European Journal of Pharmaceutics and Biopharmaceutics 95 (2015) 128135

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research Paper

Mucoadhesive cellulosic derivative sponges as drug delivery system for vaginal application

Tania Furst, Marie Piette, Anna Lechanteur, Brigitte Evrard, GÕraldine Piel

Laboratory of Pharmaceutical Technology and Biopharmacy, CIRM, University of LiÒge, Belgium

ARTICLE INFO

Article history:
Received 14 November 2014
Accepted in revised form 23 January 2015
Available online 4 February 2015

Keywords:
Vaginal
Cellulosic derivative
Lyophilization
Hydrogel
Sponge
Mucoadhesion

ABSTRACT

Vaginal delivery of active drugs has been largely studied for local and systemic applications. It is well known that vagina is a complex route, due to physiological and non-physiological changes. Therefore, in order to achieve a prolonged local effect, these variations have to be considered.

The aim of this study was to formulate and to characterize a solid system, called sponges, obtained by lyophilization of cellulosic derivative (HEC 250M) hydrogels. These sponges have to meet particular criteria to be adapted for vaginal application: they have to adhere to the vaginal cavity and to be rehydrated by the small amount of vaginal fluids. Moreover, they have to be easily manipulated and to be stable.

Three freezing temperatures have been tested to prepare sponges (15˚C, 25˚C, 35˚C). By SEM analyzes, it was observed that the pores into the sponges were smaller and numerous as the freezing temperature decreases. However, this temperature did not have any influence on the rehydration speed that was rather influenced by the HEC concentration. Viscosity and mucoadhesive strength of hydrogels and corresponding sponges were also measured. It appeared that these parameters are mainly dependent on the HEC concentration.

These mucoadhesive sponges can be considered as potential drug delivery systems intended for vaginal application.

1. Introduction

It is well known that the vaginal route is an excellent way for mucosal drug delivery for both systemic and local applications. The avoidance of the gastrointestinal absorption and of the hepatic first-pass metabolism, the presence of homogeneous and dense vascularization and the high mucosal permeability have made this route widely used for the administration of many drugs (sexual hormones, peptides, antimicrobials, antimycosics, etc.) [13].

However, despite these advantages, the vaginal route has some particularities that can lead to variations in the bioavailability of drugs. Vaginal conditions are subject to changes because of numerous physiological and non-physiological factors, like the menstrual cycle of women or menopause, but also after sexual intercourses or depending on personal hygiene. The vaginal pH value and the vaginal fluid characteristics are two critical parameters to consider for the formulation of a vaginal drug delivery system. The normal vaginal pH value is generally comprised between 3.5 and 5 but can vary significantly in the different conditions previously mentioned. Composition, volume and rheological properties of the vaginal fluids are also influenced in these conditions [25]. Another important parameter which can interfere with the performance of these delivery systems is the natural clearance process of vaginal secretions, commonly called mucus [6,7]. Although considered as a mucosal tissue, the vagina does not have secretory glands. Vaginal secretions are formed by a mixture of fluids (cervical fluid and secretions from Bartholins glands) which coat the vaginal walls [810]. This mucus holds important physiological functions such as lubrication and formation of a physical barrier to protect the vagina against pathogens. Its continuous production and elimination can be considered as a relatively constant flux, leading out of the body vaginal flora metabolites and external pathogens. The mucus plays an important role and can significantly impact drug absorption or action. Accordingly, the medication system could rapidly be eliminated and not be effective. With the purpose to achieve a sustained local effect and to retain the dosage form in the vagina, it is necessary to consider and overcome all these variations.

Abbreviations: HEC, hydroxyethylcellulose; PEG, polyethylene glycol; CVM, cervicovaginal mucus; TA, Texture Analyzer.

Corresponding author. University of LiÒge, Laboratory of Pharmaceutical Technology and Biopharmacy, CHU B36, Tour 4, 1 avenue de lHopital, 4000 LiÒge, Belgium. Tel.: +32 4 366 43 07.

E-mail address: tania.furst@ulg.ac.be (T. Furst).

http://dx.doi.org/10.1016/j.ejpb.2015.01.019
0939-6411/

A large variety of vaginal forms have been developed and commercialized (tablets, gels, creams, rings, foams, films, etc.) [3]. In order to have a prolonged contact between drug and mucosal surface and to enable a sustained drug release, solid or semi-solid systems are generally preferred. Among these systems, hydrogels are largely studied. These three-dimensional polymeric matrices are made of small quantities of gelling excipient in relatively large amounts of liquid. They are easy to prepare and to use but depending on the polymer, they can be rapidly eliminated with the flux of vaginal fluids. Thus, to increase the residence time, a bioadhesive and particularly mucoadhesive excipient is required [11,12]. The most commonly used mucoadhesive polymers are polyacrylates (carbomer), alginates, chitosan and cellulosic derivatives (hydroxyethylcellulose, hydroxypropylmethylcellulose, carboxymethycellulose, etc.) [2]. This paper explains the development of a solid delivery system which will be retained in the vaginal cavity and will allow a prolonged drug delivery. The solid form was chosen to enable the preservation of sensitive molecules such as oligonucleotides and for its ease of handling and application. In addition, this solid system must meet different criteria such as adhesion to the vaginal mucosa and rehydration with the little amount of available vaginal fluids to return to hydrogel with an appropriated viscosity [1,1316]. Based on previous results obtained in our laboratory in the framework of the development of vaginal HEC sponges containing cidofovir as active ingredient [17] (COLVIR Patent), we focused on cellulosic derivatives hydrogels which can be lyophilized in order to form sponges [1821].

The aim of this study was thus to formulate cellulosic derivatives lyophilized sponges and to characterize them in terms of mucoadhesion, rehydration ability and stability.

2. Materials and methods

2.1. Materials

Hydroxyethylcellulose (HEC) 250 M was purchased from Ashland (Covington, USA) and polyethylene glycol (PEG) 400 was purchased from Fagron (Waregem, Belgium).

Urea, dried porcine gastric mucin (type III), guar gum, methylparaben, propylparaben, dibasic potassium phosphate (K_2HPO_4) and monobasic potassium phosphate (KH_2PO_4) were purchased from SigmaAldrich (Schnelldorf, Germany).

GynodaktarinÛ was purchased from Janssen Pharmaceutica (Beerse, Belgium) and LubrilanÛ was purchased from Pannoc (Olen, Belgium).

2.2. Methods

2.2.1. Preparation of hydroxyethylcellulose derivative hydrogels

Hydrogels (6 g) were prepared by a homogenous aqueous dispersion of HEC 250M polymer and PEG 400. The compositions of the hydrogels are shown in Table1. Briefly, the polymer was gradually dispersed in around 4 mL of water for 1 h, at room temperature and under continuous magnetic stirring. Then, PEG 400 and water were added to obtain 6 g of hydrogel. The homogenization was still performed for 2 h. All the hydrogels were prepared into 4 cm diameter glass containers.

Table1
Composition of hydrogels.

	HEC 250M (mg)	PEG 400 (mg)
A	100	25
B	200	25
C	200	50

2.2.2. Sponge preparation

Sponges were prepared by lyophilization of hydrogels in a freeze dryer (Heto-Holten, model DW 8030) using a vacuum pump (Vacuubrand RZ8). Initially, the samples were frozen from room temperature to 15°C (cycle 1) or to 25°C (cycle 2) or to 35°C (cycle 3) over a period of 3 h and 30 min. After that, primary drying was performed at 15°C for 3 h (under 0.8 bar pressure), and then at 10°C for 12 h (under 0.1 bar pressure). Finally, secondary drying was carried out at 10°C for 5 h (under 0.1 bar pressure). The entire freeze-drying cycle took 23 h and 30 min (Table2).

Once the process was completed, the lyophilized sponges were removed and weighed. They were sealed with aluminum and plastic film and stored at room temperature in glass desiccators.

The water loss was calculated using the following equation:

$$\text{Water loss } (\%) = (W_H - W_S)/W_H \times 100 \quad (1)$$

where W_H is the weight of the hydrogel and W_S is the weight of the sponge [19].

2.2.3. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was performed using a JEOL JSM 840-A microscope after metallization with platinum (30 nm) (Balzers, model SCD30).

Surface areas (m^2) of the pores were calculated from SEM images using the ImageJÛ Software.

Results are expressed as the median˜ 1090 percentile of 3 experiments for each condition. The data were analyzed with a Kruskal Wallis Test (nonparametric test) followed with a Dunns Multiple Comparison Test, using GraphPad PrismÛ software and a *p-value* < 0.05 was considered to be significant.

2.2.4. Preparation of synthetic cervicovaginal mucus (CVM)

CVM was prepared following the method described by BrÚd etal. [6]. Briefly, guar gum (1.00 g), dried porcine gastric mucin (type III) (0.50 g), urea (0.30 g), methylparaben (0.15 g), propylparaben (0.02 g), K_2HPO_4 (0.26 g) and KH_2PO_4 (1.57 g) were added to 90 g of water and stirred until uniform dispersion. The pH was then adjusted to 4.5 using hydrochloric acid (0.1 M) and the total weight was adjusted to 100 g with water.

2.2.5. Rehydration study

The ability of HEC 250M sponges to reform hydrogel was evaluated. To simulate the dilution that may occur after a vaginal application, 2 mL of CVM were added on the top of sponges. Pictures were taken at different times to assess their rehydration speed.

2.2.6. Evaluation of the viscosity

The viscosity of hydrogels was measured using a Brookfield Digital viscometer (model DV-2+) with a Helipath E95 probe.

Table2
Freeze-drying conditions (cycles 1, 2 and 3).

Cycle 1	Cycle 2	Cycle 3	Duration
Freezing step			
15°C	25°C	35°C	3h30
Temperature	Pressure		Duration
Primary drying step			
15°C	0.8 bar		3h00
10°C	0.1 bar		12h00
Secondary drying step			
10°C	0.1 bar		5h00
Total			23h30