Evaluation of Polycaprolactone Matrices for Sustained Vaginal Delivery of Nevirapine in the Prevention of Heterosexual HIV Transmission

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ABSTRACT: Nevirapine (NVP) was loaded in polycaprolactone (PCL) matrices to produce vaginal inserts with the aim of preventing HIV transmission. NVP dispersions in PCL were prepared, at 10% (w/w) theoretical loading, measured with respect to the PCL content of the matrices, in the form of (1) NVP only, (2) a physical mixture of NVP with polyethylene glycol (PEG) 6000 or (c) a solid dispersion (SD) with PEG produced by co-dissolution in ethanol. Characterisation of SD by differential scanning calorimetry and attenuated total reflectance–Fourier transform infrared spectroscopy suggested transformation of the crystalline structure of NVP to an amorphous form which consequently increased the dissolution rate of drug. A low-loading efficiency of 13% was obtained for NVP-loaded matrices and less than 20% for matrices prepared using physical mixtures of drug and PEG. The loading efficiency was improved significantly to around 40% when a 1:4 NVP–PEG SD was used for matrix production. After 30 days, 40% of the drug content was released from NVP-loaded matrices, 55% from matrices containing 1:4 NVP–PEG physical mixtures and 60% from matrices loaded with 1:4 NVP–PEG SDs. The *in vitro* anti-viral activity of released NVP was assessed using a luciferase reporter gene assay following the infection of HeLa cells with pseudo-typed HIV-1. NVP released from PCL matrices in simulated vaginal fluid retained over 75% anti-HIV activity compared with the non-formulated NVP control. In conclusion, 1:4 NVP–PEG SDs when loaded in PCL matrices increase drug loading efficiency and improve release behaviour. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2107–2115, 2014
Keywords: nevirapine; solid dispersion; microbicides; polycaprolactone matrix; vaginal delivery; biomaterial; drug delivery systems; controlled release

INTRODUCTION

The global AIDS epidemic remains one of the world's most serious health challenges with 35.3 million (32.2–38.8 million) people living with HIV worldwide at the end of 2012.[1] However, the health burden varies considerably between countries and regions. Sub-Saharan Africa, for example, remains the most severely affected region, with nearly one in 20 adults infected with HIV and accounting for around 70% of the global incidence. Most HIV infections occur through heterosexual intercourse and young women between the ages of 15 and 24 are at least three times more likely to be HIV-positive than men.[2] The scale of the AIDS epidemic, the absence of a vaccine and the major gender imbalance in infection rate has heightened awareness of the need for more effective preventative measures that reduce virus transmission and are controlled by women independently of a sexual partner. As a consequence, research on vaginal delivery of microbicides that kill or inactivate viruses at the mucosal barrier has been receiving increasing attention in recent years.[3,4] Early work employed non-specific microbicides, including anionic polymers such as cellulose acetate phthalate, and carbohydrate-binding polymers such as carrageenan that bind to viral envelope proteins (gp120 and gp141 in HIV) and inhibit virus entry into host target cells ($CD4^+$-T cells, macrophages and dendritic cells) in the genital sub-mucosal region. However, the ability of these "first generation" microbicides to prevent HIV transmission has not been clinically proven.[5] Several antiretroviral drugs, including the nucleoside reverse transcriptase inhibitor (NRTI), tenofovir and the non-nucleotide reverse transcriptase inhibitor (NNRTI), dapivirine, are currently being evaluated as "specific" vaginal micro-bicides.[6] Tenofovir, formulated in a 1% hydroxyethylcellulose vaginal gel, was reported to be modestly effective in a Phase IIb trial (CAPRISA-004) when applied in a pre-exposure prophylaxis study.[7] However, tenofovir gel was found to perform no better than placebo in the subsequent, larger-scale Phase IIb VOICE trial.[8] The ineffectiveness of the formulation was ascribed to poor adherence of the trial participants to the dosing regimen.[9] The negative trial outcomes tend to highlight the major disadvantages of semi-solid vaginal formulations; they are inherently "messy" to apply, they tend to leak, and concerns exist over ineffective coverage of the epithelium. In addition, such formulations often need to be applied prior to sexual intercourse to reduce the risk of infection and this can entail use of an application device. As a consequence, intra-vaginal ring (IVR) devices are being investigated intensively for the delivery of anti-virals[10–12] to improve user compliance and to maintain therapeutic concentrations of the drug in vaginal fluid over long time periods. Conventional silicone elastomer or thermoplastic poly(ethylene vinyl acetate) IVRs are normally confined to the delivery of low molecular weight; hydrophobic compounds such as dapivirine and IVR manufacture involves the curing of silicone elastomer

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at 80°C or hot-melt extrusion of poly(ethylene vinyl acetate) at 110°C, which may degrade heat-sensitive bioactives.

We have previously shown the potential utility of microporous polycaprolactone (PCL) matrices for vaginal delivery of hydrophilic antibacterial (ciprofloxacin) and hydrophobic anti-fungal agents (miconazole) in the treatment of gonorrhea and candida.[13] More recently, we incorporated the hydrophilic NRTI, tenofovir, in PCL matrices and achieved gradual release over 30 days in simulated vaginal fluid (SVF) with over 70% retention of anti-viral activity against pseudo-typed HIV-1 viruses.[14] In this study, we describe the development of PCL matrices incorporating a hydrophobic NNRTI, nevirapine (NVP), as part of an on-going evaluation of the materials for prevention and treatment of sexually transmitted infections. NVP is an attractive option as an anti-retroviral drug because it is inexpensive and is widely used in resource-poor areas for treating both HIV and preventing mother-to-child transmission. However, low-water solubility of NVP (0.7046 mg/L[15]) can hinder drug dissolution and thus release into vaginal fluid from an IVR. In addition, it has been pointed out in our previous study[13] that the use of a drug with high solubility in organic solvents, such as NVP, could result in a low loading efficiency in the matrices because of elution into methanol during the solvent extraction stage of matrix production. Polyethylene glycol (PEG) 6000 exhibits limited solubility in methanol[16] but can act as a hydrophilic carrier for NVP in a solid dispersion (SD) to improve dissolution rate of drug in aqueous environment. Thus, physical mixtures and SDs of PEG6000 and NVP were incorporated in the matrices with the aim of improving drug loading and release properties.

MATERIALS AND METHODS

Materials

Polycaprolactone (MW 115,000 Da, Capa 650) was obtained from Solvay Interox (Warrington, UK). NVP (MW 267 g/mol) was supplied by Huahai Pharmaceutical Company (Zhejiang, China). PEG6000 was obtained from BDH Laboratory Supplies (Leicestershire, UK). Sodium chloride, potassium hydroxide, calcium chloride, bovine serum albumin, glucose, glycerol, urea, lactic acid and acetic acid were purchased from Sigma–Aldrich (New South Wales, Australia). All solvents [acetone, methanol, ethanol and dichloromethane (DCM)] were of analytical grade and obtained from Sigma–Aldrich. SVF (pH 4.2) was prepared following the method of Owen and Katz[17] and consisted of 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 in 1 L of distilled water.

Preparation of SDs of NVP

Solid dispersions of NVP–PEG6000 in weight ratios of 1:1; 1:2 and 1:4 (labelled as SD1:1, SD1:2 and SD1:4, respectively) were prepared by co-dissolution in ethanol. The appropriate amount of PEG6000 (1, 2 or 4 g) was added to a solution of NVP (1 g) in ethanol (50 mL), the solvent was removed under reduced pressure at 40°C and the NVP–PEG dispersion was dried under vacuum at room temperature for 5 h. The samples were ground using a mortar and pestle and 0.05–0.25 mm particle size fractions were obtained by sieving. Physical mixtures of NVP and PEG6000 were prepared by grinding process followed by manually mixing appropriate amounts of the 0.05–0.25 mm particle size fractions of each powder to obtain NVP–PEG6000 ratios of 1:1, 1:2 and 1:4 (labelled as PM1:1, PM1:2 and PM1:4, respectively).

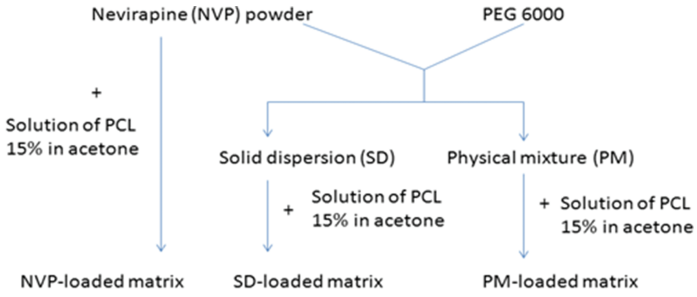

Figure 1. A diagram of matrix production.

Preparation of NVP-loaded PCL Matrices

A PCL solution (15%, w/v) was prepared by dissolving the polymer in acetone at 50°C. NVP powder or NVP in the form of a SD or a physical mixture with PEG6000 was then dispersed in the PCL solution to produce theoretical drug loadings equivalent to 10% (w/w) of the PCL content. The resulting suspensions were poured into a polypropylene syringe body (3 mL) and rapidly cooled in ethanol at −80°C for 2 h to allow crystallisation of PCL. Following the hardening process, the matrices were removed from the moulds and immersed in methanol for 12 h to extract the residual acetone. Solvents (acetone and methanol) remaining in the matrices were removed by evaporation under ambient conditions for 24 h. A schematic diagram illustrating matrix production is presented in Figure 1.

Thermal Analysis

Thermal analysis of NVP and PEG6000 in isolation, as physical mixtures, or as SDs, was performed using differential scanning calorimetry (DSC) (Mettler Toledo DSC1 Star System; Mettler-Toledo Ltd., Victoria, Australia). Samples (5–10 mg) were heated in hermetically sealed aluminium pans at a heating rate of 10°C/min from −80°C to 300°C under a nitrogen atmosphere. Thermal analysis of PCL matrices loaded with NVP or physical mixtures of NVP and PEG6000 or SDs of NVP and PEG6000, respectively, was also carried out under the same conditions.

Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy

A Nicolet Fourier Transform Infrared Spectroscopy (FTIR) Spectrometer (Thermo Scientific, Brisbane, Australia) with Diamond Attenuated Total Reflectance (ATR) was employed for investigations of NVP and PEG6000 in isolation, as physical mixtures and as SDs. Spectra were obtained at a resolution of 4 cm^{-1} in the 4000—5000 cm^{-1} range.

Determination of the NVP Content of PCL Matrices by HPLC Analysis

Sections (∼100 mg) were cut from matrix samples, weighed and dissolved in DCM (2 mL). Precipitation of PCL was induced by adding 5 mL of methanol, followed by vortex mixing (Vibrax VXR; IKA, Werke Staufen, Germany) at 1000 rpm overnight to allow DCM to evaporate and the drug content to partition into the methanol phase. The residue was washed twice with

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methanol (10 mL) to extract the residual drug and the washings were combined. NVP concentrations in methanol were assayed using an Agilent 1200 HPLC system (Agilent Technologies, Victoria, Australia) equipped with a binary pump and photodiode array detector. Separation was carried out using an Eclipse XDB-C18 (150 × 4.6 mm^2 ID, 5 μm) column (Agilent Technologies). The solvent system comprised 0.1% (v/v) trifluoroacetic acid in water (eluent A) and acetonitrile (eluent B). A gradient elution programme was performed as follows: initial condition, 95% eluent A and 5% eluent B for 3 min (flow rate 1 mL/min), followed by 70% eluent A and 30% eluent B for 3.5 min (flow rate 1.5 mL/min). The column was then returned to the initial condition and equilibrated for 5.5 min. The limit of quantification of NVP was 0.44 μg/mL and the concentration of standard solutions ranged from 0.44 to 220 μg/mL with R^2 value of 0.9999. The sample injection volume was 10 μL and detection of NVP was performed at 280 nm. The NVP concentration was determined by reference to standard curves. The measured (actual) drug loading (w/w) of the matrices was compared with the theoretical loading to provide a measure of loading efficiency (%). Experiments were conducted using triplicate samples.

In Vitro Release of NVP from PCL Matrices

Cylindrical samples of NVP-loaded PCL matrices (~6.7 mm diameter, 15 mm in length, 0.15 g in weight), taken from the central section of the moulding, were subjected to a drug release study. Both ends of the samples were coated with a thin film of PCL by dipping in a 5% (w/v) solution of PCL in acetone followed by drying in air at room temperature. The release study was performed on triplicate samples. Individual samples were placed in 10 mL SVF, incubated at 37°C and shaken at 300 rpm using a Heidolph Titramax Inkubator 1000 (Heidolph Instruments GmbH and Company KG, Schwabach, Germany). The release media were collected and replaced with fresh SVF every day for a period of 30 days. The drug concentration in the release media was analysed using HPLC as described above. Cumulative drug release (%) is calculated with respect to the actual (real) drug content of the matrices. Then drug release behaviour was expressed as cumulative release (%) versus time. Separate release samples were stored at 0°C prior to testing the anti-HIV activity of released drug.

Morphology of PCL Matrices

A JEOL JSM-6610LV scanning electron microscope (SEM) (Jeol Ltd., Tokyo, Japan) was used to examine the morphology of the surface and interior of drug-free PCL matrices and PCL matrices loaded with NVP–PEG SDs or NVP–PEG physical mixtures before and after drug release. Specimens were mounted on SEM stubs using carbon tabs and sputter coated with platinum using an Eiko–Sputter coater automatic mounting press (Eiko Ltd., Tokyo, Japan) prior to examination in the SEM at a voltage of 15 keV.

Anti-HIV Activity Testing

Anti-HIV activity of NVP released from PCL matrices were assayed using luciferase-expressing, VSV-G pseudo-typed HIV-1 as previously described.[14] Briefly, HeLa cells (ATCC number CCL-2) were cultured in RPMI 1640 (Invitrogen Corporation, Melbourne, Australia) supplemented with 10% fetal bovine serum (Life Technologies Australia Pty Ltd., Victoria, Australia), 100 mg/mL streptomycin and 100 U/mL penicillin at 37°C and 5% CO_2. HeLa cells (10^5 cells/mL) were seeded in 24-well plates to achieve confluence above 80% after 24 h. Aliquots (100 μL) of the SVF release medium collected at day 1, 7, 14 and 23 which had contained drug-free matrices or NVP-loaded matrices were filtered through a 0.22-μm membrane and fivefold dilutions were added to each well. HIV (10 ng of CAp24 equivalent per well) and hexadimethrine bromide (0.8 μg/mL, for enhancing HIV infection) were added. At day 1, post-infection, the culture was harvested, the cells were lysed in 100 μL Glo Lysis Buffer (Promega Corporation, New South Wales, Australia) and the amount of luciferase activity [expressed in relative light unit (RLU)] in 20 μL of each lysate was determined using the Steady-Glo Luciferase Assay System (Promega Corporation) and luminescence-compatible spectrophotometer (Synergy H4 Hybrid Reader; Biotek, Victoria, Australia).

The HIV used in the assay has a luciferase gene inserted into the *nef* open reading frame. If a target cell is successfully infected, luciferase activity expressed by the luciferase gene is an indicator of HIV infection. Thus, the percentage reduction in luciferase activity correlating with the anti-viral activity of NVP was determined using Eq. (1):

$$\%\,\text{Reduction RLU} = \left(1 - \frac{\text{RLU}_{\text{test}} - \text{RLU}_{\text{cell}}}{\text{RLU}_{\text{virus}} - \text{RLU}_{\text{cell}}}\right) \times 100 \quad (1)$$

RLU_{test}: RLU of wells containing infected HeLa cells and NVP released in SVF

RLU_{virus}: RLU of control wells containing infected HeLa cells and drug-free SVF

RLU_{cell}: RLU of control wells containing uninfected HeLa cells

For the analysis of test results, a sigmoidal dose-response model was fitted using non-linear regression analysis (GraphPad Prism 6) to the plot of percentage reduction of RLU versus the log-transformed concentration data (X).[18] On the basis of this model, IC_{50} values, which represent the concentration at which the anti-viral agent reduces HIV infection to 50% of the control value, were calculated. Non-formulated NVP, NVP in SDs or physical mixtures and each release sample were prepared in five diluted concentrations to obtain their IC_{50} values. Each concentration of NVP was tested in duplicate. Relative activity, defined as the ratio of the IC_{50} value for non-formulated NVP to the IC_{50} of the NVP-formulated samples, was used to compare the activity amongst test samples.

$$\text{Relative activity}\,(\%) = \frac{\text{IC}_{50}\,(\text{NVP in non} - \text{formulated samples})}{\text{IC}_{50}\,(\text{NVP in formulated samples})}$$

RESULTS AND DISCUSSION

Characterisation of NVP SDs

Solid dispersions composed of a drug incorporated in a hydrophilic carrier or matrix are frequently employed to improve the rate of dissolution of the drug entity in aqueous environments. PEG6000 was selected as the hydrophilic carrier for NVP in a SD because it is one of the most frequently investigated hydrophilic polymeric carriers and was expected to form a dispersed water soluble phase within the PCL matrix to assist the uptake of SVF, dissolution of NVP and drug transport from the PCL matrix. PEG also exhibits limited solubility in methanol and was therefore expected to reduce loss of NVP

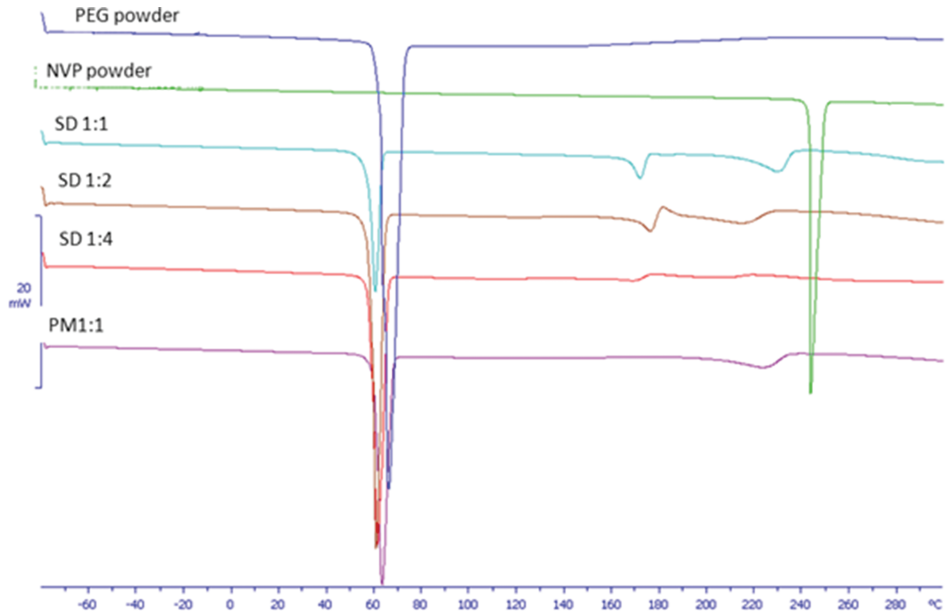

Figure 2. Differential scanning calorimetry thermogram of PEG6000 powder, NVP powder, SDs of NVP and PEG6000 at ratios of 1:1, 1:2 and 1:4 (SD1:1, SD1:2 and SD1:4, respectively), and physical mixture of NVP and PEG6000 at a ratio of 1:1 (PM1:1).

from the matrices during the solvent-extraction stage of matrix production which utilises methanol.

Thermal Analysis

Samples of NVP, PEG6000, SDs and corresponding physical mixtures of the drug with PEG6000 were analysed by DSC (Fig. 2) to investigate whether NVP was incorporated in amorphous or crystalline form. The melting point (T_m) of NVP and PEG6000 in isolation was measured at 243.6°C and 66.2°C, respectively, corresponding to literature values of 244°C.[19] and 62.5°C.[20] All physical mixtures of NVP and PEG6000 at the various drug–PEG ratios investigated (1:4, 1:2 and 1:1) displayed the T_m of the PEG carrier and the drug corresponding to separate crystalline phases of each component. In contrast, SDs of NVP–PEG6000 at the ratio 1:4 only showed the melting peak of PEG6000, indicating that NVP was present in the amorphous form. When the ratio of NVP in SDs increased to 1:2, a small additional peak was observed at 181.6°C and the melting point of NVP was lowered to 216°C. This additional peak became more prominent and separated from the melting peak of non-formulated NVP (243.6°C) when the NVP–PEG6000 ratio was changed to 1:1. These results indicate that in 1:4 drug–PEG SDs, NVP was partially molecularly mixed with PEG6000 and partially distributed as amorphous clusters. However, in SDs containing higher amounts of NVP (1:1 and 1:2 drug–PEG), the PEG chains are inadequate to fully suppress crystallisation of the total drug content but disrupt crystal formation, resulting in a reduction of melting point.

ATR–FTIR Spectroscopy

Fourier transform infrared spectra of physical mixtures and SDs were recorded (Fig. 3) to investigate the possible intermolecular interactions between NVP and PEG6000. The FTIR spectrum obtained for NVP reveals characteristic bands at 1643 and 1585 cm^{-1} (C=O stretching) and 3185 and 3064 cm^{-1} (N–H stretching). A characteristic band at 2881 cm^{-1}, corresponding to CH_2 stretching, was observed in the spectrum of PEG6000. FTIR spectra of the physical mixtures were effectively a combination of the individual NVP and PEG6000 spectra. This indicates that no interaction was occurring between the drug and the polymer. Similar data were obtained in the spectra of SDs of NVP and PEG6000 at ratios of 1:1 and 1:2. However, the FTIR spectra of the 1:4 SD did not show the characteristics of NVP, only peaks representative of PEG6000 without positional changes. The above observations are in agreement with DSC thermograms of 1:4 SDs which showed an absence of the melting peak corresponding to crystalline NVP.

Morphology

Morphology of Particles

Irregular-shaped crystals of NVP with dimensions of 5–50 μm were observed in SEM images of the drug powder (Fig. 4a), whereas PEG6000 particulates were larger, in the 20–200 μm size range (Fig. 4b). Physical mixtures of NVP and PEG6000 (Fig. 4c) comprised crystalline aggregates of size 10–50 μm because of the grinding process. In contrast, SDs were observed in the form of uniform-sized particles with lamellar morphology (Fig. 4d) and evidence of particle aggregation, which was not observed for the drug and PEG particulates alone.

Morphology of PCL Matrices

Rapid cooling of PCL solutions in acetone-containing dispersed particles of NVP, physical mixtures or SDs with PEG6000 resulted in uniform, flexible mouldings, approximately 6.7 mm in diameter on drying, which were free of cracks and voids in the surface and interior. Figure 4 illustrates the irregular porous morphology of matrices formed by crystallisation and hardening of the PCL phase. The surface of drug-free matrices (Fig. 5a) and drug-loaded matrices (Figs. 5b–5d) generally exhibited a nodular morphology with pore dimensions of 3–5 μm (drug-free matrices) and 5–10 μm (drug-loaded matrices).

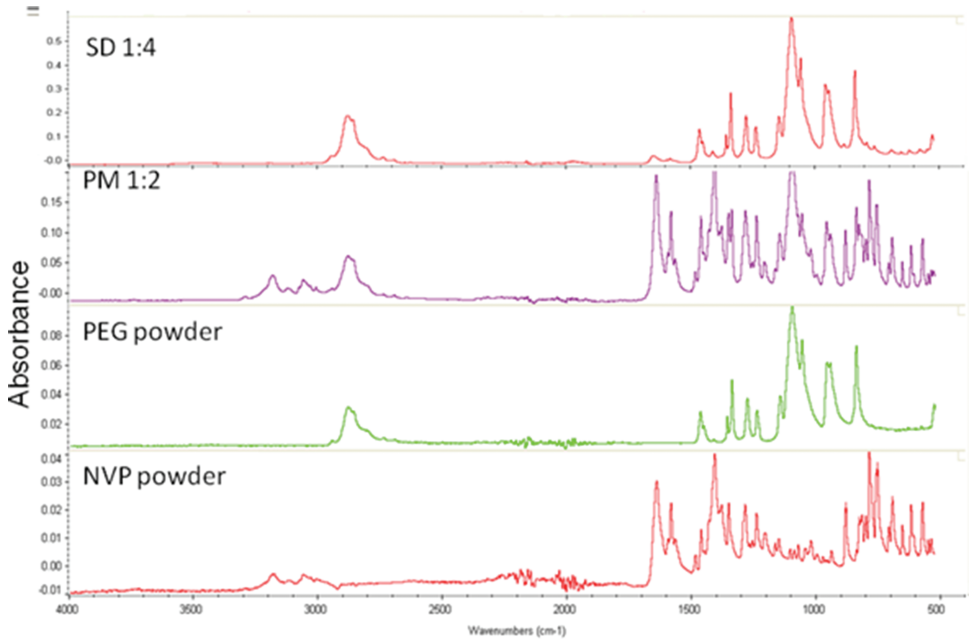

Figure 3. Fourier transform infrared spectra of PEG6000, NVP, SDs of NVP and PEG6000 at a ratio of 1:4 (SD1:4), and physical mixtures of NVP and PEG6000 at a ratio of 1:2 (PM1:2).

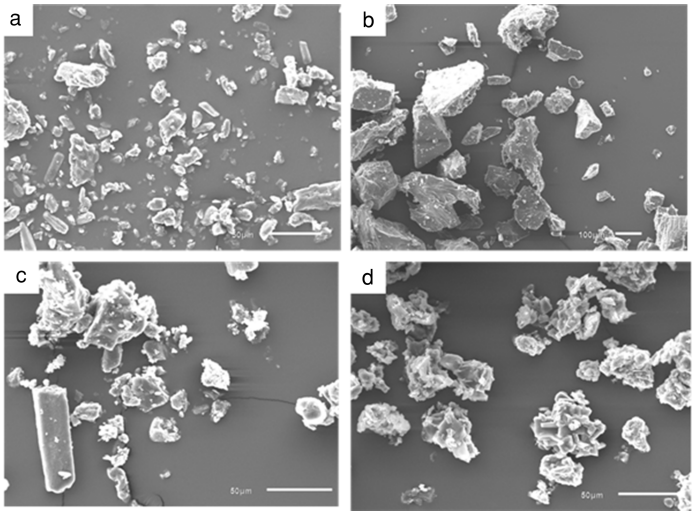

Figure 4. Scanning electron micrographs of (a) NVP powder, (b) PEG6000 powder, (c) physical mixture of NVP and PEG6000 at a ratio of 1:4 and (d) SD of NVP and PEG6000 at a ratio 1:4.

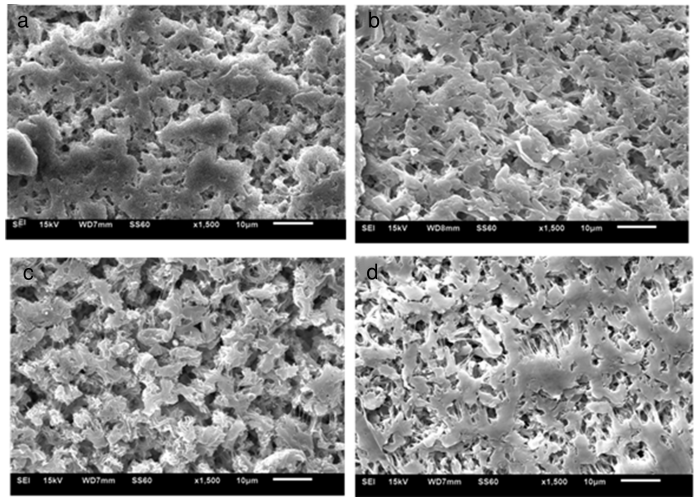

Figure 5. Scanning electron micrographs of the surface of: (a) drug-free PCL matrix, (b) NVP-loaded matrix, (c) PCL matrix loaded with a SD of NVP and PEG at a ratio of 1:4 and (d) PCL matrix loaded with a physical mixture of NVP and PEG at a ratio of 1:4.

Drug Loading of PCL Matrices

The loading efficiency of NVP in the PCL matrices is displayed in Figure 6. All matrices were produced using 15% PCL solution with theoretical drug loading of 10% (w/w). The use of drug powder suspensions to produce NVP-loaded matrices resulted in low drug loading of 1.3% (w/w) and loading efficiency of 13%. This behaviour is explained by the high solubility of NVP in methanol (939 mg/mL)[15] which is used for extraction of acetone from the matrices. The use of physical mixtures of NVP and PEG6000 improved drug loading efficiency to a level of around 22%. However, a doubling of loading efficiency to 44% was achieved when matrices were prepared using 1:4 SDs of NVP and PEG6000. These improvements may be explained by the low solubility of PEG6000 in methanol.[16] In the case of physical mixtures, the presence of PEG in the matrix is expected to impede drug transport through the micro-porous PCL phase during the solvent extraction stage of matrix production. The SDs are expected to present a further barrier to drug transport by a micro-encapsulation effect.

In Vitro Release Behaviour

The release profiles of NVP from PCL matrices in SVF at 37°C are presented in Figure 7. Over the first 24 h, the 1.3% (w/w) NVP-loaded matrices released the lowest percentage of drug

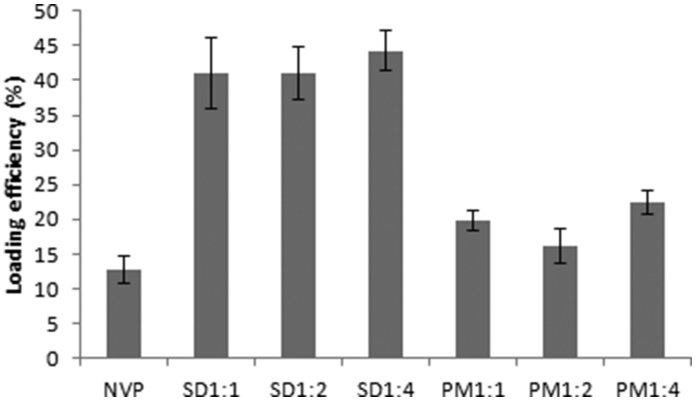

Figure 6. Loading efficiency (%) of NVP in PCL matrices (NVP: matrices loaded with NVP only; SD1:1, SD1:2 and SD1:4: matrices loaded with SDs of NVP and PEG6000 at ratios of 1:1, 1:2 and 1:4, respectively; PM1:1, PM1:2 and PM1:4: matrices loaded with physical mixtures of NVP and PEG6000 at ratios of 1:1, 1:2 and 1:4, respectively).

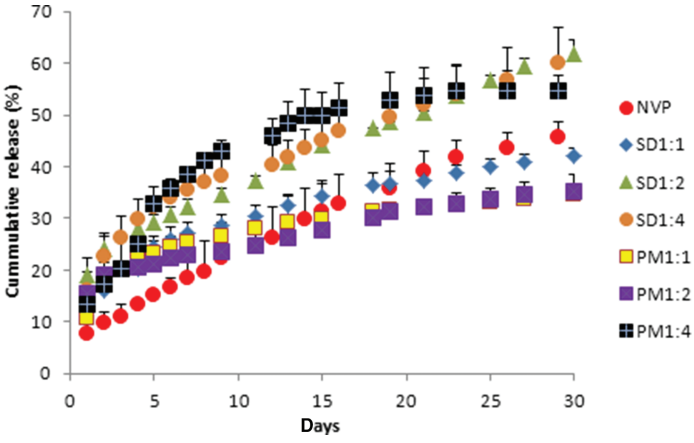

Figure 7. Release profile of NVP from PCL matrices into SVF at 37°C (NVP: matrices loaded with NVP only; SD1:1, SD1:2 and SD1:4: matrices loaded with SDs of NVP and PEG6000 at ratios of 1:1, 1:2 and 1:4, respectively; PM1:1, PM1:2 and PM1:4: matrices loaded with physical mixtures of NVP and PEG6000 at ratios of 1:1, 1:2 and 1:4, respectively).

(7.6%), followed by gradual release of approximately 45% of the drug load over a 30-day period. When 1:1 and 1:2 physical mixtures of NVP and PEG6000 were incorporated in the matrices, (corresponding to 2% and 1.6% drug loading) only around 35% of the initial drug content was released from the matrices during the 30-day release period, suggesting that the PEG phase is impeding drug availability, possibly because of the formation of a high-viscosity fluid within the pore structure. The use of 2.2% NVP-loaded matrices, produced using higher quantities of PEG in 1:4 physical mixtures, resulted in the release of 54% of the drug load (Fig. 7). This behaviour may arise from an increase in matrix porosity as a consequence of the higher PEG phase content (26.7% of the matrix structure). When NVP was loaded in the form of a 1:2 and 1:4 SD with PEG6000, around 60% of the drug content was gradually released over 30 days. The lower release (42% of drug content after 30 days) measured for 1:1 SDs may reflect both the reduced PEG content of the matrix and a higher crystalline content of the drug phase (detected by DSC analysis; Fig. 2) which reduces drug dissolution rate.

The kinetics of drug release from matrix-type devices containing dispersed drug particles, where release occurs through fluid-filled pores in the matrix, is frequently controlled by Fickian diffusion and often interpreted using the simplified Higuchi equation[21]:

$$M = K_d t^{0.5}$$

where M is the amount of drug released in time (t) and K_d is a release rate constant.

A dependency of drug release is found on the square root of time, along with a decrease in drug release rate over time, because of the drug depletion in the matrix and a corresponding increase in the diffusion path length. Analysis of the release data in Figure 7 suggested that the release kinetics of PCL matrices loaded with NVP alone or SDs of NVP and PEG6000 can be described effectively by the Higuchi square root of time model ($R^2 > 0.98$; Table 1). Only the release kinetics for matrices loaded with 1:2 physical mixtures of NVP and PEG is poorly defined by this model ($R^2 = 0.912$).

The Korsmeyer–Peppas model was applied in a further attempt to correlate the release data with underlying controlling factors and to identify release kinetics arising from multiple phenomena:

$$M_t/M_\infty = kt^n$$

where M_t is the amount of drug released at time t, M_∞ is the total amount of drug in the matrices, k is a kinetic constant characteristic of the drug/polymer system and n is an exponent which characterises the mechanism of drug release.[21] Drug delivery from a cylindrical matrix is considered to be governed by Fickian diffusion when $n = 0.45$, whereas relaxation behaviour (zero-order release) is suggested when $n = 1$. Values of n between 0.5 and 1 indicate anomalous (non-Fickian) kinetics. The exponent values (0.31–0.57) obtained using the Korsmeyer–Peppas model were close to 0.45 for all matrices, providing further support for a diffusion-controlled release mechanism in the systems investigated.

The Korsmeyer–Peppas relationship was applied to calculate the diffusion coefficient (D) of NVP in PCL matrices for those systems where diffusion-controlled release behaviour was indicated:

$$\frac{M_t}{M_\infty} = \frac{2\sqrt{D_t}}{\delta}$$

where M_t/M_∞ is the fractional amount of drug released at time (t) and δ is the radius of the cylindrical PCL matrix.[22]

Similar values of diffusion coefficient (D) were calculated for NVP in PCL matrices (6.7×10^{-9} cm^2/s) and for matrices incorporating 1:4 physical mixtures of NVP and PEG6000 (7.1×10^{-9} cm^2/s) (Table 2). The diffusion coefficient decreased by around 50% to 3.2–3.6 $\times 10^{-9}$ cm^2/s when SDs were used to prepare the matrices. The amount of drug released in time (t) from a matrix system containing dispersed drug particles according to the Higuchi equation[21] is influenced by the initial drug concentration (C), the drug solubility in the release medium (C_s), the diffusion coefficient of drug molecules in the release medium (D), the tortuosity of the pore system (τ) and

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Table 1. Modelling of NVP Release from PCL Matrices in SVF at 37°C

		Zero-Order Kinetics		Higuchi Model		Korsmeyer–Peppas		
	Drug Loading (%, w/w)	k_0	R^2	k_d	R^2	n	k	R^2
NVP	1.3	0.05	0.98	0.334	0.99	0.567	0.125	0.983
SD1:1	4.1	0.0329	0.91	0.221	0.982	0.352	0.31	0.992
SD1:2	4.1	0.056	0.98	0.318	0.988	0.34	0.286	0.971
SD1:4	4.4	0.075	0.94	0.306	0.990	0.358	0.251	0.988
PM1:1	2.0	0.02	0.80	0.503	0.99	0.306	0.386	0.919
PM1:2	1.6	0.0239	0.91	0.165	0.912	0.421	0.241	0.958
PM1:4	2.2	0.042	0.78	0.155	0.985	0.494	0.25	0.968

Zero order: $M_t = k_0 t$; k_0, zero-order release constant (mg/day); M_t, amount of drug released at time t.
Higuchi: $M_t = K_d t^{0.5}$; K_d, release rate constant (mg/day$^{0.5}$).
Korsmeyer–Peppas: $M_t/M_\infty = kt^n$; M_t, the amount of drug release at time t; M_∞, the total drug released over a long time period; k, kinetic constant; n, release exponent.

Table 2. Diffusion Coefficient (D) of NVP in PCL Matrices Prepared Using NVP Powder, Physical Mixtures or SD of NVP and PEG6000 at (w/w) Ratios 1:1, 1:2 and 1:4

	Drug Loading (%)	D ($\times 10^{-9}$ cm^2/s)
NVP	1.3	6.67
SD1:1	4.1	3.31
SD1:2	4.1	3.19
SD1:4	4.4	3.61
PM1:1	2.0	4.69
PM1:2	1.6	4.73
PM1:4	2.2	7.05

matrix porosity (ε).

$$Q = \frac{\sqrt{tDC_s\varepsilon(2C - \varepsilon C_s)}}{\tau}$$

Thus, the expected increase in the porosity of matrices containing SDs of NVP and PEG combined with the higher drug loading and anticipated improvement in NVP dissolution rate resulting from decreased drug crystallinity may compensate for the reduction in drug diffusion coefficient in the matrix resulting from the formation of a viscous PEG environment (about 100 cP at 0.5% concentration). As a result, the release process for NVP is gradual and prolonged.

The highest efficiency of NVP delivery (expressed as percent cumulative release) was obtained when PCL matrices were prepared using 1:4 drug–PEG SDs, as drug release tended to increase over time rather than plateau as was observed for physical mixtures (Fig. 7). Therefore, release media obtained using PCL matrices loaded with 1:4 NVP–PEG SDs were selected to test the anti-HIV activity of released NVP in comparison with matrices loaded with 1:4 NVP–PEG physical mixtures and matrices loaded with NVP alone.

Anti-HIV Activity of NVP Released from PCL Matrices

The luciferase gene reporter assay using pseudo-virus has been demonstrated to be a rapid assay with high reproducibility and sensitivity.[23] Therefore, the use of the luciferase gene reporter assay conveyed by HIV-1 pseudo-virus has become routine in screening for inhibitory activity of anti-viral agents. The VSV-G envelope glycoprotein improves the stability and infectivity of the virus population, enabling greater ease of use and sensitivity of the assay.[24] Figure 8 shows the inhibitory activity of NVP

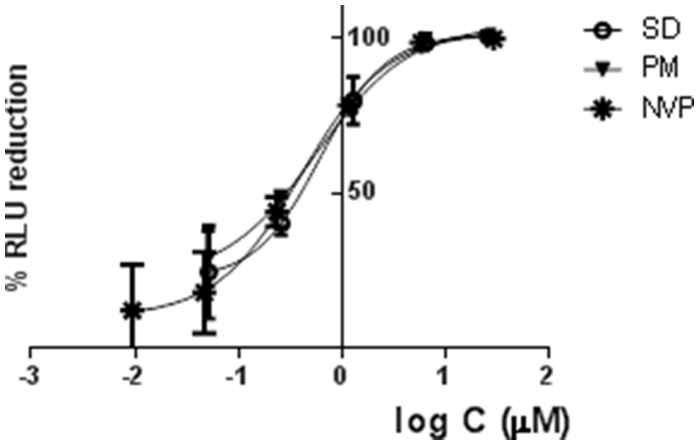

Figure 8. Inhibition of infectious HIV-1 activity (expressed by percent RLU reduction) by solutions (expressed by logarithms of concentration of NVP) of non-formulated NVP, or SD of NVP and PEG6000 at a ratio of 1:4 or PM of NVP and PEG6000 at ratio 1:4.

standard prepared in SVF; a log concentration-response model being subsequently fitted to the data ($R^2 = 0.9998$). An IC_{50} value (the concentration of NVP required for 50% inhibition of virus infectivity) of 40.6 nM was obtained which is similar to reported IC_{50} values for wild-type HIV-1 ranging from 15 to 40 nM.[25]

The IC_{50} value of NVP in SVF was subsequently used as a reference for comparing the activity of release samples obtained from PCL matrices loaded with NVP powder, 1:4 SDs or 1:4 physical mixtures of NVP and PEG6000 after 1, 7, 14, and 23 days immersion in SVF at 37°C. The comparison was then expressed as relative anti-viral activity.

When NVP was prepared as a SD or a physical mixture, the IC_{50} values were increased slightly to 42.8 and 41.2 nM, respectively. NVP in a SD or physical mixture with PEG retained activities of 95% and 99%, respectively, and the presence of PEG6000 did not contribute to the anti-HIV activity of the samples. NVP released from PCL matrices loaded with drug powder into SVF at 37°C exhibited relative anti-viral activities ranging from 75% to 95% (Fig. 9). Release samples obtained from PCL matrices loaded with SDs retained high relative activity from 88% to 94%. Meanwhile, the activity of NVP released from PCL matrices-loaded with physical mixtures of NVP and PEG6000 was almost the same as the NVP standard, with relative activity ranging from 92% to 96%. The anti-viral activity of NVP

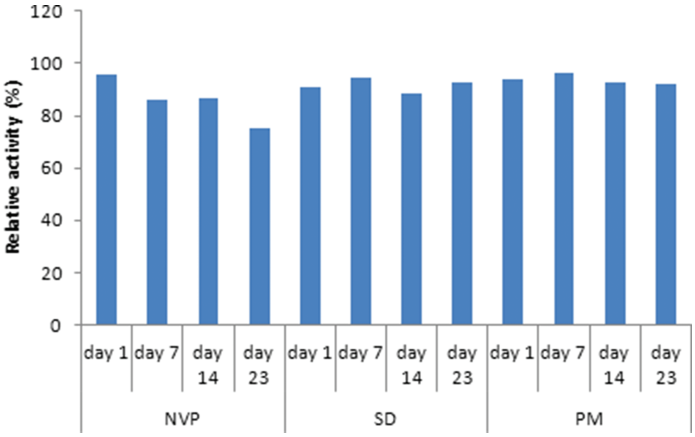

Figure 9. Relative activity of NVP released from PCL matrices loaded with NVP powder, or with SDs of NVP and PEG6000 at a ratio of 1:4 (SD), or with physical mixtures of NVP and PEG6000 at a ratio of 1:4 (PM) after 1-, 7-, 14- and 23-days immersion in SVF at 37°C.

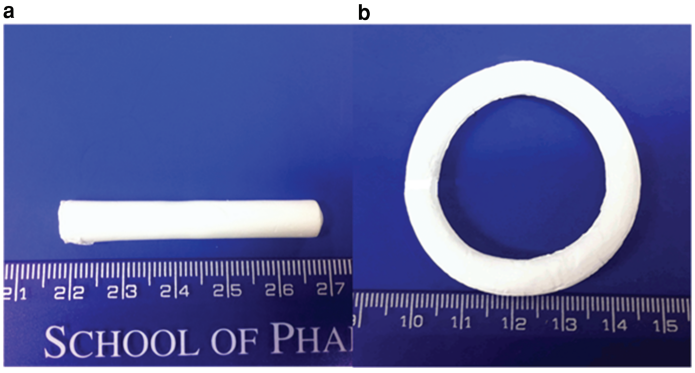

Figure 10. (a) Polycaprolactone matrix and (b) Polycaprolatone IVR produced using the rapid cooling technique.

released from PCL matrices loaded with SD or physical mixtures was decreased slightly (3%–7%) compared with NVP in a corresponding SD or physical mixture. Neither the loading of NVP in the matrices in the forms of SD or physical mixture nor the release time had a significant effect on relative anti-viral activity. In addition, SVF and PEG solution prepared in SVF and release medium, which had contained drug-free matrices, did not give rise to anti-HIV activity (data not shown). Thus, NVP may be loaded into PCL matrices and released in SVF over 23 days with significant effect on anti-viral activity, which renders the material a promising candidate for application as IVRs for prevention of heterosexual transmission of HIV.

In addition to the high levels of *in vitro* anti-viral activity shown above, the predicted concentrations of NVP, which would be released from a PCL IVR into vaginal fluid, exceeded the reported IC_{50} value for HIV-1. This assessment is based on the linear dimensions of an IVR (Fig. 10) of 150 mm (outer diameter 58 mm, inner diameter 38 mm) and weight (1.5 g) being 10-fold greater than that of the studied matrices (15 mm, 0.15 g) reported here. The minimum release amount of 10 μg NVP/day (Fig. 11) corresponds to a drug release rate from a PCL IVR in excess of 100 μg/day. Assuming 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,[17] then the expected minimum concentration of

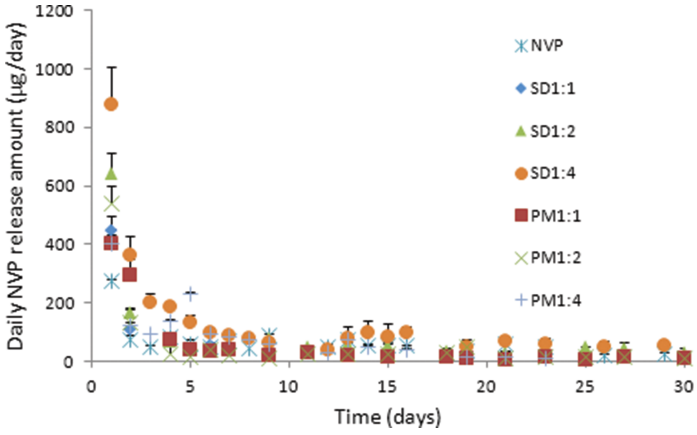

Figure 11. Amount of NVP release (μg/day) from PCL matrices loaded with NVP only, or with solid dispersions of NVP and PEG6000 at ratios of 1:1, 1:2 and 1:4 (SD1:1, SD1:2 and SD1:4, respectively), or with physical mixtures of NVP and PEG6000 at ratios of 1:1, 1:2 and 1:4 (PM1:1, PM1:2 and PM1:4).

NVP in vaginal fluid is approximately 12.5 μg/mL or 44 μM. These estimates do not take account of the complex changes in vaginal fluid volume and biochemical composition over time or the possibility of systemic uptake of drugs. However, the released drug concentrations are much higher (by a factor of 1000) than the reported IC_{50} of NVP against HIV-1 (40 nM).

CONCLUSION

The use of SDs of NVP in PEG6000 improved the drug loading efficiency of PCL matrices to 44% compared with 13% when matrices were prepared using drug powder. Gradual NVP release from PCL matrices over 30 days in SVF followed the Higuchi model and provided estimates for the diffusion coefficient (D) of 3.2–7.1 $\times$ 10^{-9} cm/s. Relative anti-viral activity in excess of 75% against pseudo-typed HIV-1 was retained by released NVP. The predicted *in vivo* release concentrations from a PCL IVR in vaginal fluid are higher than the IC_{50} against HIV-1 by a factor of more than 1000. These findings demonstrate the potential of PCL matrices loaded with SDs of NVP and PEG6000 for providing intra-vaginal devices for the control of heterosexual HIV transmission.

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