig. 4. DSC traces for the 5% w/w DSF-loaded thermoplastic vaginal rings immediately after manufacture (A), 1 month after manufacture (B) and 3 months after manufacture (C).

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3.4. Content uniformity and drug stability of 5% w/w DSF-loaded PEVA vaginal rings

Fig. 3A demonstrates that the Elvax 150 and Elvax 40 vaginal rings, which were manufactured at 80 °C had a DSF content statistically similar ($p \geqslant 0.05$) to their theoretical content, while the Elvax 40 rings manufactured at 65 °C had a DSF content significantly ($p < 0.05$) lower than their theoretical content. The reason for this is that at 80 °C the DSF has melted and is easier to compound into the PEVA during the extrusion process, while at 65 °C the DSF is in its solid form, which makes compounding it into the PEVA much more difficult.

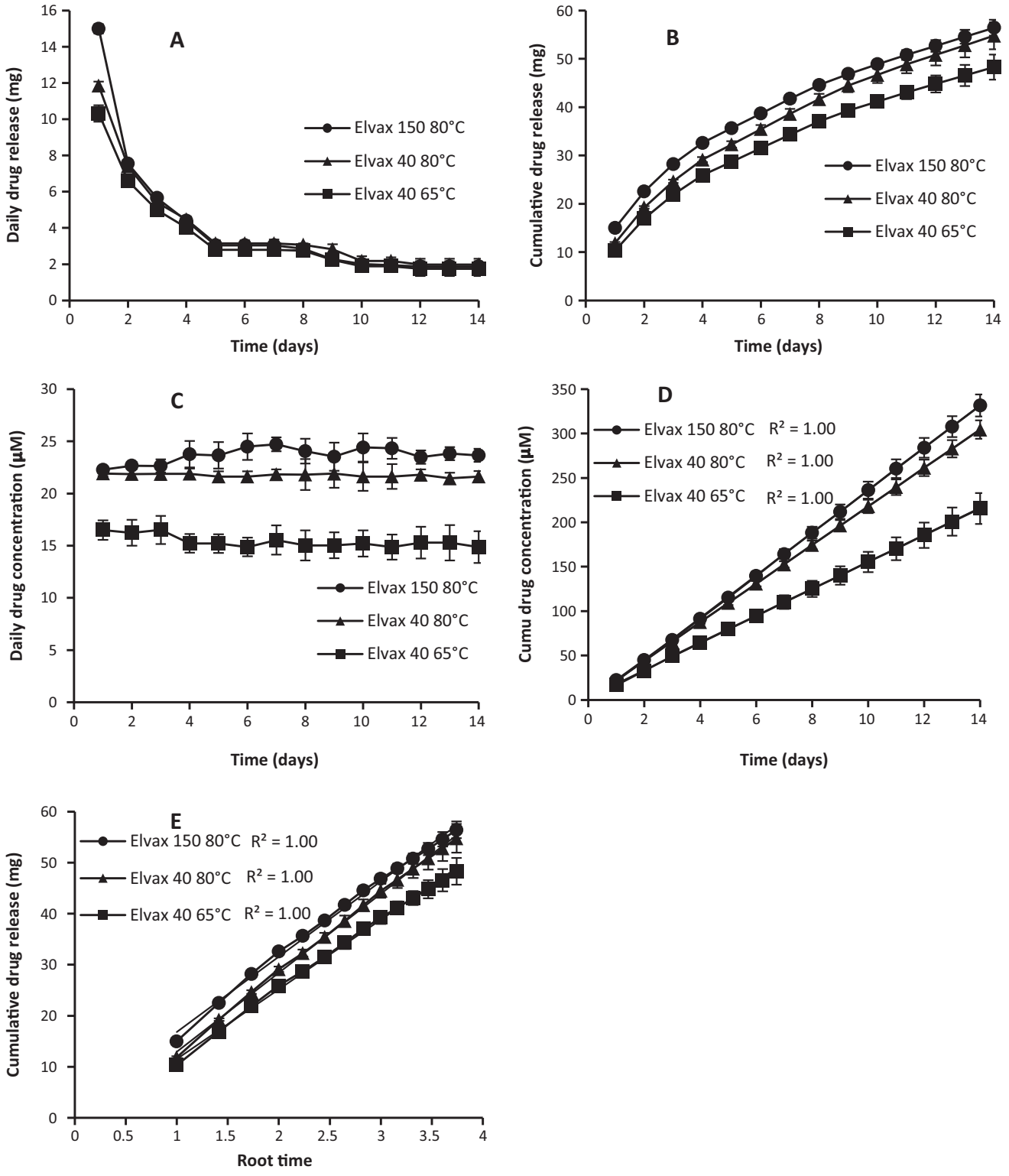

Fig. 5. *In vitro* daily (A) and cumulative (B) release of DSF from 5% w/w DSF-loaded thermoplastic vaginal rings into sink conditioned media. *In vitro* daily (C) and cumulative (D) release of DSF from 5% w/w DSF-loaded thermoplastic vaginal rings into non-sink conditioned media. Cumulative DSF release vs. root time plot for the 5% w/w DSF-loaded thermoplastic vaginal rings into sink conditioned media.

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Fig. 3B is a representative chromatograph for the thermal degradation of DSF. It demonstrates that upon thermal degradation DSF degrades into two main products diethyldithiocarbamate (DDC) (retention time 3.0 min) and an unknown impurity (retention time 4.8 min). Fig. 3C is a representative chromatograph from the content uniformity study and demonstrates that there was no DSF degradation during manufacture as none of the chromatographs for any of the rings contained peaks at 3.0 and 4.8 min.

3.5. Determination of the physical state of DSF in 5% w/w DSF-loaded PEVA vaginal rings

DSC analysis was used to determine the physical state (amorphous or crystalline) of the DSF within the vaginal rings. Fig. 4A demonstrates that the Elvax 150 and Elvax 40 rings manufactured at 80 °C contained an amorphous form of DSF immediately after manufacture. This is because the rings were manufactured at temperatures above the melting point of DSF, converting it to its amorphous form and the subsequent solidification of the molten polymer stabilises the DSF in its amorphous form. We know the DSF is mostly in its amorphous form and not molecularly dispersed, because the glass transition temperature of the polymers is the same for both the DSF-loaded rings and the controls. If the DSF was molecularly dispersed, then the glass transition temperature of the polymer in the rings would shift to the right. The fact that the DSF is in its amorphous form should result in an increase in the overall release rate particularly into the non-sink conditioned media, as the amorphous form of a drug has a greater solubility compared to the crystalline form as the energy required to solubilise the drug is reduced due to the drug molecules no longer being in a crystal lattice [49–53]. However, the Elvax 40 vaginal ring manufactured at 65 °C had a peak at 70 °C, representative of crystalline DSF, which when compared with the DSF heat flow (5.317 J/g) of the control sample corresponds to approximately 35% (1.85 J/g) crystalline structure. The reason for this is that these rings were manufactured at temperatures below the melting point of DSF, making it much more difficult for it to be solubilised in the polymer. Some drugs can recrystallise out of the polymer as their crystalline state is more stable than the amorphous state. Therefore, we evaluated the physical state of DSF in the rings at 1 month (Fig. 4B) and 3 months (Fig. 4C) after manufacture. At both 1 and 3 months the DSF in the Elvax 40 vaginal rings manufactured at 80 °C remained completely in the amorphous state and thus we would expect limited recrystallisation after 6 or more months on storage. However, the DSF in the Elvax 150 rings manufactured at 80 °C began to recrystallise after 1 month having approximately a 15% (0.81 J/g) crystalline structure, while at 3 months it had an approximate 33% (1.75 J/g) crystalline structure. The Elvax 40 rings manufactured at 65 °C continued to recrystallise having approximately 57% (3.04 J/g) and 79% (4.20 J/g) crystalline structure after 1 month and 3 months respectively.

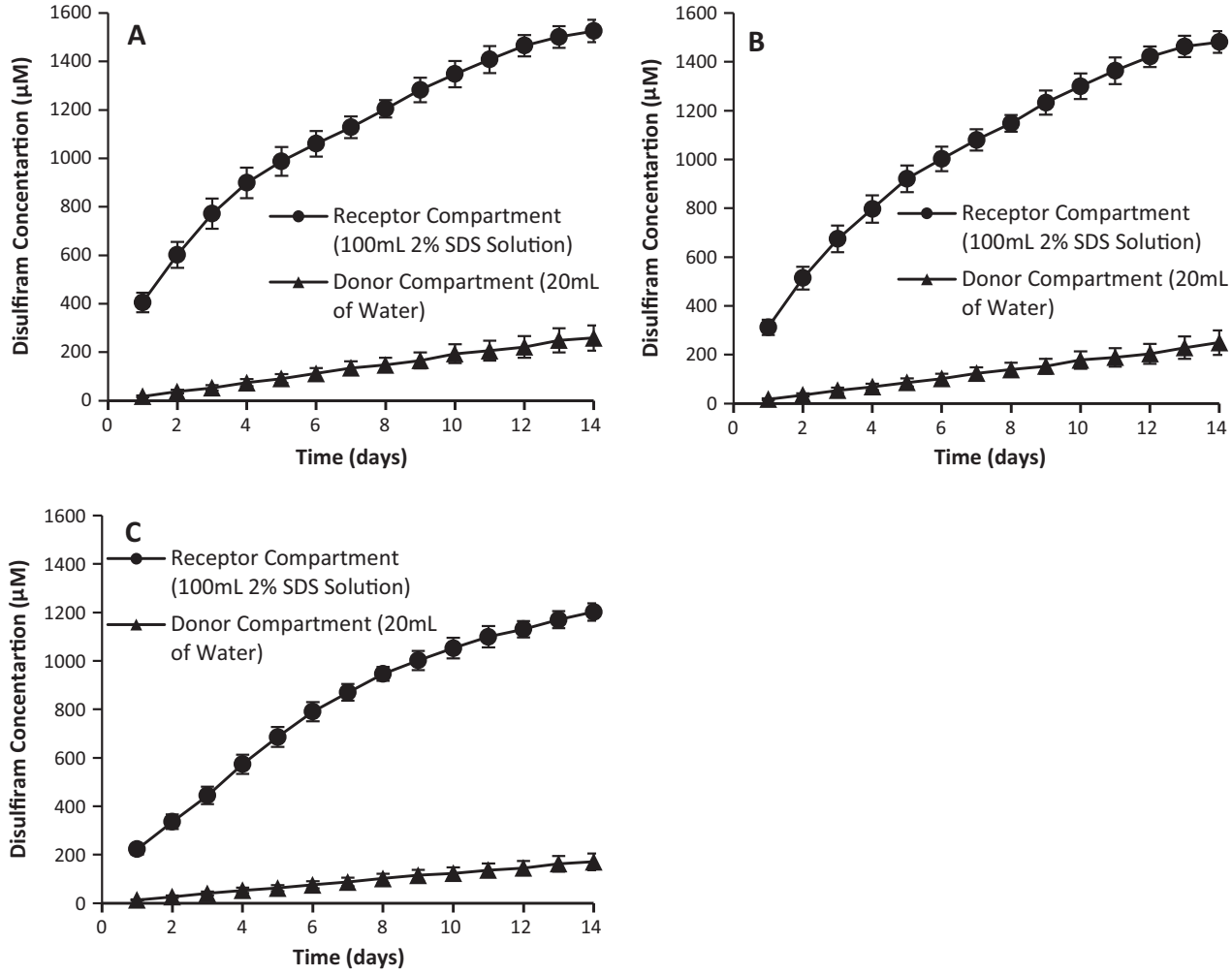

Fig. 6. *In vitro* DSF release from Elvax 150 (A) and Elvax 40 (B) 5% w/w DSF-loaded thermoplastic vaginal rings manufactured at 80 °C and Elvax 40 rings manufactured at 65 °C (C) using a dual chambered release model.

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3.6. In vitro release of 5% w/w DSF-loaded PEVA vaginal rings

Fig. 5A and B shows the daily and cumulative *in vitro* release of DSF from the various DSF-loaded PEVA vaginal rings into sink-conditioned release media. As would be expected the three rings have similar release profiles, except that the Elvax 150 ring has a slightly higher day 1 burst. The Elvax 150, Elvax 40 (80 °C) and Elvax 40 (65 °C) released a total of 56.4, 54.8 and 48.3 mg of DSF over 14 days. The reason for the similarity in release is due to the fact the release media is sink conditioned and thus the limited aqueous solubility of DSF has no effect on the release rate. However, if we compare this to the non-sink conditioned *in vitro* release (Fig. 5C and D) we see that the Elvax 150 and Elvax 40 vaginal rings manufactured at 80 °C have a significantly ($p < 0.05$) higher release rate than the Elvax 40 vaginal rings manufactured at 65 °C. The reason for this is that the release study was started immediately after ring manufacture and consequently the DSF in the vaginal rings manufactured at 80 °C is in its amorphous form (Fig. 4A), while the DSF in the rings manufactured at 65 °C has approximately 35% crystalline nature and has been mentioned previously the amorphous form of a drug has a greater solubility compared to the crystalline form as the energy required to solubilise the drug is reduced due to the drug molecules no longer being in a crystal lattice [49–53]. The DSF concentration has been used to plot the non-sink conditioned *in vitro* release (Fig. 5C and D) and it demonstrates that even in an aqueous environment the vaginal rings are capable of delivering DSF at levels well in access of its IC50 value (124.3 nM) for the HeLa cervical cancer cell line. The Elvax 150 and Elvax 40 rings manufactured at 80 °C have an average daily release of approximately 23.7 and 21.7 μM and release a total DSF concentration of 331.5 and 304.4 μM respectively over 14 days, while the Elvax 40 vaginal rings manufactured at 65 °C have an average daily release rate of 15.4 μM and release a total DSF concentration of 215.6 μM over 14 days.

3.7. In vitro release of 5% w/w DSF-loaded vaginal rings using a dual chambered release model

The vagina is a much more dynamic system that the single compartment release models described above. The vaginal vault contains aqueous vaginal fluid, but it is a collapsed space surrounded by vaginal tissue which is much more hydrophobic. Therefore, to represent the two compartments (aqueous vaginal vault surrounded by hydrophobic tissue) we used a more dynamic dual chambered release model consisting of a balloon filled with water to represent the aqueous vaginal vault, which was submerged in 100 mL of 2% SDS solution to represent the hydrophobic tissue and systemic up take.

Fig. 6 shows that the cumulative release profile of DSF concentration from all three rings, into the balloon, which represents the aqueous vaginal vault, is similar to their cumulative release profiles in Fig. 5D, with each ring releasing between 170 and 260 μM of DSF over 14 days. This is to be expected as the release medium used in both experiments was non-sink conditioned. However, Fig. 6 also shows that a much greater concentration of DSF is diffusing across the balloon wall (which represents the vaginal wall) and into the 2% SDS solution (which represents tissue absorption). This dual chambered model demonstrates that the Elvax 150 rings, the Elvax 40 rings manufactured at 80 °C and the Elvax 40 rings manufactured at 65 °C could potentially achieve DSF cervical tissue concentrations (1525.5, 1480.6 and 1201.8 μM respectively) 9668.5–12,000 times greater than its IC50 value for HeLa cervical cancer cells. The ring is not the only thing influencing the DSF concentrations in each compartment, the renewal rate of the release medium will also influence the DSF concentrations and must be taken into consideration. However, this study does demonstrate that the rings have the potential to deliver an effective level of DSF to the cervical tissue, which would need to be tested using an *in vivo* model.

4. Conclusion

This paper demonstrates the development of DSF-loaded thermoplastic vaginal rings, which have the potential to be used as a localised treatment of cervical cancer. It shows that due to its four sulphur atoms DSF is unsuitable for formulation into a silicone vaginal ring as it inhibits the curing process. Furthermore, as a result of its thermal instability, low melt thermoplastics are required, so as not to degrade the drug during manufacture. The processing temperature of the rings can significantly affect the physical state of the drug within the rings, with those rings being manufactured at temperatures above DSF's melting point having a significantly lower crystalline nature compared to those rings manufactured below its melting temperature. This in turn influences the *in vitro* release of the DSF into non-sink conditioned release media, with the rings containing the lower amount of crystallinity having the higher release rate due to an increase in solubility as a result of a lower solvation energy requirement. We believe that if successfully tested in an *in vivo* model these ring formulations offer a non-invasive alternative to the current cervical cancer treatments available, while localised delivery to the cervix will reduce the dose of drug needed to provide a therapeutic effect and minimise the systemic side effects associated with other chemotherapeutic delivery options.

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Please cite this article in press as: P. Boyd et al., Development of disulfiram-loaded vaginal rings for the localised treatment of cervical cancer, Eur. J. Pharm. Biopharm. (2014), http://dx.doi.org/10.1016/j.ejpb.2014.08.002

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