Evaluation of polycaprolactone matrices for the intravaginal delivery of metronidazole in the treatment of bacterial vaginosis

Meenakshi Pathak, Mark Turner, Cheryn Palmer and Allan GA Coombes

The online version of this article can be found at:

http://jba.sagepub.com/content/29/3/354

Published by:

$SAGE

http://www.sagepublications.com

Additional services and information for **Journal of Biomaterials Applications** can be found at:

Email Alerts: http://jba.sagepub.com/cgi/alerts

Subscriptions: http://jba.sagepub.com/subscriptions

Reprints: http://www.sagepub.com/journalsReprints.nav

Permissions: http://www.sagepub.com/journalsPermissions.nav

Citations: http://jba.sagepub.com/content/29/3/354.refs.html

>> Version of Record - Aug 18, 2014

OnlineFirst Version of Record - Mar 28, 2014

What is This?

Evaluation of polycaprolactone matrices for the intravaginal delivery of metronidazole in the treatment of bacterial vaginosis

Meenakshi Pathak[1], Mark Turner[2], Cheryn Palmer[3] and Allan GA Coombes[1,4]

Abstract

Microporous, poly (ε-caprolactone) (PCL) matrices loaded with the antibacterial, metronidazole were produced by rapidly cooling suspensions of drug powder in PCL solutions in acetone. Drug incorporation in the matrices increased from 2.0% to 10.6% w/w on raising the drug loading of the PCL solution from 5% to 20% w/w measured with respect to the PCL content. Drug loading efficiencies of 40–53% were obtained. Rapid 'burst release' of 35–55% of the metronidazole content was recorded over 24 h when matrices were immersed in simulated vaginal fluid (SVF), due to the presence of large amounts of drug on matrix surface as revealed by Raman microscopy. Gradual release of around 80% of the drug content occurred over the following 12 days. Metronidazole released from PCL matrices in SVF retained antimicrobial activity against *Gardnerella vaginalis in vitro* at levels up to 97% compared to the free drug. Basic modelling predicted that the concentrations of metronidazole released into vaginal fluid *in vivo* from a PCL matrix in the form of an intravaginal ring would exceed the minimum inhibitory concentration of metronidazole against *G. vaginalis*. These findings recommend further investigation of PCL matrices as intravaginal devices for controlled delivery of metronidazole in the treatment and prevention of bacterial vaginosis.

Keywords

Bacterial vaginosis, intravaginal rings, polycaprolactone, metronidazole

Introduction

Bacterial vaginosis (BV) is one of the most common genital conditions occurring in women of child bearing age[1] and is caused by the displacement of normal vaginal lactobacilli by other species notably *Gardnerella vaginalis*, *Mycoplasma homonis* and anaerobic bacteria such as peptostretococci, *Prevotella* spp. and *Mobiluncus* spp.[2] BV is associated with adverse pregnancy outcomes including post-partum, post-abortion and post-hysterectomy infections. BV increases women's risk of acquiring pelvic inflammatory disease and potentially some sexually transmitted infections (STIs) such as chlamydia, gonorrheae and HIV/AIDS by inducing changes in the mucosal immune environment of the vagina.[2–4] BV is estimated to have a mean prevalence of 14% when considering both developed and developing countries, but as the microflora of the vaginal ecosystem changes throughout the menstrual cycle, under the influence of exogenous hormones and during reproductive life in most women it is difficult to estimate the true prevalence or impact of BV.[5] In the United States, BV affects approximately 80,000 pregnant women per year, resulting in an increased

[1]The University of Queensland, Pharmacy Australia Centre of Excellence, Woolloongabba, Brisbane, Queensland, Australia
[2]The University of Queensland, School of Agriculture and Food Sciences, St. Lucia, Queensland, Australia
[3]Princess Alexandra Hospital, Dept. of Sexual health, Queensland, Australia
[4]The International Medical University, School of Pharmacy, Bukit Jalil, Kuala Lumpur, Malaysia

Corresponding author:
Allan GA Coombes, The International Medical University, School of Pharmacy, No. 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.
Email: AllanCoombes@imu.edu.my

incidence of preterm delivery or low-birth weight. Treatment can reduce these risks and may in turn reduce the number of associated perinatal deaths and neurologic abnormalities in infants.[6]

BV, when treated, is generally managed by using metronidazole (MTZ), which belongs to the nitro-imidazole class of antibiotics and exhibits broad spectrum activity against most Gram-negative and Gram-positive anaerobic bacteria.[7] MTZ is particularly attractive for the treatment of BV because it also eradicates any coexisting trichomoniasis, an STI caused by the protozoa, *Trichomonas vaginalis*.[3] MTZ is generally administered orally in tablet form (2 g single dose or 400 mg, 12-hourly for 7 days) but this route is often associated with adverse gastrointestinal side effects, nausea, headache, anorexia and occasionally vomiting. The bitter or metallic taste of oral dosage forms presents a further disadvantage. The side effects have been avoided by administration of MTZ as solid vaginal formulations such as pessaries and tablets but the residence time is short, necessitating frequent administration.[8] Semi-solid intravaginal gels incorporating MTZ (0.75%) require daily administration of approximately 5 g gel for 5 days but semi-solid preparations are messy to apply, prone to leakage and concerns exist over effective coverage of the vaginal epithelium. These factors have contributed to the recent upsurge of interest in intravaginal ring (IVR) devices for sustained delivery of antiviral[8,9] and antibacterial microbicides.[10] Recently, polyanionic dendrimers constructed from L-lysine with precise configurations of oligomeric moieties (e.g. naphthalene disulfonate) have attracted attention as microbicides.[11] Dendrimer SPL-7013 in particular has been reported to display selective antibacterial activity against *G. vaginalis* at a concentration of 5 mg/mL using the agar dilution method and low activity against normal vaginal *Lactobacillus* flora thus holding out promise as a future therapy or prophylaxis for BV. However, vaginal formulations of dendrimer in Carbopol gel may need to contain 40–100 mg dendrimer in 4–5 g of gel for effective daily dosing and application may be required for 3–4 days per week which renders the approach inconvenient for the user.[12,13]

IVRs offer advantages of low and continuous dosing over extended time periods, reduced side effects, self-administration and improved patient compliance. Conventional IVRs produced from silicone elastomer or poly (ethylene vinyl acetate) (pEVA) have been used clinically for many years for delivery of oestrogen (hormone replacement therapy) and etonogestrel and ethinyl oestradiol (contraceptive purposes).[14] IVRs are being evaluated for sustained release of the non-nucleoside reverse transcriptase inhibitor, Dapivirine as an anti-HIV microbicide and acyclovir for herpes prophylaxis.[15,16] Conventional IVRs do however display a number of disadvantages for microbicide delivery; they are generally restricted to delivery of low molecular weight, hydrophobic drugs such as Dapivirine. In addition, manufacture involves heating at 80°C for silicone elastomer or 140°C for pEVA which could degrade thermally-sensitive compounds. These problems have been circumvented by introducing more complex IVR designs and manufacturing techniques, whereby drug-containing pods are inserted into preformed rings.[17] The synthetic polyester, poly (ε-caprolactone) (PCL) has been investigated extensively for many years for production of a range of drug delivery systems including, microparticles, nanoparticles, films and fibres.[18] PCL nanoparticles loaded with the immunosuppressant cyclosporine, for example, have been reported to decrease the nephrotoxicity of the drug and efficiently target lymphocytes.[19] We have previously shown that microporous PCL matrices prepared by precipitation casting[20] or rapid cooling techniques[13] are effective for sustained delivery of small hydrophobic drug molecules (progesterone),[21] hydrophilic entities (gentamicin sulphate)[20] and macromolecules such as enzymes with retained activity.[22] More recently, the potential utility of these materials was demonstrated for vaginal delivery of the antibacterial, ciprofloxacin, in the treatment of gonorrhoea. Drug loadings of 7.3–15% were obtained and drug released into SVF retained high antibacterial activity against *N. gonorrhoeae*.[10] Here we describe sustained delivery of MTZ from PCL matrices intended for production of IVRs in the treatment of BV.

Materials and methods

Materials

PCL (Mw 115,000 Da, CAPA 6500) was obtained from Solvay Interox, Warrington, UK. MTZ, glucose, urea, bovine serum albumin, potassium hydroxide, calcium chloride, glycerol, lactic acid and acetic acid were purchased from Sigma-Aldrich, Australia. *G. vaginalis* stock culture in glycerol broth was supplied by Micromon, Monash University, Clayton, Victoria, Australia. Horse blood agar, heart infusion broth (HIB), CO_2 generation kit and antimicrobial susceptibility blank discs were obtained from Oxoid, Basingstoke, UK.

Production of MTZ-loaded PCL matrices

PCL solutions of concentration 15% w/v were prepared by dissolving the polymer in acetone at 50°C. MTZ was ground to a fine powder and added to the PCL solution to produce suspensions of concentrations 5%, 10%,

15% and 20% w/w of the PCL content. The resulting suspension was homogenized for 30 s at 5000 rpm using a Silverson SL27 homogenizer (Silverson Machines, Chesham, Bucks, UK). The suspension was poured into a polypropylene syringe body (3 mL) which was used as a mould and cooled in ethanol at −80°C for 2 h to allow crystallisation of PCL. The hardened matrices were removed from the moulds and immersed in 10 mL ethanol for 24 h to extract acetone by solvent exchange. Samples were removed from ethanol and left to dry under ambient conditions to evaporate residual solvents. The final matrices were in the form of cylinders of diameter 6.5 ± 0.5 mm and length 45.0 ± 5 mm.

Determination of MTZ content of PCL matrices

Four sections were cut from the top, upper-middle, lower-middle and base of cylindrical PCL matrices to determine the uniformity of drug distribution with in the matrices. Samples were weighed and dissolved in 2 mL of dichloromethane (DCM). Precipitation of PCL was induced by adding 5 mL of 30% methanol followed by shaking overnight (Vibrax VXR, IKA, Werke Staufen, Germany) to evaporate DCM and obtain partitioning of the drug into the methanol phase. The MTZ content of the methanol phase was determined by using UV spectrophotometry (Varian, Cary 50 Bio, Agilent Technology, USA) at an absorbance wavelength of 319 nm by comparison with a calibration curve constructed using a series dilution of MTZ in 30% methanol (4–20 μg/mL). Experiments were performed in triplicate to obtain values of actual drug loading and loading efficiencies of PCL matrices.

Morphology of MTZ-loaded PCL matrices

The morphology of the surface and interior of drug-free and drug-loaded PCL matrices was examined using a JSM 6460LA scanning electron microscope (SEM, JEOL, Japan). Specimens were mounted on aluminium SEM stubs using carbon tabs and sputter coated with platinum using an Eiko-Sputter coater automatic mounting press, prior to examination at a voltage of 5 kV.

Differential scanning calorimetry

The thermal characteristics of PCL matrices were investigated using differential scanning calorimetry (DSC) (DSC 1 STARe System, Mettler Toledo, Switzerland) under a nitrogen atmosphere. Samples of drug-free and MTZ-loaded PCL matrices were weighed, placed in sealed aluminium pans and heated over the temperature range −100°C to 150°C at a rate of 10°C/min. The peak melting point, glass transition temperature (T_g) and heat of fusion data were obtained using the DSC software facility. The crystallinity of PCL matrices loaded with different amounts of MTZ was examined to identify any changes with drug loading. Crystallinity (%) was estimated using a value of 139.5 J/g for the heat of fusion of fully crystalline PCL.[20]

Hardness testing of PCL matrices

Hardness testing of drug-free and MTZ-loaded matrices was carried out using a CT3 Texture Analyzer (Brookfield Engineering Laboratories Inc., Middleboro, MA). As-moulded cylinders were mounted horizontally and compressed locally at a speed of 0.1 mm/min to a depth of 2.0 mm using a 2 mm diameter, flat-ended, cylindrical probe (TA39). The hardness (or indentation resistance) of each sample was calculated from the applied force measured at a depth of 2 mm. A pEVA IVR (Nuvaring®, Schering-Plough Pty limited, New South Wales, Australia) was subjected to the same test procedure for comparison.

In vitro release of MTZ from PCL matrices

Cylindrical sections of MTZ-loaded PCL matrices (length 45 mm) were subjected to a release study in SVF. Prior to testing both ends of each sample were sealed by dipping in 5% w/v solution of PCL in acetone followed by drying in air. Experiments were performed in triplicate. Each sample was placed separately in 10 mL of SVF and retained at 37°C in an incubator. SVF was prepared according to the method of Owen and Katz[23] and contained 3.51 g NaCl, 1.40 g KOH, 0.222 g Ca(OH)$_2$, 0.018 g bovine serum albumin, 2.00 g lactic acid, 1.00 g acetic acid, 0.16 g glycerol, 0.4 g urea and 5.0 g glucose up to 2 L of distilled water. The pH was adjusted to 4.2 using 10% HCl. The release media were collected and replaced with fresh media daily for 12 days. The concentration of drug in the release medium was analysed by UV spectrophotometry (Varian, Cary 50 Bio, Agilent technology, USA) at 319 nm by comparison with a standard curve produced using a series dilution of MTZ in SVF. Separate release samples were stored at 4°C for antimicrobial testing.

Raman spectroscopy

Raman mapping of 10% MTZ-loaded PCL matrices was performed to characterise the drug distribution for correlation with drug release behaviour. A 1 mm thick disk was taken from the middle of drug-loaded PCL matrices, placed on a glass slide and scanned using

a Raman microscope (Nicolet Almega XR Dispersive Raman, Thermoscientific, USA) at spatial intervals of 100 μm.

In vitro assay of antimicrobial activity

The antimicrobial activity of MTZ released from the PCL matrices was assayed with *G. vaginalis* using the disc diffusion method. *G. vaginalis* was stored as stock cultures in 40% glycerol at −80°C. *G. vaginalis* was grown for 48 h at 37°C on horse blood agar plates and the colonies of bacteria were then scraped from the agar surface using a spreader and HIB. The cell suspension was then diluted in HIB and plated onto horse blood agar plate to get approximately 100 colony forming units (CFUs) per plate. A blank disc (Oxoid) was placed at the centre of each inoculated plate and 100 μL of drug standard solution in SVF or release medium containing MTZ was added to the disc. The plates were incubated at 37°C for 48 h under anaerobic conditions and the diameter of the zone of inhibition surrounding the disc was measured. The relative antibacterial activity of MTZ released from PCL matrices was calculated by comparison with the zone of inhibition obtained using non-formulated drug solutions of MTZ of the same concentration. SVF and release media used for incubation of blank PCL matrices were used as controls.

Results and discussion

Morphology of PCL matrices

MTZ-loaded PCL matrices prepared by rapidly cooling suspensions of drug powder in PCL solution exhibit flexibility, uniformity of structure and an absence of large cracks and voids in the sample surface and interior. SEM examination of drug-free PCL matrices revealed a nodular type of morphology and irregular shaped pores with dimensions of 2–4 μm (Figure 1a). The surface of MTZ-loaded matrices exhibited a flat texture (Figure 1b), probably, resulting from contact of the matrix with the mould wall. Fine fissures were observed in certain areas along with evidence of trapezoidal-shaped drug crystals 1–2 μm in size. The internal structure of MTZ-loaded PCL matrices exhibited a woven, lamellar type of morphology (Figure 1c) and the characteristic microporous morphology of the
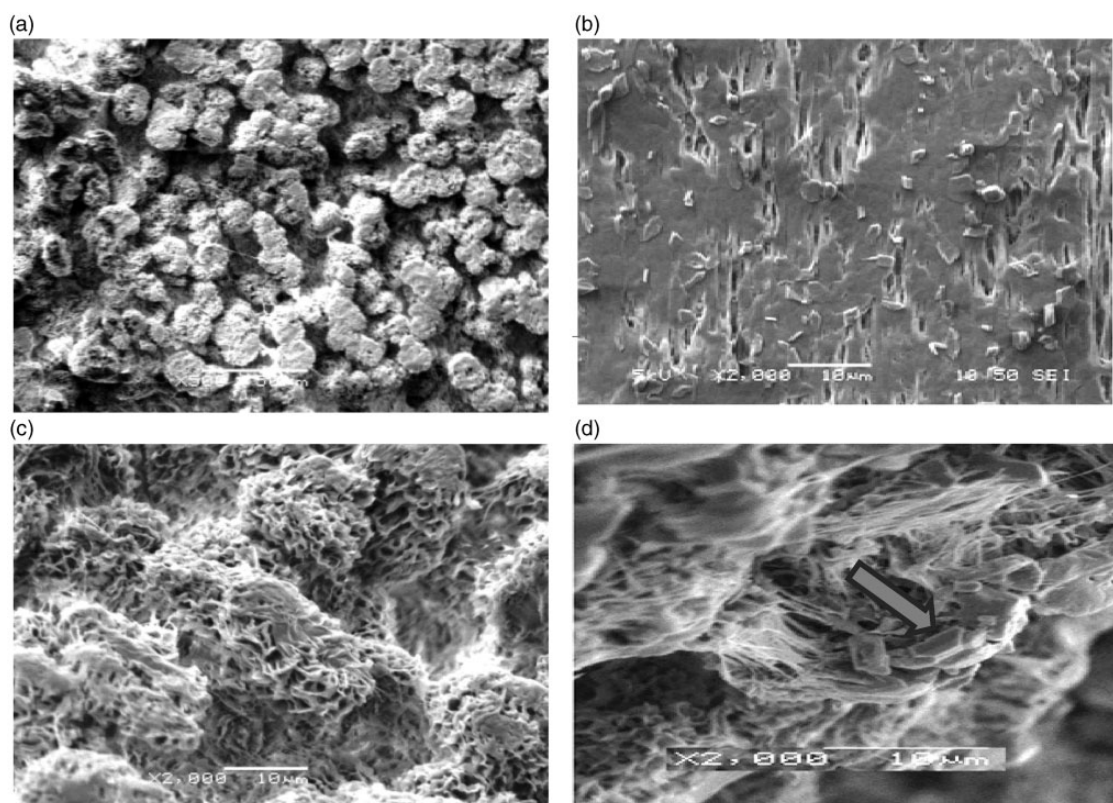

Figure 1. Morphology of drug-free and 5.4% metronidazole (MTZ)-loaded PCL matrices. (a) Interior of drug-free PCL matrix, (b) surface of MTZ-loaded PCL matrix, (c) interior of MTZ-loaded PCL matrix and (d) interior of MTZ-loaded PCL matrix showing presence of drug crystals (arrowed). PCL: polycaprolactone.

PCL phase consisting of 2–5 μm pores. Lozenge-shaped or trapezoidal drug crystals are visible at higher magnification (Figure 1d, arrowed).

MTZ loading of PCL matrices

The actual loading of MTZ in PCL matrices and the corresponding theoretical loading are presented in Table 1. Actual drug loading tended to increase towards the base of the moulding, indicating sedimentation of larger drug particles before crystallisation and hardening of the PCL phase occurred during cooling to −80°C. The variation of drug loading throughout samples was typically confined to 1–2% but a larger spread in actual drug loading (6–10%) was observed for PCL matrices prepared using 15% w/v PCL solution.

Average MTZ loadings of 2.0%, 5.4%, 8.1% and 10.6% w/w were measured in PCL matrices corresponding to theoretical loadings of 5%, 10%, 15% and 20% w/w, resulting in fairly low incorporation efficiencies of 40–54% (Table 1). These findings may be explained by the relative solubility of MTZ in the solvents used for matrix production. The solubility of MTZ in methanol, acetone and ethanol is 32.2, 20.7 and 5.0 mg/mL,[24] respectively. The solubility of MTZ in the ethanol phase used to extract acetone from the hardened PCL matrices results in partition of MTZ and elution from the matrix. Despite loss of drug from the matrix, the solvent extraction stage is essential to avoid shrinkage of the PCL matrix following crystallisation and drying which can result in cracking of the material. Wang et al. found that the use of ethanol instead of methanol for acetone extraction resulted in an increase in catalase loading of PCL matrices[22] due to the lower solubility of the enzyme in ethanol. Ethanol was also used instead of methanol in the present study to exploit the lower solubility of MTZ in ethanol and thus reduces drug loss.

Thermal analysis of matrices

DSC analysis revealed more than doubling of the crystalline content of PCL matrices from around 33% to 76% with increasing MTZ loading of the material from 0 to 8.1% w/w (Table 2). Previous studies by Chang et al. revealed crystallinity levels of 50–75% for drug-free PCL matrices and both increases and decreases in PCL crystallinity depending on the type of drug molecule incorporated in the matrix.[21] Progesterone inclusion (10%) resulted in a major reduction of crystallinity of around 12% from 66% to 54%,[21] whereas gentamicin sulphate particulates increased the crystallinity of PCL matrices by 4–8%. Progesterone particulates were considered to inhibit PCL crystal nucleation and growth, while gentamicin sulphate particles acted as nucleating agents to enhance PCL crystallisation. The significant increase in crystallinity of the PCL phase with MTZ loading measured in the present study indicates the strong effect of the dispersed drug particles on nucleation and crystal growth of PCL. Detailed investigations of the influence of processing conditions on drug particle crystallinity, to control drug dissolution behaviour and bioavailability, for example, have been reported extensively in the scientific literature.[25,26] However, we have found no reports, apart from our previous studies which document the effect of drug presence on the crystallinity of excipients used in the formulation of drug delivery devices. The particles of MTZ appear to promote heterogeneous or epitaxial crystallisation of PCL, which is known to be influenced by similarities in the crystal lattice of the substrate and crystallising polymer and also by the surface topography of the substrate (defects, steps and terraces).[27] The lower crystallinity of the drug-free PCL phase in the present study compared with samples produced by Chang et al. reflects rapid cooling of the PCL solution compared with the room temperature, precipitation technique employed by Chang et al.[21] Rapid cooling

Table 1. Loading and incorporation efficiency of metronidazole in PCL matrices prepared using the rapid cooling technique.

Theoretical drug loading (% w/w)	Actual loading (% w/w)	Incorporation efficiency (%)
5	2.0 ± 0.5	40.0 ± 6.6
10	5.4 ± 0.8	54.0 ± 8.3
15	8.1 ± 1.3	54.0 ± 8.8
20	10.6 ± 0.7	53.0 ± 3.4

PCL: poly (ε-caprolactone).

Table 2. Thermal analysis of metronidazole-loaded PCL matrices.

Theoretical drug loading (% w/w)	Actual drug loading (% w/w)	% Crystallinity	Glass transition temperature (T_g) (°C)
0	0	33.4 ± 2.3	−60.1 ± 0.6
5	2.9	42.4 ± 1.9	−58.1 ± 0.4
10	5.4	56.1 ± 2.2	−56.5 ± 0.4
15	8.1	74.8 ± 1.6	−55.6 ± 0.5
20	10.6	75.6 ± 1.2	−54.5 ± 0.6

PCL: poly (ε-caprolactone).

restricts polymer chain mobility and crystal growth. The glass transition temperature indicates the reversible change in the amorphous regions of a polymer from a hard and relatively brittle condition to a viscous or rubbery state. The increase in T_g found with increasing MTZ loading (Table 2) indicates an interference or restriction of PCL chain mobility due to the presence of the dispersed drug particles in the matrix.

Hardness testing of PCL matrices

The hardness values determined by texture analysis for MTZ-loaded PCL matrices are shown in Table 3. Matrices containing low drug loadings (2.0% w/w) exhibited similar hardness to unloaded samples. However, the matrix hardness decreased significantly by a factor of almost 2.5 when the drug loading was increased to 5.4%. The highest loaded samples (10.6%) exhibited a further decrease in hardness to around 900 mN/mm^2 indicating that excessive drug loading causes deterioration and weakening of the matrix structure, probably by micro-cracking effects which are also influential in relation to drug release behaviour.[28] The hardness of the pEVA IVR was found to be 9280 mN/mm^2 which is almost 2.5 times more than the 2% MTZ-loaded PCL samples. Thus microporous PCL IVRs potentially offer scope for improving user comfort compared with conventional materials. The work of indentation (determined by measurement of the area under the force/displacement curve) essentially reveals a major (65%) deterioration in material toughness compared with drug-free matrices at relatively low levels of drug incorporation around 5% w/w. This factor requires careful consideration when optimising the properties of IVR devices, based on MTZ-loaded PCL matrices since adequate mechanical properties are required to withstand the flexural loads experienced during insertion and during device residence in the vagina and thus ensure successful clinical performance.

In vitro release of MTZ from PCL matrices

Drug release from PCL matrices featuring dispersed drug particles is governed by a number of factors including drug loading, uptake of release medium and drug solubility in the fluid phase, matrix porosity and the rate of drug diffusion in fluid-filled pores of the material. Since PCL exhibits a bioresorption time in excess of two years, the detailed pore structure (pore size, connectivity and tortuosity) exerts a major influence on drug transport from the matrix. The release profiles of MTZ from PCL matrices in SVF at 37°C are presented in Figure 2.

Following a large burst release phase in day 1, gradual drug release occurs over the following 12 days giving rise to an almost linear profile. The magnitude of the burst release phase increases with drug loading of the matrix from around 35% for 2.0% loaded matrices to almost 60% for the most highly loaded PCL matrix (10.6%). This behaviour suggests the presence of large amounts of drug particles at or close to the matrix surface and is supported by the SEM observations described above (Figure 1b). In the case of low drug loadings, gradual release of MTZ is expected to occur predominantly through interconnected pores and channels inherent in the microporous PCL matrix since the separation of drug particles will not favour formation of interconnected macropores by dissolution of contacting drug particles. Increasing numbers of interconnected macropores, fissures and channels are expected in the highly loaded systems, due to contact of drug particles and micro-cracking effects,[28] which facilitate entry of release medium and enhance drug dissolution and extraction. Around 80% of the drug load is released from all samples by day 12 demonstrating in general high pore interconnectivity and thus delivery
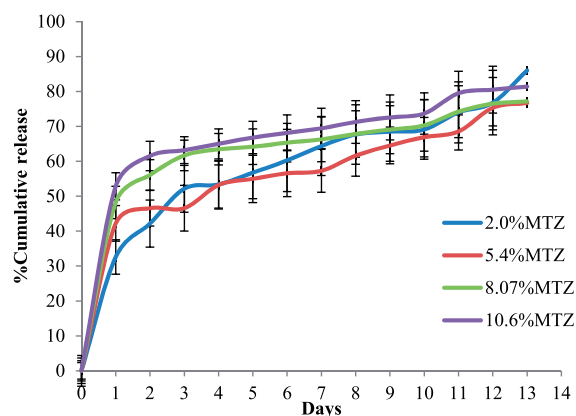
Table 3. Hardness testing of PCL matrices.

Drug loading (% w/w)	Hardness (mN/mm^2)	Work done (mJ)
0	3986 ± 210	14.3 ± 2.0
2.0	3841 ± 190	13.2 ± 1.5
5.4	1522 ± 75	5.0 ± 0.7
8.1	1268 ± 62	4.6 ± 1.1
10.6	888 ± 32	2.9 ± 0.9

PCL: poly (ε-caprolactone).

Figure 2. Cumulative (%) release of MTZ from PCL matrices in SVF at 37°C. MTZ: metronidazole; PCL: polycaprolactone; SVF: simulated vaginal fluid.

efficiency. The amount of drug released daily from the PCL matrices is presented in Figure 3. A minimum release amount of 51 μg of MTZ per day was measured at day 10.

Analysis of drug distribution using Raman spectroscopy

The Raman spectra of PCL show a characteristic peak in the region of 3000 cm^{-1} due to C–H stretching; small peaks at around 1800–1700 cm^{-1} are assigned to C=O stretching, while the peak at 1500 cm^{-1} is attributed to δCH_2 and that at 1200–1280 cm^{-1} is due to ΩCH_2.[29] The spectra of PCL matrices revealed an absence of

those molecules characteristic of the solvents used (acetone, ethanol) in matrix preparation.

Raman peaks for MTZ at 1500–1650 cm^{-1} are due to C=N stretching. In-plane and out-of-plane deformation vibrations are normally observed as sharp but weak to medium intensity bands in the region 1300–750 cm^{-1}. Small peaks around 800 cm^{-1} are due to NO_2 group scissoring, wagging, rocking and twisting.[30]

The Raman spectra presented in Figure 4 were generated at radial positions starting from the centre of a 5.4% MTZ-loaded PCL matrix disk and moving toward the edge. The distinct peaks in the region of 3000 and 1000–1500 cm^{-1} are due to the presence of the polymer and drug, respectively. The traces clearly indicate the change in peak intensity corresponding to differences in drug concentration at different positions within the sample, which reflects the dispersion of drug powder within the PCL matrix. The high intensity Raman spectra obtained at points along the sample edge for the 5.4% MTZ-loaded PCL matrix (Figure 5) confirm that high concentrations of drug are present at the matrix surface. These findings support the SEM images of drug crystals located at the sample surface and explain the high burst release behaviour of MTZ at day 1 of release testing (Figure 2).

Antibacterial testing

G. vaginalis is an anaerobic, β-haemolytic, oxidase-negative, catalase-negative, gram variable bacterium, which is detected in all women diagnosed with BV and plays an important role in its pathogenesis.[5]
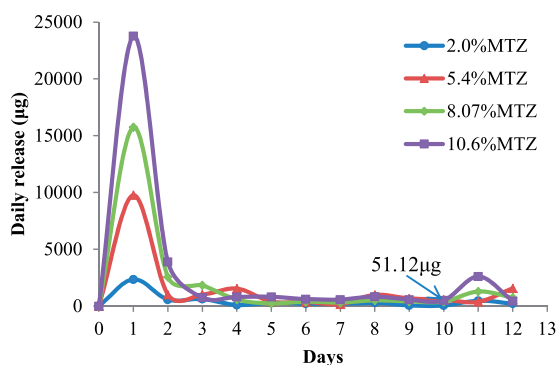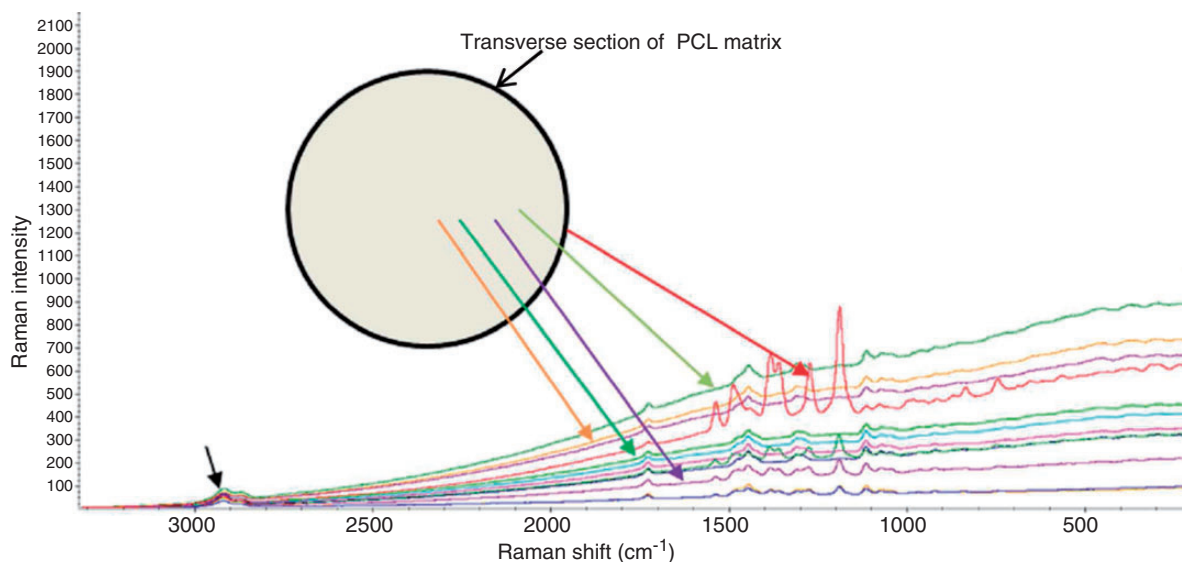

Figure 3. Amount of metronidazole (MTZ) release (μg) from PCL matrices in SVF at 37°C. PCL: polycaprolactone; SV: simulated vaginal fluid.

Figure 4. Raman spectra obtained at different radial positions in a transverse section of 5.4% MTZ-loaded PCL matrix. MTZ: metronidazole; PCL: polycaprolactone.
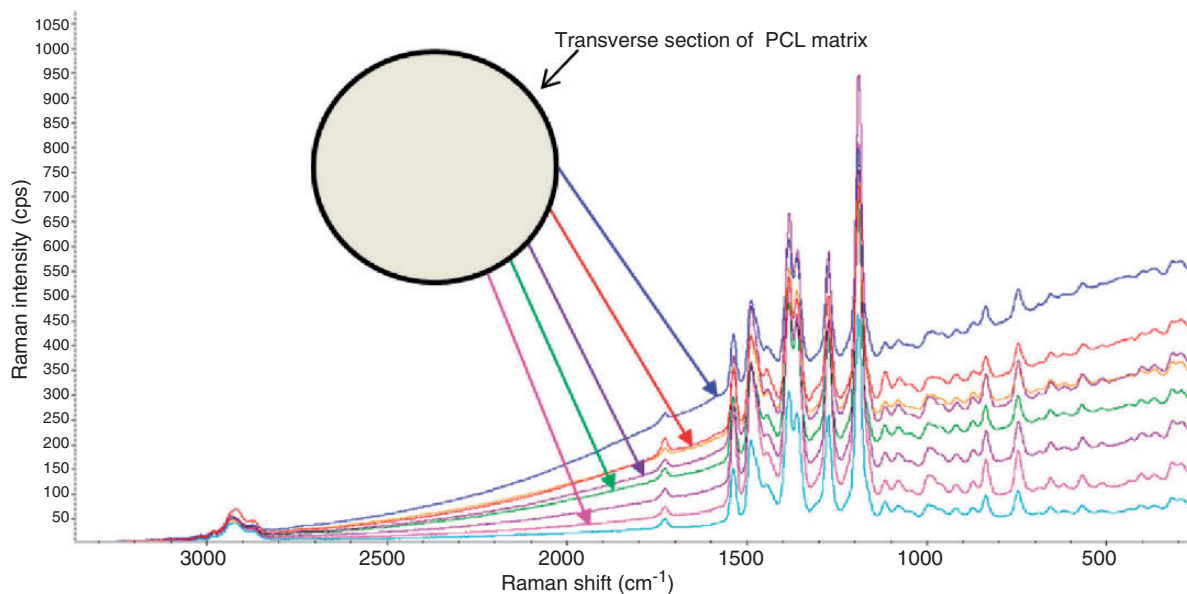
Figure 5. Raman spectra obtained at different edge points in a transverse section of 5.4% MTZ-loaded PCL matrix. MTZ: metronidazole; PCL: polycaprolactone.
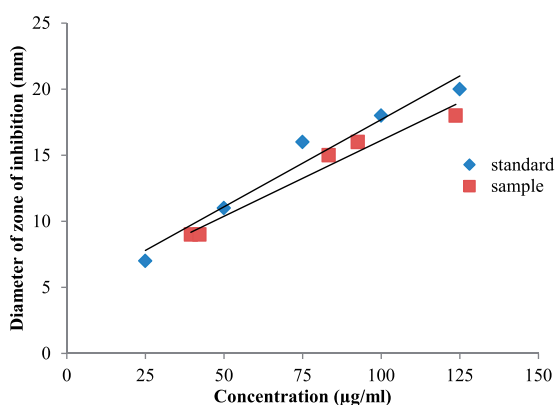

Figure 6. Relationship between concentration of MTZ and diameter of zone of inhibition against *G. vaginalis*. MTZ: metronidazole; *G. vaginalis*: *Gardnerella vaginalis*.
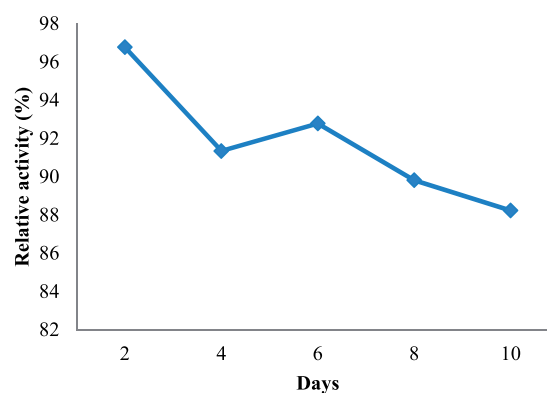

Figure 7. Relative activity (%) of MTZ released from 5.4% drug-loaded PCL matrices in SVF at 37°C over 10 days. MTZ: metronidazole; PCL: polycaprolactone; SVF: simulated vaginal fluid.

The antibacterial activity of non-formulated MTZ and drug released from PCL matrices into SVF against *G. vaginalis* was investigated using a disc diffusion assay to determine the effect of matrix formulation and matrix residence time in SVF on drug activity and to assess any implications for *in vivo* performance and dosing regimens A zone of inhibition increasing from 7 to 20 mm diameter was observed for control drug solutions in SVF with increasing concentration from 25 to 125 μg/mL (Figure 6). No zone of inhibition was observed in the case of control samples comprising SVF alone or release media obtained from drug-free PCL matrices.

Release media of days 2, 4, 6, 8 and 10 have been selected for the comparison with the standard drug. A linear relationship was observed between MTZ concentration and the diameter of the zone of inhibition, with a high correlation coefficient ($R^2 = 0.962$). The relative antibacterial activity (%) of MTZ released from 5.4% drug-loaded PCL matrices into SVF was obtained by comparing the diameter of the zone of inhibition obtained for standard MTZ solution and MTZ-containing release media at equivalent drug concentrations. A high relative antibacterial activity (88–97%) was exhibited by released drug over a 10-day release period (Figure 7). The relatively small decrease in

antibacterial activity over the course of the study may be due to prolonged exposure of the drug within the PCL matrix to elevated temperature (37°C) and the fairly complex biochemical environment presented by the SVF.

Based on the minimum daily amount of MTZ released from PCL matrices (51 µg released into 10 mL SVF at day 10 from a 5.4% drug-loaded matrix) the predicted concentrations of drug which would be released from an intra-vaginal ring (produced from a PCL matrix) into vaginal fluid are above the minimum inhibitory concentration (MIC) against *G. vaginalis* (2–12.8 µg/mL).[31] This assessment is based on the linear length of an intra-vaginal ring of 150 mm (outer diameter 58 mm, inner diameter 38 mm) and weight (1.5 g) being approximately 3.5 times that of the studied matrices (45 mm, 0.4 g). The minimum release amount of 5.1 µg/mL/day corresponds to a drug release rate from a PCL IVR of around 18 µg/mL/day. It is further assumed that the *in vitro* release rate from PCL matrices is similar to the *in vivo* release rate from a PCL vaginal ring and a maximum vaginal fluid turnover rate of 8 mL/day applies. These estimates do not take into account the complex variations in vaginal fluid volume and biochemical composition over time, or the possibility of systemic uptake of drug.

Conclusion

The basic modelling approach applied predicts that PCL matrices incorporating MTZ are potentially capable of eradicating *in vivo* one of the principal microorganisms implicated in the pathogenesis of BV. IVRs produced from microporous PCL matrices would offer advantages over MTZ vaginal gels in terms of reduced frequency of application and oral dosage forms in terms of avoidance of side effects and as a result warrants further investigation.

Conflict of interest

Funding

References

1. Marie P, Fethers Kath A and Bradshaw CS. Bacterial vaginosis: more question than answer. *Aust Fam Physician* 2009; 38: 394–397.
2. Wilson J. Managing recurrent bacterial vaginosis. *Sex Transm Infect* 2004; 80(1): 8–11.
3. Marrazzo JM. Interpreting the epidemiology and natural history of bacterial vaginosis: are we still confused? *Anaerobe* 2011; 17(4): 186–190.
4. O'Brien G. Bacterial vaginosis. *Pediatr Rev* 2008; 29(6): 209–211. (discussion 211).
5. Keane F, Ison CA, Noble H, et al. Bacterial vaginosis. *Sex Transm Infect* 2006; 82(Suppl 4): iv16–iv18.
6. Carey JC, Klebanoff MA, Hauth JC, et al. Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *N Engl J Med* 2000; 342(8): 534–540.
7. Filho DSC, Diniz CG and Silva VL. Bacterial vaginosis: clinical, epidemiologic and microbiological features. *HU Revista Juiz de Fora* 2010; 36: 223–230.
8. Friend D. Advances in vaginal drug delivery. *Drug Deliv Transl Res* 2011; 1(3): 183–184.
9. Fetherston SM, Malcolm RK and Woolfson AD. Controlled-release vaginal ring drug-delivery systems: a key strategy for the development of effective HIV microbicides. *Ther Deliv* 2010; 1(6): 785–802.
10. Dang NT, Turner MS and Coombes AG. Development of intra-vaginal matrices from polycaprolactone for sustained release of antimicrobial agents. *J Biomater Appl* 2013; 28(1): 74–83.
11. Tomalia DA, Christensen JB and Boas U (eds). Dendrimers, dendrons and dendritic polymers. *Discovery, application and the future*. Cambridge University Press, New York, USA, 2012.
12. Fairley JK, Lewis GR, Paull JRA, et al. *Method of treatment or prophylaxis of bacterial vaginosis*. Patent WO2012155172A1, 22 November 2012.
13. Rupp R, Rosenthal SL and Stanberry LR. VivaGel (SPL7013 Gel): a candidate dendrimer–microbicide for the prevention of HIV and HSV infection. *Int J Nanomed* 2007; 2(4): 561–566.
14. Dezarnaulds G and Fraser IS. Vaginal ring delivery of hormone replacement therapy–a review. *Expert Opin Pharmacother* 2003; 4(2): 201–212.
15. Romano J, Variano B, Coplan P, et al. Safety and availability of dapivirine (TMC120) delivered from an intra-vaginal ring. *AIDS Res Hum Retroviruses* 2009; 25(5): 483–488.
16. Moss JA, Malone AM, Smith TJ, et al. Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. *Antimicrob Agents Chemother* 2012; 56(2): 875–882.
17. Malcolm RK, Edwards KL, Kiser P, et al. Advances in microbicide vaginal rings. *Antiviral Res* 2010; 88(Suppl 1): S30–S39.
18. Woodruff MA and Hutmacher DW. The return of a forgotten polymer—polycaprolactone in the 21st century. *Prog Polym Sci* 2010; 35(10): 1217–1256.
19. Varela MC, Guzman M, Molpeceres J, et al. Cyclosporine-loaded polycaprolactone nanoparticles: immunosuppression and nephrotoxicity in rats. *Eur J Pharm Sci* 2001; 12(4): 471–478.
20. Chang HI, Perrie Y and Coombes AG. Delivery of the antibiotic gentamicin sulphate from precipitation cast

matrices of polycaprolactone. *J Control Release* 2006; 110(2): 414–421.

21. Chang HI, Williamson MR, Perrie Y, et al. Precipitation casting of drug-loaded microporous PCL matrices: incorporation of progesterone by co-dissolution. *J Control Release* 2005; 106(3): 263–272.

22. Wang Y, Chang HI, Li X, et al. Delivery of bioactive macromolecules from microporous polymer matrices: release and activity profiles of lysozyme, collagenase and catalase. *Eur J Pharm Sci* 2009; 37(3–4): 387–394.

23. Owen DH and Katz DF. A vaginal fluid simulant. *Contraception* 1999; 59(2): 91–95.

24. Cho MJ, Kurtz RR, Lewis C, et al. Metronidazole phosphate–a water-soluble prodrug for parenteral solutions of metronidazole. *J Pharm Sci* 1982; 71(4): 410–414.

25. Perez CJ, Vázquez A and Alvarez VA. Isothermal crystallization of layered silicate/starch-polycaprolactone blend nanocomposites. *J Therm Anal Calorim* 2008; 91(3): 749–757.

26. Fornes TD and Paul DR. Formation and properties of nylon 6 nanocomposites. *Polímeros* 2003; 13: 212–217.

27. Okada KN and Hikosaka M. Polymer nucleation. In: Piorkowska E and Rutledge GC (eds) *Handbook of polymer crystallization*. Hoboken, NJ: John Wiley & Sons, Inc., 2013. DOI:10.1002/9781118541838.ch4.

28. Wang Y, Wertheim DF, Jones AS, et al. Micro-CT analysis of matrix-type drug delivery devices and correlation with protein release behaviour. *J Pharm Sci* 2010; 99(6): 2854–2862.

29. Diem M. *Raman spectral imaging for the determination of structure and dynamics of human cells*. Laboratory for Spectral Diagnosis, Department of Chemistry and Chemical Biology, North-eastern University, Boston, 2009.

30. Chamundeeswari SPV, Samuel ERJJ and Sundaraganesan N. Theoretical and experimental studies on 2-(2-methyl-5-nitro-1-imidazoyl)ethanol. *European Journal of Chemistry* 2011; 2(2): 136–145.

31. Jones BM, Geary I, Alawattegama AB, et al. In-vitro and in-vivo activity of metronidazole against *Gardnerella vaginalis*, Bacteroides spp. and Mobiluncus spp. in bacterial vaginosis. *J Antimicrob Chemother* 1985; 16(2): 189–197.