International Journal of Pharmaceutics 503 (2016) 16–28

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Poly(ethylene glycol) enclatherated pectin-mucin submicron matrices for intravaginal anti-HIV-1 drug delivery

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ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form 25 February 2016
Accepted 27 February 2016
Available online 2 March 2016

Keywords:
Anti-HIV-1
Zidovudine
Pectin
Mucin
Poly(ethylene glycol)
Intravaginal delivery
Submicron matrices

ABSTRACT

This paper explores the potential of polyethylene glycol enclatherated pectin-mucin (PEG-*encl*-PEC:MUC) submicron matrices (SMMs) as an intravaginal drug delivery system capable of delivering an anti-HIV-1 agent (zidovudine; AZT) over a prolonged duration. A three factor and three level (3^3) Box-Behnken statistical design was employed to optimize the SMMs. Optimized PEG-*encl*-PEC:MUC SMMs prepared as a stable W/O emulsion (determined by the degree of reversible colloidal phenomena) were spherical with a mean particle size of 270.6 ± 5.533 nm and mean zeta potential of -34.4 ± 0.539 mV. The microencapsulation of AZT and the hydrogen bonding mediated shielding of AZT by SMMs was confirmed by Fourier Transform Infrared (FTIR) analysis. The thermochemical (differential scanning calorimetry and thermogravimetric analysis) data proposed that Ca^{2+}-based macromolecular ionic crosslinking as well as the intermolecular interactions may be responsible for the thermal stability of the delivery system. The partially amorphous nature of drug-loaded SMMs, as confirmed by X-ray diffraction patterns, further strengthened the matricization of AZT into the pectin-mucin matrix. *In vitro* drug release studies from the SMMs showed approximately 91% zidovudine release in simulated vaginal fluid (SVF) and 94% in phosphate buffered saline (PBS) in 24 h. The mean dissolution time (MDT) of zidovudine from the SMMs was 5.974 h. The attainment of required dimensional structure and drug release profiles from SMMs highlights the potential of their inclusion into a secondary carrier system for extended and controlled intravaginal stay.

1. Introduction

Focus on preventing vaginal HIV-1 transmission has led to the development and usage of a physical polymeric barrier/sheath commonly known as a condom which is worn during coitus to prevent the mixing of male and female reproductive fluids thus preventing HIV-1 infection. Though largely effective in preventing HIV-1 transmission, the condom has been hampered by inappropriate, infrequent usage and to some extent it has suffered problems of cultural and religious acceptability. As an alternative to the condoms, contemporary research is being done on microbicides. These are anti-HIV-1 agents that may be applied topically to the vaginal or rectal cavity to prevent HIV-1 transmission (Stone, 2002; Woolfson et al., 2010). A keen interest in using antiretroviral drugs as microbicides has also emerged given their success in treating HIV/AIDS (Klaase et al., 2008). Although microbicides hold much promise and their concept is sound, clinical trials conducted up-to-date have failed to demonstrate efficacy (Hendrix et al., 2009). Several questions have been raised and some answers postulated to try end explain why anti-HIV-1 agents effective *in vitro* have failed to demonstrate efficacy *in vivo*. One common possible explanation given is microbicide formulations did not take into consideration and did not address some fundamental anatomical, physiochemical and physiological principles involved in vaginal HIV-1 transmission. Such factors include; the need to maintain or augment the vaginal mucosal barrier properties, maintenance of the HIV-1 prohibitive acidic pH (~4.5) of the vaginal cavity, presence of the anti-HIV-1 agent in appropriate quantities in the vaginal cavity where it has to be distributed extensively and exhaustively on the vaginal mucosa, anti-HIV-

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http://dx.doi.org/10.1016/j.ijpharm.2016.02.046
0378-5173/

1 agent presence in the sub-epithelia to counteract HIV-1 that would have transcytosed the vaginal epithelia and having the microbicide stays at the site for a longer duration than HIV-1 (Hendrix et al., 2009).

The development of topically applied chemical agents that may effectively prevent vaginal HIV-1 transmission (microbicides) has been a major challenge for pharmaceutical scientists (Hendrix et al., 2009; Adams and Kashuba, 2012; Pillay et al., 2012). The difficulties have included; microbicide acceptance as an alternative HIV-1 prevention modality, pharmacokinetic inadequacy whereby the microbicide has to be distributed throughout the vaginal mucosa in sufficient quantities to prevent HIV-1 transmission and mucosal toxicity which diminishes the vaginal mucosa's barrier properties resulting in easy HIV-1 mucosal transcytosis and transmission (Adams and Kashuba, 2012; Pillay et al., 2012). Despite having vast potential as a preventative tool against vaginal HIV-1 transmission, research conducted thus far has failed to provide an effective anti-HIV-1 microbicide product to the market. Various formulations have been prepared to try and mitigate these concerns, among them; gels, creams, films, tablets and rings (Hendrix et al., 2009). Gels, creams and films are indiscrete, sticky and are short acting thus require frequent application before and after every coital act whilst tablets and rings are discrete and may be formulated to deliver the anti-HIV-1 agent over an extended duration (Klaase et al., 2008). In this study, poly(ethylene glycol) enclatherated pectin-mucin submicron matrices (SMMs) were formulated for potential inclusion into a polymeric caplet eventually forming a composite polymeric drug delivery system as a potential microbicide drug delivery system. The submicron matrices were formulated from biocompatible materials; pectin, porcine gastric mucin and polyethylene glycol. These polymers formed the framework structure of the submicron matrices enabling the encapsulation and controlled release of zidovudine, the model antiretroviral used in this study.

2. Materials and methods

2.1. Materials

Commercial grade pectin (PEC), GENU® pectin type USP/100 [Degree of esterification (DE) 55–65%] was obtained from CP Kelco ApS, Lille Skensved, Denmark. Porcine gastric mucin type III (MUC) with 1–1.5% bound sailic acid, polyethylene glycol of M_w 400 (PEG) and Kollidon® SR (KSR) and phosphate buffered saline (PBS, pH 7.4) were purchased from Aldrich® (Sigma–Aldrich Inc., St. Louis, USA). Poly (D,L-lactide) was purchased from Boehringer Ingelheim, Ingelheim, Germany. Carbopol 794 P NF was obtained from Noveon Inc., Cleveland, OH, USA. The model anti-HIV-1 active pharmaceutical ingredient (API), zidovudine (AZT) was obtained from Glaxo Smith Kline, Middlesex, UK. Other materials and excipients including; calcium chloride, magnesium stearate (MS), glucose and cyclohexane were of analytical grade and were utilized as obtained. Simulated vaginal fluid (SVF, pH 4.5) was prepared from analytical grade reagents in accordance to Owen and Katz's formulation (Owen and Katz, 1999).

2.2. Box-Behnken design approach for the preparation of PEG-encl-PEC:MUC SMMs

A three-factor, three-level (3^3) Box-Behnken statistical design on MINITAB® (V14, State College, Pennsylvania, USA) was employed to optimize the preparation of AZT-loaded PEG-*encl*-PEC:MUC SMMs (Karnachi and Khan, 1996; Box and Behnken, 1960). Upper and lower levels of three independent parameters that included; ultrasonication time (ST), surfactant concentration (SC) and drug:polymer (D:P) ratio were chosen due to their high significance in the fabrication of the SMMs. The dependent parameters or responses that comprised; particle size (PS), zeta potential (ZP) and mean dissolution time (MDT) were sought, as presented in Table 1. Fifteen formulations were generated from the Box-Behnken design (Table 2). These formulations were prepared and experimentally tested and the results obtained were fed into the MINITAB® design software which then computed the optimized formulation's independent parameter and expected response values.

Table 1
Independent parameters and responses from the Box-Behnken statistical design used to optimize AZT-loaded PEC-MUC-PEG SMMs.

Independent parameter	Levels	
	Lower	Upper
ultrasonication time (min)	5	10
surfactant concentration%($^{v}/_{v}$)	1.5	2
drug:polymer ratio	0.5	1
Response	Objective	
particle size (nm)	minimize	
zeta potential (mV)	minimize	
mean dissolution time (hours)	maximize	

2.3. Preparation of AZT- loaded PEG-encl-PEC:MUC SMMs

AZT-loaded PEG-*encl*-PEC:MUC SMMs where prepared using a crosslinking-emulsion technique. PEC, MUC, PEG and AZT (360 mg) were subsequently dispersed and dissolved in MilliQ water, obtained from a MilliQ® gradient water purification system (Millipore SAS, Molsheim, France), whilst stirring for 15 min to form an aqueous phase which was then crosslinked by drop-wise addition of calcium chloride. The PEC:MUC ratio used was 9:1, PEG was 0.1 mLs and drug (AZT):polymer (MUC, PEC and PEG) ratios used were in accordance with the Box-Behnken-design. A water-in-oil (W/O) emulsion was prepared by ultrasonication using a high intensity ultrasonic processor (Sonics Vibracell VCX 130, Sonics Materials INC, Newtown, CT, USA) with the crosslinked PEG-*encl*-PEC:MUC-AZT dispersion acting as the aqueous phase and cyclohexane as the oil phase. The w/o ratio was 1:4 and span 85 was added as the surfactant in accordance with the Box-Behnken design. The emulsion was centrifuged at 4000 rpm for 1 min and thereafter excess cyclohexane was decanted. The remaining concentrated SMM emulsion was frozen at −80 °C for 12 hrs before being lyophilized for 48 h.

2.4. Determination of the stability of the optimized SMM emulsion

The dispersion state of the W/O emulsion obtained in the formulation of SMMs was measured using a Turbiscan Lab® (Turbiscan Lab™, Formulaction SA, L'Union, France) which assessed the degree of reversible colloidal phenomena such as creaming and sedimentation as well as irreversible phenomena such as coalescence and flocculation (Celia et al., 2009). The Turbiscan Lab® Expert software was used to analyze transmitted (T) and backscattered (BS) light according to Eq. (1) and in reference to the Mie theory represented by Eq. (2).

$$BS = 1/\sqrt{\lambda^*} \tag{1}$$

$$\lambda^*(d, \Phi) = 2d/[3\Phi(1-g)Q_s] \tag{2}$$

where λ^* is the photon transport mean free path, Φ is the volume fraction of the particles, d is the particle mean diameter and g