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RESEARCH ARTICLE

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The vaginal sheet: an innovative form of vaginal film for the treatment of vaginal infections

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ABSTRACT

Objective: To develop and characterize a new form of vaginal film.

Significance: This formulation is intended to overcome some known limitations of traditional dosage forms. It has an absorptive intention to control symptoms and to improve the treatment of vaginal infections characterized by excessive fluid. The vaginal sheet is a thick drug delivery system easy to manipulate, nontoxic and composed by biocompatible macromolecules and polymers, such as gelatin and chitosan.

Methods: The sheets were prepared by formulating gelatin or chitosan based gels isolated or in combination, in association with a plasticizer. Gels were subsequently lyophilized. Different proportions of polymer:plasticizer were tested. Lactose was used as a surrogate to study powder incorporation in the formulation. All formulations were analyzed regarding their organoleptic characteristics, texture (hardness and resilience), *in vitro* absorption efficiency of vaginal fluid simulant – VFS (pH 4 and 5), pH and acid-buffering capacity.

Results: Different properties were obtained by varying polymer and plasticizer proportions. Combinations including gelatin with propylene glycol showed the best organoleptic characteristics. The best proportions were 4:3 and 4:5. Up to 10% of powder was successfully incorporated in the formulation. Hardness and resilience of formulations were largely dependent on the concentration of plasticizer. Absorption of vaginal fluid was found to be highly efficient, especially at pH 5. Buffering capacity, upon dilution in normal saline and VFS, was generally higher for VFS pH 4.

Conclusions: The vaginal sheet is a promising solid drug delivery system able to further incorporate drugs to treat vaginal clinical conditions characterized by excessive fluid.

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Vagina; vaginal sheet; dosage form; vaginal infections

Introduction

Female urogenital infections are highly prevalent [1] and the available dosage forms present some disadvantages from the patients' point of view [2]. The vagina provides a route for drug administration with not only local but also systemic effect, due to its large surface area, high blood supply, evasion of the first pass effect and relative permeability to some drugs [3,4]. Vaginal ecology is influenced by factors such as glycogen content in epithelial cells, glucose, pH, hormone levels, trauma caused by sexual intercourse, contraceptive methods, age and antimicrobial treatments. Normal microflora produces enough lactic acid to maintain the vaginal environment at a pH range of 3.5–4.5 [5,6]. This vaginal feature is mainly due to *Lactobacillus spp.* which convert glycogen from exfoliated epithelial cells into lactic acid. Vaginal pH changes occur with age, menstrual cycle, infections and sexual arousal. The daily production of vaginal fluid is estimated to be approximately 6 g, and 0.5–0.75 mL being present at each moment in the vagina [7]. These physiological factors should be taken into account during the development and evaluation of vaginal delivery systems [8].

Bacterial vaginosis (BV) affects 10–15% of women in reproductive age and it is estimated that around 50% of women are asymptomatic [9]. BV is a complex condition that occurs as a function of change in the normal dominant flora from Lactobacillus *spp.* to a polymicrobial community of other aerobes and anaerobes. It is estimated that more than 30% of women have a recurrence within 3 months after treatment [10,11]. BV is symptomatically characterized by the excess of vaginal fluid produced with increased pH and unpleasant (rotten fish) odor. In clinical practice, the conjugation of the scarcity in therapeutic options, the drug resistance and the high recurrence of the infections clearly reflects the need to develop new treatments [12].

The vaginal dosage forms most frequently used are suppositories (ovules), tablets and semisolids [13]. However, their use may be limited by some disadvantages such as short retention time, due to the vaginal self-purifying mechanism; uncomfortable application; and multiple daily administrations [13]. These characteristics generally lead to a decrease in the acceptability of women to use these pharmaceutical forms which, in turn, may affect compliance with the therapeutic regimen [6]. Vaginal films are a recently

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developed dosage form composed by solid thin polymeric preparations that rapidly dissolve in contact with vaginal fluids, and so they are unlikely associated with leakage and messiness [14–22]. Some studies have actually showed that film-like formulations are more easily accepted by women than other pharmaceutical forms, such as gels, foams and ovules [15,16,23–27]. Formulation of vaginal films with bioadhesive polymers has been privileged for compliance purposes [16,23,28–30].

The mucoadhesion phenomena is a bioadhesion branch, whose biological surface is a mucous membrane and, therefore, interactions are made with the mucus [31,32]. Mucoadhesive polymers are capable of swelling when placed in an aqueous medium, and consequently, exhibiting a controlled release profile. Including mucoadhesive polymers in formulations can improve the therapeutic efficacy of the locally acting drugs, as there is a prolonged availability of drug in the target membrane. Generally, this type of polymers have high molecular weight and hydrophilic functional groups [3], furthermore, they are easily applied to several drug delivery systems for vaginal administration, such as gels, tablets, films, emulsions or ovules [5,6,33].

Considering the specific characteristics of BV it has been hypothesized that its treatment should be madewith a vaginal dosage form presenting the ability to absorb the excess amount of fluid (instead of dissolving in these biological fluids), while neutralizing its odor and eventually releasing an antibacterial drug, therefore controlling symptoms while treating the infection.

The main objective of this work was to develop and characterize ann innovative solid pharmaceutical form for vaginal application as a variation of vaginal films – the vaginal sheet. The target product profile of the vaginal sheet was defined as a solid vaginal dosage form which consistency should be rigid, but malleable, flexible and soft enough so that it can be introduced without discomfort while maintaining enough structure to be removed after absorption of excessive fluids. Figure 1 reflects the sheet shape and how it should be handled before its administration. Typical excipients expected for this formulation are shared with the overall qualitative composition of vaginal films: defined as water-soluble polymers, plasticizers, diluents, colorants and flavorings. The polymers to be used must be nontoxic, nonirritating, have minimal impurities and should become easily wet and dispersed. The choice of the polymer and its molecular weight are expected to have a significant impact on the final formulation properties', such as strength and disintegration time. The plasticizers should allow for acceptable flexibility and texture [34–36]. During the vaginal sheet development, some important parameters were considered: rate of hydration in a limited volume of fluid (to be similar to the physiological amount); drug delivery mechanisms and local tolerability; size and shape of the product; water content of the product; conditions of packaging and storage. The vaginal sheet is a variation of vaginal films because unlike vaginal films it is supposed not to dissolve quickly, is bigger and thicker, and can carry higher quantities of powders (active substances and excipients) that could be either dissolved or suspended in it.

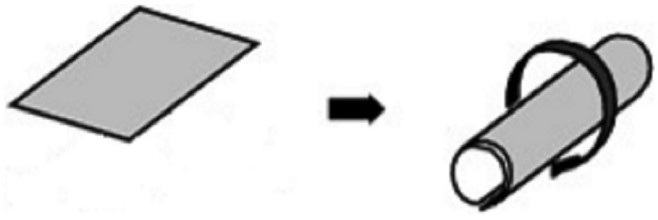

Figure 1. Schematic representation of the expected vaginal sheet formulation and folding movement before administration for further expansion on the vaginal cavity.

The process of manufacturing was designed to be similar to the films: preparing the mass of the film (gel-type formulation), pouring the mass on a mold, drying step, cutting and separating the sheets individually and packaging [15].

Materials and methods

Materials

For the preparation of the vaginal sheet the following excipients were used: gelatin (Fagron and Guinama, Spain); propylene glycol (Farma-Química Sur S.L., Spain); glycerin (Acofarma, Spain), all with pharmaceutical grade; high and medium molecular weight chitosan (Aldrich Chemistry, Germany) with 310–375 KDa and 161 KDa, respectively; lactose monohydrate (Acofarma, Spain); 85% lactic acid (Aldrich, Germany); anhydrous sodium sulfate (Fluka, Germany) and MilliQ water (obtained *in house* through a Merck Milli-Q® Reference equipment). For the preparation of the vaginal fluid simulant (VFS), sodium chloride (Panreac, Spain), potassium hydroxide (Prolab VWR, France), sodium hydroxide (Acros Organics, UK), bovine serum albumin (Sigma, Germany), lactic acid (Aldrich, Germany), acetic acid (Panreac, Spain), glycerol (HiMedia, India), urea (Sigma, Germany) and glucose (Sigma, Germany). For the determination of buffer capacity, 0.9% NaCl solution (B-Braun, Germany) was used as the control. All other chemicals and reagents were of analytical grade.

A commercial vaginal film formulation available in the U.S. market (the *Vaginal Contraceptive Film*, VCF®, Apothecus Pharmaceutical) was acquired in a pharmacy in the USA to be used as control formulation for absorption efficiency testing.

Vaginal sheets preparation

To prepare the gelatin based formulations, MilliQ water was first heated to 50 °C (in a water bath). The plasticizer was the first ingredient to be added, and then, after dissolving the gelatin (50 °C, water bath), all the remaining constituents were incorporated. Stirring was minimized to avoid the excessive incorporation of air bubbles in the forming gel. When all the excipients were dissolved, the gel was poured into standard plastic Petri dishes (9 cm diameter, pre-marked 2 mm high, which makes up approximately 10 g of gel). Formulations were allowed to cool down and were frozen (at −20 °C or −80 °C), overnight. The plates were then freeze-dried during 24 h using a Scanvac CoolSafe™ freeze drier (temperature reached −110 °C; pressure 0.019 hPa). For formulations containing both gelatin and chitosan, a gelatin solution was first prepared and then chitosan was added to it, under heating (water bath). Two different types of chitosan were used: high molecular weight (HMW) and medium molecular weight (MMW), Low molecular weight chitosan was not selected because a high viscosity gel was envisaged to form the sheet after drying. For chitosan formulations, the solvent used was a lactic acid aqueous solution (2% v/v) previously prepared and heat was not required for dissolution. Solubilization was performed under a helical stirrer. For these sheets, the percentage of propylene glycol was always half of chitosan concentration [37].

Formulations

The concentrations to be used of each one of the excipients were defined based on the available vaginal safety data for each

Table 1. Qualitative and quantitative composition of different vaginal sheets, during the formulation development step (Codification of formulations represent the combinations of polymer/macromolecule and plasticizer within each group).

Formulation (F)	Experimental Strategy	Polymer/Macromolecule % (w/w)	Plasticizer % (w/w)	Powder % (w/w)	Solvent (q.s.ad 100)
F1 (F1.1–2)	Test the maximum amount of gelatin	Gelatin 20, 40%			Water
F2 (F2.1–10)	Insertion of a plasticizer to obtain malleability	Gelatin 20, 25, 30, 40%	Propylene glycol 10, 15, 25%		Water
F3 (F3.1–8)	Introduction of a model (inert) powder – lactose	Gelatin 20, 25, 30%	Propylene glycol 10, 15, 25%	Lactose 10%	Water
F4 (F4.1–6)	Introduction of a highly hygroscopic powder – anhydrous sodium sulfate	Gelatin 20, 25, 30%	Propylene glycol 10, 15%	Anhydrous sodium sulfate 10%	Water
F5 (F5.1–13)	Substitution of propylene glycol with glycerin	Gelatin 20, 25, 30%	Glycerin 10, 15, 25%	Lactose 10% (only for F5.2 and F5.8–13)	Water
F6 (F6.1–12)	Glycerin and gelatin at lower concentrations and combination with lactose	Gelatin 5, 10, 15%	Glycerin 6.25, 7.5, 11.25, 12., 18.75%	Lactose 10%	Water
F7 (F7.1–10)	Chitosan as polymer	Chitosan (HMW) 1, 3% (MMW) 1.5, 2, 2.5%	Propylene glycol 0.5, 0.75, 1, 1.25, 1.5% Glycerin 0.75, 1, 1.25%		Lactic acid 2%
F8 (F8.1–5)	Polymer combination	Gelatin 13.5, 18.5% combined with Chitosan 1.5% (HMW or MMW)	Propylene glycol 10, 15, 25%		Lactic acid 2%

q.s.ad: quantum satis ad (the amount necessary to complete the total mass).

substance and on the approved concentrations for vaginal products. The usual excipients concentrations for soft gelatin vaginal capsules were taken as the starting point. Although gelatin vaginal capsules are designed to be totally dissolved on vaginal fluids while the vaginal sheet should only swell, it is not known from clinical practice that they do not always disintegrate completely after a 12-h usage period. Since the objective of this work is to reach a non-dissolvable but swelling-hygroscopic formulation, gelatin capsules were used as references, but the excipient concentrations were reduced to accomplish optimization of textural properties while complying with reduced toxicity and lower interference with the vaginal milieu. These capsules have been described as containing 40% gelatin in the wet mass of molten gel and that their plasticizers (glycerin, sorbitol and propylene glycol or combinations) represent 20–30% of the gel in the wet formulation [38]. Qualitative and quantitative composition of the different formulation groups are described in detail in Table 1. Preparations were numbered according to the general feature of formulations and their specific characteristics (e.g. F1.1). The detailed composition of each formulation is described in the Supplementary Material.

Organoleptic characterization

The organoleptic characterization was performed from a technical and pharmaceutical point of view, but also taking into account the envisaged clinical use. The sheets were carefully analyzed for the following characteristics: appearance (color, opacity and homogeneity), odor, softness to touch and malleability (which encompasses easiness of winding and subsequent ability of the sheet to return to its initial form – as plausible indicator of the ability to cover the surface of the vaginal wall).

Thickness evaluation

The thickness was evaluated on the vaginal sheets F2.1, F3.8 and F5.2. These formulations were prepared in petri dishes of 5.5 cm diameter which have a base area of 23.76 cm^2. Proportionality was applied to calculate the amount of gel to be added to these petri dishes (smaller molds). In previous experiments the formulations had been prepared in 9.0 cm petri dishes (base area of 63.62 cm^2). Petri dishes were pre-marked 2 mm high, so the volume of gel is 12.72 cm^3. This volume corresponds to approximately 10 g of gel. So, applying the proportionality, petri dishes of 5.5 cm should have approximately 3.73 g of gel. Once approximately 10 g of gel occupy a volume of 12.72 cm^3, 3.73 g predictably occupy a volume of 4.75 cm^3, which correspond to a height of 0.199 cm. Thus, petri dishes of 5.5 cm were pre-marked 2 mm high. Gel was placed on the petri dishes up to approximately 2 mm high. After the freeze dying process, the thickness of vaginal sheets was measured with a ruler at three different points of the edges in three vaginal sheets of each formulation. The thickness of the vaginal sheet was compared to the gel height in the mold (2 mm). Additionally, each petri dish was weighed empty, after putting the gel and after the freeze drying process.

Lyophilization efficiency

The efficiency of the process was calculated by the following equation for the comparative study between freeze-drying efficiency (%) with pre-freezing at −20 and −80 °C:

$$\text{Weight loss} = [(M_i - M_{fd})/M_i] \times 100 \quad (1)$$

$$\text{Lyophilisation efficiency} = (S_{fd}/S_i) \times 100 \quad (2)$$

Equation 1 calculates the formulation weight loss (%) after the lyophilization process. M_i (g) is the initial weight of the formulation and M_{fd} (g) is the weight after lyophilization. In Equation 2 S_{fd} represents the solvent mass (g) lost by the freeze drying (corresponding to $M_i - M_{fd}$) and S_i is the initial mass (g) of the solvent in the formulation.

Texturometric analysis

The vaginal sheets were analyzed regarding their hardness and resilience using a method already described in the literature, with some modifications [38,39]. A TAXT Plus (Stable Micro Systems,

United Kingdom) was used. Hardness was determined by the maximum force (F_{max}) exerted by the 2 mm needle shape probe (P2N) on the formulation sample placed on a heavy duty platform under the following test conditions: a test speed of 3 mm/s, a penetration distance of 1 mm and a trigger force of 0.05 N. Resilience was measured using a P2 (2 mm) flat probe and a ring platform to avoid movements of the formulation. Test speed was 3 mm/s, distance was 5 mm and trigger force was 0.05 N. Both tests were performed on return to start mode. Resilience is the ability of a sample to regain its shape after a force has been exerted on it. In this case, the parameter evaluated (resilience) results from the measurement of two areas of the graph obtained. The resilience was then calculated by the following expression:

$$\text{Resilience}\,(\%) = (\text{Area 2}/\text{Area 1}) \times 100 \quad (3)$$

Area 1 (Nsec) represents the force exerted by the probe by shifting/pushing the formulation through the hole of the platform, and thus reflects the resistance of the formulation to deformation. On the contrary, Area 2 (Nsec) represents the force exerted by the formulation on the probe, while returning to its starting point (no forces are applied by the equipment). Thus, the mathematical expression 3 becomes representative of the behavior of the formulations, as it reflects both the strain resistance component and the ability of the component to acquire its initial state. For each sheet, hardness and resilience were measured at three different points, and the mean and standard deviation of these three measurements were calculated. Two sheets were analyzed for each formulation, made at room temperature.

Bioadhesion

The bioadhesion of vaginal sheets F2.1, F3.8 and F5.2 was measured. Formulation adhesiveness to the porcine vaginal tissue was evaluated using a texturometer TAXT Plus (Stable Micro Systems, United Kingdom). The tissue was excised from vaginal tubes from approximately 6 months' years old animals, kindly provided from a local slaughterhouse. The vaginal tubes were cut longitudinally, washed with a sodium chloride solution (0.9% w/w) wrapped in aluminum foil, and preserved at −20 °C (less than 6 months). For the experiment, vaginas were thawed at room temperature and washed with sodium chloride solution.

The vaginal sheets were cut into circular portions with 10 mm diameter (corresponding to the diameter of the probe base). On the upper side of each circular portion 53 µL of VFS_m (Vaginal Fluid Simulant with Mucin) were added. After 2 h and 30 min all the VFS_m had been absorbed by the sheets. This volume of VFS_m was calculated considering the proportionality between the area of sheet and the area of circular portions. Vaginal sheet area was 16.8 cm^2 and the circular portion area was 0.785 cm^2. Previous studies report that 0.5–0.75 g are present at every moment in the vagina [7,12,40]. However, women with BV present an increase of the vaginal fluid volume [9,41]. So it was considered that 1.125 mL was present in the vagina at every moment in this pathology, representing an increase of 50%. The volume of 53 µL was defined proportionally, considering the area of the vaginal sheet circular portions.

The vaginal epithelium samples were fixed using a mucoadhesion rig (Stable Micro Systems) which was placed on the equipment's base. The whole system composed of the mucoadhesion rig with the tissue and the probe with the formulation was kept at 37 ± 1 °C by means of an oven. The vaginal tissue fixed in the rig was hydrated with 50 µL of VFS_m immediately before the beginning of the determination, since mucin is the protein most likely to be responsible for bioadhesion. A double-sided adhesive tape was used to attach the hydrated sheet portions to the probe.

The software was used in mucoadhesive mode. The pretest speed was 0.5 mm/s with a trigger force of 0.02942 N to allow for sensitive detection of the tissue. The test speed and the post-test speed were 0.1 mm/s. The contact/hold time was 180 s, and the force applied was 2.5 N [8,16]. In total, three porcine vaginal tubes were used. Each vaginal tube was divided into small portions. The 3 formulations ($n = 2$) and the control (no formulation, $n = 2$) were tested on adjacent portions of epithelium from the same vagina. The evaluation of mucoadhesion was performed in two different points of three vaginal sheets of the same formulation. The parameters maximum force of detachment (F_{max}), debonding distance (D_{dist}), work of adhesion (W_{ad}) were used to access the bioadhesive potential of vaginal sheets F2.1, F3.8 and F5.2. These parameters were compared to the control, consisting of a cellulose acetate membrane fixed in the double-sided adhesive tape, tested two times in each vagina used ($n = 6$).

Absorption efficiency of vaginal fluid simulant

The test of contact with the VFS was developed within this project to clarify the absorption capacity of the vaginal sheets, considering a physiological/pathological volume of fluid. A defined volume of VFS was added at both pH 4 (representing the normal physiological vaginal pH) and 5 (typical of BV pathology). The sheets tested were weighed before and after the addition of VFS, and their percentage of absorption was calculated, corresponding to the percentage increase in the formulation mass. Two VFS were prepared with the following compositions1.76 g NaCl; 0.70 g KOH; 0.11 g $Ca(OH)_2$; 0.009 g Bovine Serum Albumin; 1.00 g lactic acid; 0.50 g acetic acid; 0.08 g glycerol; 0.2 g urea; 5.0 g glucose; and MiliQ water to complete 500 mL. Finally, pH was adjusted to either 4 or 5 [7].

The vaginal sheets were cut to an adapted scale for this test. Its final defined size (7 × 2.4 cm) was reduced to 40%, that is, 2.8 × 0.96 cm. The simulant volume was also calculated to 40% (proportional reduction) and as a function of what is considered to be present in the vagina at random moments, in healthy conditions (0.75 mL). Since the increase in the volume of fluid in BV has not yet been estimated it was considered that a 1.5 fold could represent this condition (corresponding to 1.125 mL, 40% of which is 0.45 mL which was used for these experiments). Sheets were placed in glass Petri dishes and the VFS was poured over the samples that were monitored throughout time. When VFS at the surface was no longer detected (after 24 h) the samples were weighed, comparing this weight with its initial weight. The percentage of VFS that each sheet was able to absorb was calculated as absorption efficiency (%) by the following expression:

$$\text{Absorption efficiency}\,(\%) = (\text{VFS}_{\text{absorbed}}/\text{VFS added} \times 100) \quad (4)$$

Where VFS added represents the volume of VFS (0.45 mL for VFS pH 4 and pH5).

pH and buffer capacity

The evaluation of these parameters was based on the method described for vaginal gels and lubricants [42–44]. The vaginal sheet was dissolved at 37 °C in a 1:20 ratio (sheet weight:solvent volume). The solvents used were 0.9% NaCl (normal saline, previously used by other research groups since it exhibits a more neutral pH with low buffering capacity), VFS pH 4 and VFS pH 5 (both selected to better represent the dilutions that occur in the

vagina). The method started by measuring the initial pH of the dissolution. Then, 20 µL of 1 N NaOH (0.02 meq) were added until the pH was equal to or greater than 9. Control assays were performed only with dissolution media. The Absolute Buffering Capacity (ABC) was calculated as being the NaOH meq necessary to rise the pH one unit, from the graphical representation of values obtained in the titration.

Results

Organoleptic characterization

Concerning Formulation group 1, both sheets were pearl-Colored, opaque, homogeneous but with a deformed in shape. They were odorless and very rough to touch, having no malleability. Thus, these sheets were considered not to be interesting for the intended application.

Formulation group 2, gave rise to a very viscous gel, which was not possible to pour into the Petri dish. Thus, the formulation did not acquire the desired homogenous sheet form. Although being rough, this formulation presented some malleability due to the presence of propylene glycol. Thus it was concluded that the presence of propylene glycol is essential to confer malleability to formulations. It was also evident that it was impossible to prepare sheets with a gelatin content equal to or greater than 40%, even if they contained propylene glycol. Furthermore, sheets containing 25% of propylene glycol and increasing concentrations of gelatin (20, 25 and 30%), had increasing amounts of entrapped air bubbles due to high gel viscosity. After drying, these formulations were even harder. Lower propylene glycol concentrations were also tested, with the same content of gelatin, resulting in even harder formulations, with no envisaged application in vaginal products development. It was concluded that the proportion of plasticizer and polymer should not be reduced (see Formulation 6). To sum up, from the analysis of the organoleptic characteristics of F2, the desirable propylene glycol content for formulations with a gelatin concentration of more than 20% was found to be greater than 15%, while the best formulations corresponded to gelatin content below 25%.

Formulation Group 3 corresponding to Group 2 formulations with lactose added, resulted in very distinct organoleptic characteristics. The color acquired was a homogeneous opaque beige. These formulations were softer at the surface than the similar ones without lactose, but they were less elastic. The increase in the plasticizer (25%) with 20% of gelatin, provided an excellent formulation, beige colored, opaque, homogenous, odorless, soft to the touch and malleable.

In Formulation Group 4, the substitution of lactose for a hygroscopic powder of anhydrous sodium sulfate conducted to odorless, white, opaque and slightly heterogeneous formulations. Despite an increased malleability was found in those formulations with higher amounts of plasticizer, the formulations from this batch were characterized by poor general appearance and rigidity being considered not useful for the intended purpose. Eventually, the increase in plasticizer content (e.g. 25%) could improve the malleability. Or, reversely, the decrease in the concentration of sodium sulfate, would do so. Ultimately, excessive dryness on the vaginal cavity can come up with some toxicity. Therefore, this formulation series was abandoned.

Formulation Group 5 was designed to study the replacement of propylene glycol for glycerin. These formulations had lower loss of volume during lyophilization (the propylene glycol sheets exhibit greater retraction). Also, they became more malleable with touch (a characteristic recorded within a few seconds of manipulation – which reveals glycerin hygroscopic behavior) and did not acquire as much opacity with lactose as the propylene glycol sheets (it seems to assist lactose dissolution in the formulation). The sheets that showed the best organoleptic characteristics were all composed by glycerin at 25% (as in the sheets selected with the plasticizer propylene glycol), and this amount of glycerin even allowed the incorporation of a greater amount of gelatin (30%), without prejudice to its malleability. Lower concentrations of glycerin (10 and 15%) render the sheets unviable for application.

Table 2. Lyophilization efficiency (%) after freezing at two different temperatures (−20°C and −80°C).

Formulation	Lyophilization efficiency (%) −20°C	Lyophilization efficiency (%) −80°C
F2.1 (Gelatin 20%, Propylene glycol 25%)	43.9	68.8
F2.2 (Gelatin 25%, Propylene glycol 25%)	48.2	62.9
F2.3 (Gelatin 30%, Propylene glycol 25%)	32.5	52.4
F2.4 (Gelatin 20%, Propylene glycol 15%)	48.2	67.0
F2.5 (Gelatin 25%, Propylene glycol 15%)	53.3	61.3
F2.6 (Gelatin 30%, Propylene glycol 15%)	52.6	53.9
F3.1 (Gelatin 25%, Propylene glycol 25%, Lactose 10%)	32.1	50.8

The sixth group of formulations contains lower concentration of polymer and plasticizers in order to reduce toxicity. Since the amount of excipients was reduced to half, the water content was largely increased. This modification resulted in thinner and lighter formulations due to greater water evaporation on the lyophilization process.

Group 7 represents the change from gelatin to chitosan. From this batch no sheets were selected, because they revealed a spongy/elastic appearance, with poor structure and, in general, very thin. Chitosan concentrations ranged from 1% to 3% for medium and high molecular weight, and when a plasticizer was introduced, it had a concentration of half that of the polymer, as previously described for chitosan films [37]. Some formulations were not frozen at −80 °C but only at −20 °C and refrigerated at 4 °C for 24 h prior to drying. This variation in the preparation method of the sheets destabilized the lyophilization process and conducted to porous structures easily identified by naked eye. As a general observation for all formulation groups, the lower the temperature used for pre-freezing before freeze-drying, the more efficient the drying process (Table 2) and the better the organoleptic characteristics of the sheets. Chitosan sheets were thin, white, spongy, heterogeneous and without elasticity (though malleable), with a characteristic shellfish odor. In addition, these formulations oxidize easily, resulting in a yellowish color with time. From the organoleptic point of view, no significant differences were observed between MMW and HMW chitosans formulations.

Formulation Group 8 only contains sheets combining gelatin and chitosan. The resulting organoleptic characteristics for these formulations were not ideal, although possibly with further optimization could they become promising. This combination required a further increase in the plasticizer amount beyond the limits described as safe for this excipient [45]. Additionally, it was evidenced that lower molecular weight chitosan could provide better formulations.

Figure 2 shows the general aspect of formulations.

Thickness evaluation

All vaginal sheets presented 2 mm thickness, measured in three different points. The structure of the formulation was maintained

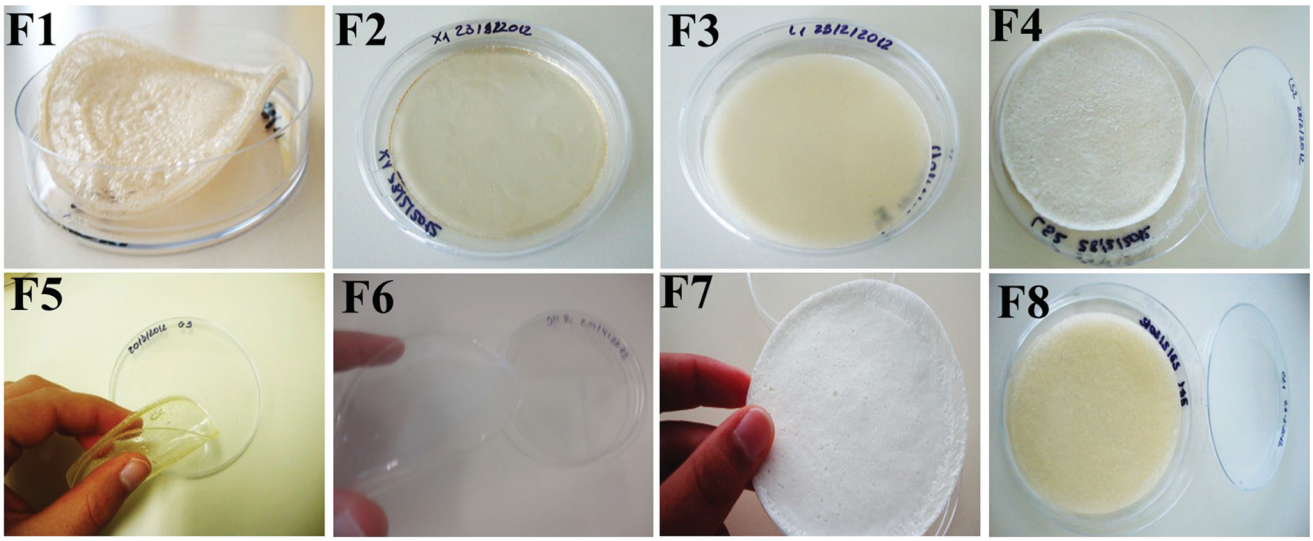

Figure 2. The general aspect of some prepared vaginal sheets concerning the seven formulation groups. From F1 the formulation represented is F1.1 (gelatin 20%). F2 is represented by F2.1 (gelatin 20%, propylene glycol 25%). In F3 the introduction of lactose is evidenced in F3.1 (gelatin 25%, propylene glycol 25%, lactose 10%). F4.2 is the representative formulation of group 4 (gelatin 25%, propylene glycol 10%, anhydrous sodium sulfate 10%). In F5 a formulation of gelatin 25% and glycerin 25% (F5.3) is represented. F6 group is represented by F6.11, a formulation that contains reduced (but proportional) amounts of gelatin and glycerin (10% and 7.5%, with no lactose in this specific example). Regarding group 7, a formulation of propylene glycol 0.5% and HMW chitosan1% is represented (F7.2). F8 shows a formulation of gelatin 13.5%, propylene glycol 15% and HWM chitosan 1.5% (F8.2).

Table 3. Weight loss (%), texturometric analysis – hardness (*N*) and resilience (%), and absorption efficiency (%) of the selected formulations.

Formulations	Weight loss (%)	Hardness (*N*)	Resilience (%)	Absorption efficiency (%) VFS pH4	Absorption efficiency (%) VFS pH5
F2.1 (Gelatin 20%, Propylene glycol 25%)	22.1 ± 2.8	0.103 ± 0.044	59.4 ± 2.9	58.8 ± 5.8	50.8 ± 8.2
F3.1 (Gelatin 25%, Propylene glycol 25%, Lactose 10%)	14.1 ± 0.0	0.052 ± 0.003	57.9 ± 4.0	57.6 ± 0.0	64.4 ± 0.0
F3.8 (Gelatin 20%, Propylene glycol 25%, Lactose 10%)	16.5 ± 1.8	0.046 ± 0.004	58.3 ± 10.5	68.2 ± 0.0	81.0 ± 11.5
F5.1 (Gelatin 20%, Glycerin 25%)	14.9 ± 0.8	0.065 ± 0.011	29.8 ± 1.2	18.2 ± 2.8	38.2 ± 0.0
F5.2 (Gelatin 20%, Glycerin 25%, Lactose 10%)	17.7 ± 2.6	0.067 ± 0.038	27.0 ± 1.5	44.0 ± 9.4	71.3 ± 0.0
F5.3 (Gelatin 25%, Glycerin 25%)	12.7 ± 0.8	0.101 ± 0.032	24.0 ± 0.3	21.3 ± 0.0	41.6 ± 0.0
F5.4 (Gelatin 30%, Glycerin 25%)	9.9 ± 1.0	0.104 ± 0.012	20.5 ± 0.6	36.6 ± 18.9	74.0 ± 0.0
F5.12 (Gelatin 20%, Glycerin 15%, Lactose 10%)	15.5 ± 2.2	0.093 ± 0.051	18.5 ± 2.3	12.6 ± 4.9	52.4 ± 0.0

Values are expressed as mean ± SD (triplicate measurements in two independent formulations).

after the freeze drying process, since the sheet thickness (2 mm) corresponds to the height of the gel added in the petri dishes. The freeze drying process removed the water, maintaining the three-dimensional structure of the gel.

Lyophilization efficiency

The freeze-drying efficiency was compared with prior freezing at −20 °C and −80 °C. The objective was to optimize the drying technique of the vaginal sheets. For this, several samples were selected. The freeze-drying efficiency characterizes the loss of water occurring during the process.

Using different pre cooling temperatures for freeze drying, the lyophilization efficiency showed to be higher when the sheets were pre-frozen at −80 °C (Table 2). Actually, the sheets frozen at −80 °C were, in advance, closer to the temperature reached by the freeze-dryer (approximately −110 °C). The formulations, freeze-dried with pre-cooling at −80 °C, were analyzed for mass loss (Equation 1). This was calculated by the change in mass after lyophilization, relative to the total mass before lyophilization (in percent). The results obtained for the formulations studied are described in Table 3.

Regarding the effect of the type of plasticizer used (propylene glycol or glycerin), it was observed that no significant differences were found regarding the loss of mass in similar formulations (regarding quantitative compositions) that also contained lactose (F3.8 and F5.2). However, for the comparison between F2.1 and F5.1 (containing only gelatin and the plasticizer) the difference was significant (ANOVA one-way, Tukeys's multiple comparisons test, $p < 0.05$). Thus, it can be concluded that the type of plasticizer may influence the lyophilization process although this effect may be hindered by the overall composition of the formulation. Both propylene glycol and glycerin are humectants and therefore it could be hypothesized that an increase in their concentration can retain more water in the formulation implying less mass loss (considering that lyophilization is only based in water loss). he analysis of the results shows that formulations that only differ in glycerin content (F5.2–25% and F5.12–15%) do have no different mass loss with increased glycerin (17.7% and 15.5%), which highlights the efficiency of the selected lyophilization method against the glycerin wetting ability.

Texturometric analysis

In accordance to what have been demonstrated during the evaluation of the organoleptic characteristics, sheets containing propylene glycol were more resilient than those containing glycerin (Table 3). This aspect was evidenced by the comparison of the results obtained with formulations that only differed in the type of plasticizer used: F2.1 and F3.8 showed resilience of 59% and

58%, respectively, compared to values of only 30% and 27% obtained with F5.1 and F5.2 (equivalent formulation where only propylene glycol has been replaced by glycerin). The sheets that had lactose in their composition (F3.1, F3.8, F5.2 and F5.12) did not have their texture characteristics markedly affected. F3.1 and F3.8 presented higher resilience due to their higher percentage of plasticizer (25%) compared to F5.12 (15%). F5.2 had 25% plasticizer – glycerin – and yet F3.8 (25% propylene glycol) had a higher resilience.

In terms of hardness, sheets having a higher gelatin content presented higher hardness (F5.4, F5.3). In fact, formulations with increasing concentration of gelatin (F5.1–20%, F5.3–25% and F5.4–30%) and 25% glycerin, presented a gradual increase in hardness (0.065, 0.101 and 0.104, respectively), while resilience decreased (29.8%, 24% and 20.5%), highlighting the influence of the polymer: plasticizer ratio on the final parameters. For F5.2 and F5.12, formulations varying only in the concentration of glycerin, the hardness did not diverge substantially (taking into account the associated standard deviation) while the resilience was 27% for F5.2 (containing glycerin in the proportion of 25%) and 18% for F5.12 (where the glycerin content is reduced to 15%). To sum up, the increase in hardness seems to be associated with increased gelatin concentration while increased resilience is related to the amount of plasticizer.

Bioadhesion

Bioadhesion represents the ability of a formulation to adhere to a biological surface, in this case, the porcine vaginal epithelium. The evaluation of this parameter is essential for the characterization of the product. Bioadhesive properties allow a more intimate and prolonged contact of the product with the vaginal surface, promoting longer residence times and reducing the leakage which is considered one of the main disadvantages of vaginal administration. The introduction of polymeric excipients in the formulations (polyacrylates, chitosans, cellulose derivatives, hyaluronic acid and derivatives, pectin, starch, and several natural gums, among others) has been a strategy used to promote bioadhesion [8,46,47].

Vaginal sheet F2.1 was the most bioadhesive among the formulations analyzed (Table 4). On the other hand, formulation F5.2 was found to be the least bioadhesive, presenting values of adhesiveness and adhesion work similar to the control. The standard deviation was quite high, but this is a difficulty associated with determinations with biological samples. In fact, there is a large variability associated with porcine vagina tissue in terms of thickness, hardness and surface irregularities of the epithelium. However, the method described resulted in reproducible results, skipping their major difficulty when working with biological surrogates. The study design considers variability between vaginas and between different portions of the same vagina, that occur *in vivo*.

Additionally, the bioadhesion potential of all formulations analyzed was superior to the bioadhesion potential of Dalacin V® (an antibacterial to treat BV) previously analyzed by the research group, using the same determination method (Dalacin V® work of adhesion = 0.020 ± 0.04 N.mm and peak force-adhesiveness 0.033 ± 0.006 N, comparing to the control work of adhesion = 0.015 ± 0.002 N.mm and peak force-adhesiveness 0.027 ± 0.012 N) [8].

Absorption efficiency of vaginal fluid simulant

It was found that all sheets presented the capacity to absorb fluids: a swelling behavior. That means that there was always an increase in mass of the sheet after contact with fluid (which reveals no impermeability, no loss of fluid, or degradation of the sheets). For all sheets except F2.1, a higher absorption efficiency at pH 5, was obtained when compared to pH 4. This is, indeed, a very interesting result, since it is exactly at a higher pH (characteristic of BV where there is also excess fluid) that it is necessary to absorb a greater amount of fluid. Furthermore, in BV, the aim is to lower the pH to lessen the growth of pathological microorganisms. In fact, the gelatin has a pH ranging from 3.8 to 7.6 [36]. However, the pH of the solution obtained is close to neutrality and outside the normal acidic pH range of the vaginal environment. aAn acidic component in the formulation, such as lactic acid, a vaginal physiological one, could be included to improve the formulation. Table 3 resumes the absorption efficiency for the selected vaginal sheets, concerning VFS at pH 4 and pH 5. It is clear that almost all formulations have high ability to absorb the fluid, and that those containing lactose, present higher absorption capacity (F3.1, F3.8, F5.2, F5.12). Generally, formulations at pH 5 have the ability of retaining approximately half of their mass in fluid. VCF was used as control in this test, but it rapidly disintegrated and was not possible to isolate and weight it after being in contact with fluids (therefore no results are shown on Table 3 for this formulation).

Table 4. Evaluation of the mucoadhesive potential of formulations F2.1, F3.8 and F5.2.

Formulation	Work of adhesion (N.mm)	Peak force (adhesiveness – *N*)	Debonding distance (mm)
F2.1 ($n = 6$)	0.043 ± 0.031	0.095 ± 0.085	1.962 ± 0.211
F3.8 ($n = 6$)	0.038 ± 0.035	0.077 ± 0.040	2.282 ± 0.443
F5.2 ($n = 6$)	0.029 ± 0.018	0.053 ± 0.033	2.000 ± 0.534
Control ($n = 6$)	0.030 ± 0.009	0.051 ± 0.019	1.315 ± 0.962

pH and acid-buffering capacity

For the purpose of testing the pH and acid-buffering capacity of the sheets, the formulations were forced to be dissolved (in the ratio of 1:20 mass:volume) in normal saline or VFS of different pH. The sheets were placed in contact with the respective solvents at 37 °C and all sheets were found to completely dissolve in this proportion of media. The buffering capacity was further determined in VFS at pH 4 and pH 5 to evaluate the change induced therein. The buffering capacity was studied in the selected sheets of Formulation Group 5 which enabled the comparison of increasing concentrations of gelatin and compared with F3.8, a formulation with propylene glycol.

Table 5 shows the absolute buffering capacities obtained for the NaCl 0.9%, VFS at pH 4, and 5. Normal Saline was shown to have very low acid-buffering capacity.However, soon after the addition of 0.02 meq of NaOH all formulations, except F5.2, reached a pH higher than 8 revealing low buffering capacity. The addition of 0.06 meq of NaOH raised the pH of all sheets (dissolved in normal saline) to values above pH 10.

For dissolutions in VFS pH 4, a clear influence of some inherent acid-buffering capacity of the VFS could be noted since the pH of the solutions prepared by dissolution of the sheets is acidified in the presence of VFS (in comparison with NaCl 0.9%). Although this property was expected it was verified that in contact with the VFS the sheets exhibit a greater acid-buffering capacity, compared with the buffering capacity of the sheets in saline solution. However, the optimal pH for vaginal sheets remains to be determined and has to be adjusted to the intended therapeutic action.

Table 5. Absolute buffering capacity (ABC – meq NaOH) of the selected vaginal sheets in NaCl 0.9%, VFS pH4, and VFS pH5 ($n = 1$).

	NaCl	VFS pH 4	VFS pH 5
F2.1 (Gelatin 20%, Propylene glycol 25%)	0.0169	0.0101	0.0004
F3.8 (Gelatin 20%, Propylene glycol 25%, Lactose 10%)	0.0040	0.0530	0.0050
F3.1 (Gelatin 25%, Propylene glycol 25%, Lactose 10%)	0.0231	0.1055	0.0299
F5.1 (Gelatin 20%, Glycerin 25%)	0.0060	0.0606	0.0049
F5.2 (Gelatin 20%, Glycerin 25%, Lactose 10%)	0.0195	0.0625	0.0320
F5.3 (Gelatin 25%, Glycerin 25%)	0.0118	0.0702	0.0165
F5.4 (Gelatin 30%, Glycerin 25%)	0.0095	0.0400	0.0135
F5.12 (Gelatin 20%, Glycerin 15%, Lactose 10%)	0.0150	0.0544	0.0202
F6.1 (Gelatin 5%, Glycerin 6.25%, Lactose 10%)	0.0075	0.0078	0.0034
F6.2 (Gelatin 10%, Glycerin 12.5%, Lactose 10%)	0.0051	0.0216	0.0031
F6.5 (Gelatin 10%, Glycerin 12.5%)	0.0089	0.0093	0.0079
F6.6 (Gelatin 15%, Glycerin 18.75%)	0.0083	0.0141	0.0074
F6.11 (Gelatin 10%, Glycerin 7.5%)	0.0029	0.0068	0.0001
F6.12 (Gelatin 15%, Glycerin 11.25%)	0.0066	0.0111	0.0059
Control NaCl	0.0084		
Control VFS pH 4	–	>0.16	–
Control VFS pH 5			0.028

Again, F5.2 presented the best acid-buffering ability, which suggests that this formulation is compatible and adequate to the intended purpose. For VFS at pH 5, the sheet behavior was different from that observed at pH4. In this assay, the sheets did not demonstrate a buffer capacity as evident as that found at pH 4. Only three additions of 0.02 meq NaOH made the pH ranged from approximately 5 to values between 9 and 11. This may be explained by the fact that the assay already starts at a higher pH. F3.8 and F5.1 revealed to have the lowest buffer capacities. F3.1 and F5.2, of similar composition (only varying in terms of the amount in gelatin, F3.1–25% and F5.2–20%) showed the best acid-buffering behavior. This data reveals that, in fact, there will be in the formulations a buffering promoting agent (e.g. Gelatin in formulations F5.1 and F5.3). It remains to be clarified, which of the constituents will have this capacity (plasticizer, gelatin or lactose) and in which concentrations will allow to conjugate the acceptability of its organoleptic characteristics with the buffer capacity. The best buffer capacity was found for F5.2 sheet (20% gelatin, 25% glycerin and 10% lactose). The simple substitution of the plasticizer led to a decrease in the buffer capacity (verified by direct comparison with F3.8), showing that glycerin may promote a better buffer capacity than propylene glycol in vaginal sheet formulations. Increased gelatin concentration (F5.1, F5.3, and F5.4) does not appear to offer an advantage regarding acid-buffering capacity and the role of lactose still needs to be elucidated (F5.2 and F5.1 only vary in the introduction of lactose and the results are very disparate).

Discussion

The development and optimization of the vaginal sheet formulation was undertaken in view of practical use, improved patient and clinical acceptability, and enhanced therapeutic and pharmaceutical characteristics for the treatment of vaginal affections related with excessive fluids.

The excipients were selected based on their prior use in other vaginal dosage forms and their toxicity profile. Other relevant performance parameters (such as known mucoadhesion) were also considered. The chosen plasticizers (propylene glycol and glycerin) are described on the literature as having additional functions, such as, being antimicrobial preservatives, solvents and co-solvents, humectants, and plasticizers [36]. Moreover, these two plasticizers are already used in vaginal films [34,36], from which the vaginal sheet represents a variation. Regarding the development of the vaginal sheet, Formulation Groups 2, 3, 4 and 8 were prepared exclusively using propylene glycol as plasticizer, while Formulation Groups 5 and 6 were prepared with glycerin to achieve a term of comparison. Glycerin and propylene glycol are widely used as excipients in products for vaginal administration, and have not been associated to toxicity when low concentrations are present in these products ($\leq$10%). However, they can cause significant toxicity when applied at higher concentrations, due to their humectant and hypertonic characteristics (5700–9900 mOsmol/Kg) [48]. Clinical data show that, compared to glycerin, propylene glycol is more associated with vaginal irritation [45], dermatitis and allergic sensitization [49]. Glycerin was therefore chosen as an alternative plasticizer for the vaginal sheet.

The vaginal sheet dimension (7×2.4 cm) was proposed since this corresponds to the mean dimension of the human vagina. Together with its shapeand mode of application it is expected to lead to an adequate coverage of the vaginal cavity and allow a homogeneous dispersion of the active substance. The bioadhesive polymers are expected to have an additional action in fixing the sheet to the vaginal cavity allowing for longer retention time [34,50].

Gelatin and chitosan were selected to fulfill the polymeric portion of the vaginal sheet. Gelatin, is a mucoadhesive macromolecule largely used in vaginal formulations [6]. It is obtained from natural origin, nontoxic, easily manipulated, allows controlled drug release and is economical [36,50]. Due to its high hygroscopic characteristics, it is expected that gelatin will promote an absorption of excessive vaginal fluid, as encountered in the BV. Chitosan is a deacetylate chitin derivative, the second most abundant natural polymer in the crustacean shells and in the fungal cell walls, and has a satisfactory biocompatibility [51]. Chitosan is a homopolymer with β (1→4) bonds between N-acetyl-D-glucosamine residues, which are positively charged providing a chemical bond with negatively charged fatty acids, lipids, cholesterol, metal ions, proteins and macromolecules [52]. There are already several pharmaceutical applications based on chitosan, taking advantage of its properties [53–55]. As excipient, chitosan has been used in various formulations such as powders, tablets, emulsions and gels. Controlled release, mucoadhesive and antimicrobial properties are expected to be characteristics imparted to formulations by chitosan [3,6,38,56,57]. This sheet represents a special variation of vaginal films specifically regarding its size, shape and its specific aim to absorb excessive vaginal fluids instead of being rapidly dissolved by them.

Like vaginal films, in general, the vaginal sheet has a great potential to overcome some of the major obstacles referred by women regarding the pharmaceutical forms of vaginal application (uncomfortable use, leakage (of the creams and gels) and non-disintegration (of the ovules and tablets)) [58]. This is because it is expected to be non-painful at insertion, comfortable to use and not prone to leakage. In fact, the vaginal sheet, unlike vaginal films, was designed not to undergo full disintegration and so that it can be removed or be naturally expelled. One of the main applications for the vaginal sheet will be the treatment of BV. In this condition, the excess fluid becomes very uncomfortable for the patient. The fact that freeze-drying allows for the overall structure of poured gels to be maintained explains why these vaginal sheets are thicker than conventional films which may actually present an advantage for vaginal insertion without requiring the use of an applicator.

The vaginal sheet, formulated with hygroscopic excipients, may as first step absorb the excess fluid, and thus predisposing the vaginal cavity to a more effective subsequent pharmacological treatment (the active substance of which may also be conveyed on a vaginal film to be inserted after). Another approach could be to include the antimicrobial substance directly in the vaginal sheet aiming at a double feature (controlling symptoms and treating the infection in one step).

The formulations were firstly prepared as aqueous gels, based on gelatin and as acidic dispersions of chitosan. To adjust the pH of formulations, the use of lactic acid in the formulations of gelatin could be a promising strategy. For chitosan formulations, one study showed that the use of more concentrated lactic acid solutions made films more elastic and easier to apply [59]… Previous studies have been published on the development of chitosan films including applications for dermal burns treatment (prepared by dissolving the polymer in lactic acid) and chitosan sponges [55–58]. Particularly, sponges for periodontal use were prepared through freeze-drying but the method applied led to the preparation of a sponge with different properties from those presented in this study [58].

The lyophilization efficiency was calculated by Equation 2, which relates the weight of solvent lost through this drying technique (the difference between the weight before lyophilization and after), and the total weight of the solvent contained in the formulation. Through the analysis of Table 2, and also considering the water content present in each formulation we can conclude that the lyophilization efficiency does not depend exclusively on the initial water content of the formulation, but also other factors might be playing an important role in this process. In any case the process is not expected to remove all water from the formulation. For example, for soft capsules, the water content constitutes 30–40% of the wet gel that is removed by controlled drying, reaching 5–8% water in the dry state [38]. The vaginal sheets have a higher initial content in water and may also retain a higher content of humidity, since they are intended to have greater malleability than soft capsules. Specifications for water content in the final formulation may, in a large-scale production of the vaginal sheet, be defined for quality control of batches.

Hardness and resilience were determined through a texturometer. Due to the reduced thickness limitation of this type of pharmaceutical form (when compared to others such as gels) determination of hardness was performed with a needle-shaped probe that penetrated the sample for only 1 mm. Although only the values obtained for the selected formulations (based on the organoleptic characterization) are herein presented, it was possible to conclude from the measurements of the remaining sheets that the harder sheets corresponded to the less resilient ones. In some cases rupture of the sheet occurred during the test. These two parameters demonstrated an inverse relationship that is in accordance with the organoleptic analysis (high hardness and very low malleability). Also, these parameters could be process control points in the sheet manufacturing process.

Since the main purpose of this formulation is the absorption of excessive fluids, the absorption efficiency of the vaginal sheet was studied. Furthermore, a Vaginal Contraceptive Film (VCF), already marketed in the USA, was used as control in this test. It is claimed as a fast-disintegrating film, and it was actually completely disintegrated after one hour at both pH conditions, unlike the vaginal sheet. Thus, through this *in vitro* test, it was clear that the behavior of the vaginal sheet *in vivo* will be quite different from that of the vaginal films. In general, the main objective of films is the dissolution and dispersion of the active pharmaceutical ingredient that they carry, while for the vaginal sheet the objective is that it is retained in the vaginal cavity exerting its action of absorption of the excess of fluid, and then it can be manually removed or naturally expelled. Some difficulties were encountered in the design of this assay, since the traditional methods described [60] and also those cited by some authors [16,29,61] do not directly evaluate the behavior of the dosage forms considering the vaginal application. These consider very high volumes (not related with physiological and pathological conditions of the vagina), since they are intended to characterize the pharmaceutical form and not to predict its behavior *in vivo*. Adaptations of methods for evaluating pharmaceutical forms for vaginal application are, therefore, essential for better characterization [8] while it would also be of main interest to define the volumes of vaginal fluid present in the various pathologies. These adaptations may contribute to the design of pharmaceutical formulations that better fit the vaginal cavity characteristics. Also, this will allow to improve the characteristics that lead to greater acceptability of the pharmaceutical forms for vaginal use.

This particular study demonstrated that it is essential to develop protocols able the evaluate the bioadhesive potential of pharmaceutical forms considering the pathological vaginal environment. This parameter should be evaluated in the early stages of product development, especially for vaginal sheets, since it will have a huge impact on their *in vivo* effectiveness. Since BV is characterized by an increased discharge, it is critical that the vaginal sheets intended to treat this pathology become bioadhesive gels while slowly absorbing the excess of vaginal fluid, overcoming the leakage associated with semi-solid traditional products and simultaneously alleviating the discomfort associated with a bad odor discharge.

Regarding manufacturing issues, particular attention had to be payed to avoid incorporating air into the gels that would appear as holes in the dried sheets. This problem could be solved by applying a sonication process to remove the air bubbles [16]. The freeze-drying technique was shown to be particularly interesting to obtain this final form and may represent a tremendous advantage over vacuum drying [61] and simple evaporation of the solvent (solvent-casting) [29] techniques (frequently used to obtain films) when heat-sensitive drugs are selected to be incorporated in the product. Another aspect that can be controlled during preparation is the pH of the formed gel and its adjustment to the desired pH [62,63].

Concerning the adaptation of methods for the vaginal sheet analysis/verification tests, we described their characterization in low volume of VFS unlike previously published works which have been based on the use of high amounts of VFS (10 and 25 mL). Therefore, it was possible to predict more rigorously the behavior of the vaginal sheet in contact with the vaginal fluid. These studies conducted to the determination of an optimal proportion of polymer:plasticizer of 20%:25%, respectively, and this composition was shown to further sustain the addition of 10% of a drying powder or an active pharmaceutical ingredient. Formulations F2.1, F3.8 and F5.2 were found to be the most promising formulations for vaginal administration.

The vaginal sheets developed within this study stand as interesting vehicles for various drugs with wide application in infectious and inflammatory vaginal affections.

Conclusion

Formulation and characterization studies were performed to successfully develop a renewed vaginal dosage form from vaginal

films: the vaginal sheet. This consists of gelatin, a plasticizer (propylene glycol or glycerin) and water. In addition, a powdered active substance (which has been tested with lactose) can be incorporated. The method of obtaining this new pharmaceutical form consisted on the preparation of a gel which, when lyophilized, gives rise to the vaginal sheet. The proportion of gelation and plasticizer was shown to be critical for textural properties with hardness and resilience presenting an inverse relation. The VFS test allowed to observe the possible behavior of the sheet when administered *in vivo*. The sheet is not expected to dissolve but rather to absorb the excess of existing vaginal fluid in pathologies such as BV. This ability is mainly attributed to gelatin, due to its higroscopic properties. The final pH of the vaginal sheets is a parameter that may be adjusted being dependent on its therapeutic purpose. Ideally, the vaginal sheet should present a good acid-buffering capacity so that vaginal pH remains at the desired acidic value and the infection may be treated more effectively. These results have shown the potential for this vehicle to be used either as a first step of treatment focused on controlling symptoms through absorption of excessive fluids or a dual strategy of fluid absorption and antimicrobial drug release Further studies under *in vitro* conditions that may represent the specific environment of BV would be particularly relevant to predict the *in vivo* performance of this formulation. To the best of our knowledge, such specific methods are not yet available.

Variations from this basic formulation to fulfill other purposes may obviously be envisaged. Its design, technology and wide applicability aim the creation of a therapeutic alternative among a group of pharmaceutical forms whose feminine acceptability and compliance is still an issue.

Disclosure statement

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