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Improvement of Tenofovir vaginal release from hydrophilic matrices through drug granulation with hydrophobic polymers

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ABSTRACT

Sustained-release vaginal microbicides hold out great hope for the prevention of sexual transmission of HIV from men to women. Tenofovir (TFV) –an antiretroviral drug– sustained-release vaginal compacts combining two release control systems (by drug-loading granules with hydrophobic polymers and incorporating them in a hydrophilic matrix) are proposed in this work as a possible microbicide. The polymers used for the drug granules are Eudragit® RS (ERS), an acrylic derivative, and Zein, a maize protein. The hydrophilic matrix is composed of a mixture of hydroxypropylmethyl cellulose (HPMC) and chitosan (CH). The thermal, microscopic, spectrophotometric and X-ray diffraction analysis showed that the drug was not altered during the granulation process. Studies of TFV release, swelling and *ex vivo* mucoadhesion were subsequently performed on simulated vaginal fluid. The formulation whereby TFV is granulated using twice its weight in ERS, and then including these granules in a matrix in which the CH predominates over HPMC, allows the sustained release of TFV for 144 h, mucoadhesion to the vaginal mucosa for 150 h and a moderate swelling, making it the most suitable formulation of all those studied. These compacts would therefore offer women protection against the sexual acquisition of HIV.

1. Introduction

In recent decades, significant efforts have been made to develop a vaginal microbicide to protect women against the sexual acquisition of the human immunodeficiency virus (HIV) (Antimisiaris and Mourtas, 2015; Doggett et al., 2015). However, the main problem with formulations capable of reducing the incidence of infection is low adherence by women to prophylactic treatment (Friend and Kiser, 2013; Gengiah et al., 2014; Marrazzo et al., 2015; van der Straten et al., 2016). It has been demonstrated that the protection offered by microbicides that have shown efficacy in phase III clinical trials varies greatly depending on the frequency of use. For example, in the CAPRISA 004 trial it was observed that the protection of the formulation varied from 28% to 54% (Abdool Karim et al., 2010; Kashuba et al., 2015; McConville et al., 2014) depending on the adherence to use, and in the ASPIRE study, infection was reduced by 27%, although when data from regions with low adherence were excluded, the percentage rose to 37% (Baeten et al., 2016). The main aim of the formulations being developed today is therefore to improve the adherence of their predecessors. To this end, the goal is to develop microbicides capable of releasing the drug over several days in order to decrease the frequency of administration. Another factor that must also be improved is women's comfort (Domanska and Teitelman, 2012; Eakle et al., 2015; Woodsong and Holt, 2015).

Nowadays there are multiple resources available to obtain extended release systems. The release rate can be controlled by increasing the particle size of the drug or by forming insoluble crystals (Chogale et al., 2016). Another notable possibility is to produce microspheres, microcapsules or microgranules in which the drug is coated with a slow-dissolving polymer; these particles are subsequently used to obtain the appropriate dosage form (tablets, capsules…) (Rambhia and Ma, 2015; Wang et al., 2013). Release can also be controlled through the design of the pharmaceutical form. Some examples are insoluble matrices (capable of controlling drug diffusion) (Bouman et al., 2016; Shojaee et al., 2016), soluble matrices (in which the drug is slowly dissolved) (Cevher et al., 2014; Maderuelo et al., 2011), coated systems (where drug delivery is controlled by a cover through which it diffuses) (Guo and Shi, 2009), and osmotic systems (in which a semipermeable membrane

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allows the water to enter and dissolve the drug, which later emerges through a small orifice) (Rastogi et al., 2013).

Hydrophilic polymers able to form a gel in an aqueous medium have been shown to be an excellent choice for the sustained release of drugs in the vaginal environment (Baloğlu et al., 2006; Sharma et al., 2006). Pharmaceutical forms can be obtained with these characteristics and developed with numerous polymers, such as hydroxypropylmethyl cellulose (HPMC) (Baloğlu et al., 2006; Hani et al., 2016; Karasulu et al., 2004; Sánchez-Sánchez et al., 2015), chitosan (CH) (El-Kamel et al., 2002; Sánchez-Sánchez et al., 2015; Szymańska et al., 2014), pectin (Baloğlu et al., 2003, 2006), guar gum (Hani et al., 2016), xanthan gum (Hani et al., 2016), alginates (El-Kamel et al., 2002; Gavini et al., 2002) and carragenates (Sánchez-Sánchez et al., 2015). The results obtained in previous studies show that the combination of HPMC and CH appears to offer the best results due to its ability to control the release of water-soluble drugs and its high mucoadhesive capacity (Notario-Pérez et al., 2018).

The most frequently used polymers for obtaining functional coatings are insoluble but permeable in aqueous medium, such as acrylic derivatives or Zein. Worth highlighting among the acrylic derivatives are those marketed as Eudragit®, comprising a wide variety of polymethacrylate-based copolymers specifically designed to obtain functional coatings, and which modify their characteristics according to the pH of the target area for the drug release (Thakral et al., 2013). Eudragit® RS (ERS) and Eudragit® RL, whose structure includes quaternary ammonium groups and are permeable but insoluble in water, are the best choice for drug release in the vaginal environment (pH 4.2) (Akhgari and Tavakol, 2016; Thakral et al., 2013). As mentioned, coatings can also be formed with Zein, a maize protein consisting mainly of hydrophobic and neutral amino acids, which is pH-stable, insoluble in water, but soluble in alcoholic solutions (Karthikeyan et al., 2012; Zhang et al., 2016). Its use for the sustained release of drugs has lately been extended to include coatings and microparticles and capsules or implants made with this polymer (Bouman et al., 2016; Karthikeyan et al., 2012; Zhang et al., 2016).

Although the most common application of these polymers is for the production of functional coatings, mainly in coated tablets, they can potentially be used to granulate drugs thanks to their adhesive properties which enable granules to be obtained in which the drug is mixed with the hydrophobic polymer. The aim of this work is to produce microgranules of tenofovir (TFV), an antiretroviral drug, combined with ERS or Zein, for their subsequent inclusion in a hydrophilic polymer matrix made from HPMC and CH, two polymers capable of forming a gel in the presence of vaginal fluid, to develop TFV sustained-release vaginal tablets.

2. Materials and methods

2.1. Materials

Tenofovir (TFV, lot: FT104801401) was supplied by Carbosynth Limited (Berkshire, UK). Eudragit RS® (ERS; lot: G120238035) was supplied by Evonik (Essen, Germany). Zein (lot: SLBL9380V) was acquired from Sigma-Aldrich (St. Louis, MO, USA). Chitosan, with 97% deacetylation and a viscosity of 92 mPa·s (CH, lot: 8826900003), was provided by Nessler (Madrid, Spain). Hydroxypropylmethyl cellulose – Methocel® K 100 M (HPMC; lot: DT352711) was kindly supplied by Colorcon Ltd. (Kent, UK). Anhydrous calcium hydrogen phosphate – Emprove® (ACDP; lot: K93487944416) was supplied by Merck (Darmstadt, Germany). Polyvinylpyrrolidone – Kollidon® 30 (PVP; lot: 98-0820) was purchased from BASF Aktiengesellschaft (Ludwigshafen, Germany). Magnesium stearate PRS-CODEX (MgSt; lot: 85269 ALP) was acquired from Panreac (Barcelona, Spain).

All other reagents used in this study were of analytical grade and used without further purification. Demineralized water was used in all cases.

Table 1
Composition of granules (content in mg per each 30 mg of drug).

Granulate	TFV	ERS	Zein
TFV1E2	30	60	
TFV1E1	30	30	
TFV2E1	30	15	
TFV1Z2	30		60
TFV1Z1	30		30
TFV2Z1	30		15

2.2. Preparation of the granules

TFV granules were prepared with ERS or Zein, with different drug/polymer ratios. This was done by mixing the powdery components, then adding sufficient ethanol to form a mass, which was possible because both ERS and Zein are soluble in ethanol and their adhesiveness eliminates the need for a binding agent. Finally, this mass was passed through a 1 mm mesh and the resulting granulate was dried at room temperature for 24 h to ensure complete evaporation of the ethanol. The granules obtained are shown in Table 1.

2.3. Characterization of the granules

2.3.1. X-ray diffraction

The X-ray diffraction patterns of pure materials and all granulated systems were recorded by using an automated Philips® X'Pert-MPD X-ray diffractometer with Bragg–Brentano geometry. Samples were irradiated with monochromatized Cu-Kα radiation (λ = 1.5406 Å) at 45 kV, 40 mA and a time per step of 2 s, and analysed between 2θ angles of 5° and 50°.

2.3.2. Infrared spectroscopy

The pure materials and prepared granules were characterised by Fourier transform infrared attenuated total reflection spectroscopy (FTIR-ATR). FTIR-ATR spectra were obtained with a Perkin-Elmer spectrophotometer instrument equipped with a MIRacle™ accessory designed for ATR measurements (Perkin-Elmer, USA).

2.3.3. Thermal analysis

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were performed in a SDT-Q 600 TA instruments TG/DTA analyser. About 5–10 mg of sample were placed in a pinholed aluminium sample pan with a lid and heated in atmospheric air to between 25° and 500 °C.

To perform hot stage microscopy (HSM), about 1 mg of sample was placed on a microscopic slide with a cover and heated at a rate of 2 °C/min on a Kofler stage. All samples were studied at between 30° and 350 °C. Microscopic examinations were made using a Thermogalen microscope fitted with the Kofler stage.

2.4. Preparation of the compacts

Batches of compacts were manufactured with a combination of hydrophilic polymers (HPMC and CH) containing the previously developed granules. Each granulated system was included in three different combinations of HPMC and CH (C1, with a ratio of 1:1 HPMC/CH; C2, with a ratio of 1.9:1 HPMC/CH; and C3, with a ratio of 1:1.9 HPMC/CH). The polymer combination was prepared by mixing HPMC, CH and ACDP, then wetting the mixture with a solution of PVP in ethanol to produce a mass, which was granulated using a 1 mm mesh and dried at room temperature for 24 h. Three granulates were obtained (C1, C2 and C3). Finally, each granulate was mixed with a sufficient amount of the TFV granules to ensure a content of 30 mg of drug and 290 mg of hydrophilic polymer per compact. MgSt was added to the total dried granulate before compaction. The final content of each batch