European Journal of Pharmaceutical Sciences 109 (2017) 556–568

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

# Vaginal semisolid products: Technological performance considering physiologic parameters

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ARTICLE INFO

*Keywords:*
Vaginal drug delivery
Semisolids
Vaginal products
Physiologic methods

ABSTRACT

Vaginal semisolid products are frequently used to treat vaginal infections and atrophy-related symptoms of menopause. Formulations composition and the methods for their characterization, especially those developed concerning the target epithelia, are key tools to predict *in vivo* results at early stages of product development. However, recent studies on this subject have been almost exclusively focused on anti-HIV preparations. The aim of this work consists on improving traditional characterization methods by using physiological parameters in order to construct predictive tools to characterize a new ideal vaginal semisolid formulation whatever target it may have. Ten vaginal antimicrobial and hormonal products already available in the market were studied (Gino-Canesten®, Sertopic®, Dermofix®, Gyno-pevaryl®, Lomexin®, Gino Travogen®, Dalacin V®, Ovestin®, Blissel®, Colpotrophine®). Furthermore, Universal Placebo gel and Replens® were used for comparison. Products were characterized in terms of: pH and buffering capacity in a vaginal fluid simulant (VFS); osmolality - directly and upon dilution in VFS; textural parameters (firmness, adhesiveness and bioadhesion) using vaginal *ex vivo* porcine epithelium; and viscosity (including VFS dilution at 37 °C and after administration on an *ex vivo* model). Interestingly, the majority of the tested commercial vaginal formulations did not present technological characteristics close to the ideal ones when tested under target biological conditions. The inclusion of such methodologic adaptations is expected to optimize cost-efficiency of new formulations development by predicting efficacy and safety profiles at early stages of product development.

## 1. Introduction

The vaginal route has been considered of great interest for drug delivery, since it enables both local and systemic drug delivery (Alexander et al., 2004; Hussain and Ahsan, 2005; Ndesendo et al., 2008; Srikrishna and Cardozo, 2013). The vaginal route provides different advantages over the oral one: it allows for high concentrations with less systemic adverse effects when local therapy is aimed, while its large surface area, rich blood supply, ability to bypass hepatic first-passage, avoidance of gastrointestinal side effects, and relatively high permeability to a wide range of molecular weight drugs represent particular physiological features that contribute to its pharmacokinetic advantages for systemic therapy (Acartürk, 2009; Choudhury et al., 2011; Hani et al., 2010; Katz et al., 1997; Katz and Dunmire, 1993; Vermani and Garg, 2000; Weber et al., 2005; Wong et al., 2014; Wu and Robinson, 1996).

Several formulations for vaginal administration are available in the market as medicines or OTC (Over-the-Counter) products, some of them classified as medical devices or cosmetics. However, especially for the later ones, there are few data on their potential to cause acute irritation and toxicity, or their suitability is poorly supported, particularly when preclinical assays were not performed on animals and clinical trials. Among all vaginal dosage forms, semisolids have been reported to be the most frequently used and preferred (Coggins et al., 1998; Hardy et al., 1998a, 1998c; Palmeira-de-Oliveira et al., 2015a). Acceptability of a vaginal product represents a major factor for its effectiveness since it clearly influences correct and consistent use, especially when long-term use is required. The development of more appropriate vaginal

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http://dx.doi.org/10.1016/j.ejps.2017.09.009
Received 7 June 2017; Received in revised form 1 August 2017; Accepted 4 September 2017
Available online 05 September 2017

**Table 1**
Studied formulations general characteristics (information provided by the manufacturer).

| Therapeutic action | Trade name/labelled dosage form | API | API concentration | Excipients | Marketing autorization holder |
|---|---|---|---|---|---|
| Antifungals | Gino-Canesten®, vaginal cream | Clotrimazole | 10 mg/g | Benzyl alcohol, Cetyl palmitate, Cetostearyl alcohol, Purified water, Polysorbate 60, Sorbitan monostearate, Octyldodecanol | Bayer Portugal, S.A |
| | Sertopic®, vaginal cream | Sertaconazole | 2 g/100 g | Ethyleneglycol and polyethyleneglycol, Stearate palmite, Saturated glycerides polyglycolized, Glycerol isostearate, Liquid paraffin, Methylparaben, Sorbic acid | Ferrer Portugal, S.A. |
| | Dermofix®, vaginal cream | Sertaconazole | 20 mg/g | Tefose 63, Labrafil, Peceol, Liquid paraffin, Nipagin, Sorbic acid | Azevedos Laboratories - Pharmaceutical Industry, S.A. |
| | Gyno-pevaryl®, vaginal cream | Econazole | 10 mg/g | Stearate pegoxol 7, Liquid paraffin, Oleic macrogolglycerides, Butylhydroxyanisole (E320), Benzoic acid, Purified water | Johnson & Johnson, Lda. |
| | Lomexin®, vaginal cream | Fenticonazole | 20 mg/g | Propylene glycol, Hydrogenated lanolina, Sweet almond oil, Polyglycol esters of fatty acids, Cetyl alcohol, Glyceryl monostearate, Sodium EDTA, Purified water | Recordati Ireland Ltd. |
| | Gino Travogen®, vaginal cream | Isoconazole | 10 mg/g | Polysorbate 60, Sorbitan stearate, Cetostearyl alcohol, Thick paraffin, White Vaseline, Purified water | Bayer Portugal, S.A. |
| Antibacterials | Dalacin V®, vaginal cream | Clindamycin | 20 mg/g | Propylene glycol, Cetostearyl alcohol, Liquid paraffin, Sorbitan stearate, Benzyl alcohol, Cetyl palmitate, Stearic acid, Polysorbate 60, Purified water | Pfizer Laboratories, Lda. |
| Oestrogens | Ovestin®, vaginal cream | Estriol | 1 mg/g | Octyldodecanol, Glycerol, Cetyl alcohol<br>Stearyl alcohol, Polysorbate 60, Sorbitan stearate, Lactic acid, Chlorhexidine hydrochloride, Sodium hydroxide, Purified water, Synthetic spermaceti | Aspen Pharma Trading Ltd. |
| | Blissel®, vaginal gel | Estriol | 50 μg/g | Glicerol (E 422), p-hydroxybenzoic acid methyl ester, p-hydroxybenzoic acidpropyl ester, Policarbophil, Carbomer, Sodium hydroxide, Hydrochloric acid, Purified water | ITF Medivida, Pharmaceutical Products, Unip. Lda. |
| | Colpotrophine®, vaginal cream | Promestriene | 1 g/100 g | p-hydroxybenzoic acid methyl ester, p-hydroxybenzoic acid propyl ester, Mono- and diglycerides of saturated fatty acids, Polyglycol ether of saturated fatty alcohols, Oleic acid decyl ester, Triglycerides of capric and caprylic acids, Glycerol, Purified water | Teva Pharma - Pharmaceutical Products, Lda. |
| Controls (Reference products) | Universal placebo, vaginal gel | Not applicable | Not applicable | Purified water, Hydroxyethylcellulose, Sodium chloride, Sobic acid, Caramel colour, Sodium hydroxide | Not applicable |
| | Replens® | Not applicable | Not applicable | Purified water, Policarbophil, Paraffin oil, Glycerin, Palm oil hydrogenated, Carbomer homopolymer type B, Sorbic acid, Sodium hydroxide | Lil'Drug Store Products, Inc. |

products must consider women's preferences that may, in turn vary, depending on their age, socio-economic status and cultural backgrounds, and on the type of product they would need to use (Hardy et al., 1998a; Nappi et al., 2006). The high prevalence of vaginal diseases and the disadvantages of contraception through the oral route seem to explain the frequent use of vaginal products among women (Palmeira-de-Oliveira et al., 2015b, 2014). Although the vaginal route is generally perceived as safe, acceptability of vaginal dosage forms is hindered by comfort issues related with unpleasant application, difficulties in insertion, leakage and interference with sexual activity (Coggins et al., 1998; J das Neves et al., 2008a, b; Joglekar et al., 1991).

Among the semisolid vaginal dosage forms creams and gels have gathered particular interest among researchers engaged in developing vaginal formulations (Allen et al., 2011). They have been pointed as the most preferred dosage forms among women although leakage has been frequently referred as their major drawback. These formulations may require multiple daily administrations in order to obtain the desired therapeutic concentration and to provide a uniform distribution of the drug (Baloglu et al., 2009; Hussain and Ahsan, 2005; Rohan and Sassi, 2009; Yoo et al., 2009).

To overcome this limitation, major research in this field has been focused on bioadhesive polymers or novel dosage forms, but by now only limited evolution has been found in commercial vaginal products. Those strategies are, however, expected to increase residence time, improving both efficacy and safety of the new delivery systems (Acartürk, 2009; J das Neves et al., 2008a, b; Machado et al., 2013; Valenta, 2005). In fact, the major challenge in vaginal dosage forms design is the ability to fulfill functional criteria such as product dispersion throughout the vagina, prolonged residence time, adequate physicochemical interaction with vaginal content, release profile of active ingredients, and effects on targets (Garg et al., 2005; Woodley, 2001).

Technological properties of vaginal semisolids have been considered to correlate with their *in vivo* performance regarding safety and efficacy. This is the case for the ability of formulations to maintain the normal vaginal pH (Garg et al., 2001a, b), to be isosmolal (Cunha et al., 2014) and to improve the retention through adhesion measurements (Caramella et al., 2015). Not only should researchers aim for better products but also to develop selective tools (i.e. characterization methods) to characterize the products more precisely (Tamburic, 1996), and so, better predict their performance. Also, new methods should address physiologic featuring's in perspective of earlier cognizance of *in vivo* possible effects. Ultimate acceptability and clinical efficacy of such preparations require that they possess optimal mechanical properties (ease of removal from the container, spreadability over the substrate), rheological properties (viscosity, elasticity, thixotropy, flowability), and other desired properties such as bioadhesion, desired drug release, and absorption (Jones et al., 1997a, b). Nevertheless, still very little attention has been given to the influence of the vaginal environment on formulation performance. Actually, formulations testing is generally carried without considering the effect of physiologic parameters such as temperature and solvency in vaginal fluids. It is well known that these variables can impact on formulations properties, especially, pH, osmolality, rheology and ultimately bioadhesion. For example, Aka-Any-Grah et al. have performed characterization studies using dilutions in physiologic fluids to determine final rheologic profiles and bioadhesion in early steps of formulations development and concluded that this tool is supposed to predict more accurately the *in vivo* behaviour (Aka-Any-Grah et al., 2010a).

In this study we aimed to develop adequate characterization assays for vaginal semisolid formulations using physiological profiling evaluations. Specifically, it is intended to perform pH and buffering capacity assays in physiologic simulants; disclose formulations osmolality, including after a physiologic dilution in vaginal fluids simulants; evaluate the formulations textural characteristics (firmness and adhesiveness); determine and compare formulations viscosity directly, when diluted in a simulant of physiologic fluids and after administration on a vaginal *ex vivo* model; and evaluate bioadhesion using a mechanic *ex vivo* model.

## 2. Materials and Methods

### 2.1. Tested Products

The semisolid products tested in this study are classified as medicines and were acquired in Portuguese community pharmacies, being also commercialized across the EU and USA (Table 1). Some of these are OTC products, not requiring a medical prescription to be dispensed at pharmacies (Gino-Canesten® and Gyno-Pevaryl®). The majority of these dosage forms are creams, except Blissel® that is a gel. All products are intended for vaginal use except Colpotrophine®, which is recommended by the manufacturer for vulvar application. Universal placebo gel (Clark et al., 2011; Garg et al., 2010; Schwartz et al., 2007; Tien et al., 2005) and Replens® (Acartürk, 2009; Adriaens and Remon, 2008; Caramella et al., 2015; Valenta, 2005) were included, since they represent widely studied formulations. The comprehensive and attentive review on vaginal products classification showed us that there is misunderstanding between vaginal and intravaginal products. We assume that when a product is intended for vaginal application it means intravaginal administration, and not for external/vulvar application, like the case of Colpotrophine®. This term inaccuracy is specially noted in the case of vaginal solutions, which should fit up for intravaginal irrigations, and that in the majority of the cases are indicated for external washes.

### 2.2. Materials

Vaginal fluid simulant (VFS) was prepared as described by Owen and Katz in 1999: sodium chloride (NaCl) 3.51 g (*JT Baker*, United States of America), potassium hydroxide (KOH) 1.4 g (*VWR Prolabo*, France), calcium hydroxide ($Ca(OH)_2$) 0.22 g (*Acros Organics*, United States of America), Bovine Serum Albumin 0.018 g (*Sigma*, Germany), lactic acid 2.00 g (*Sigma*, Germany), acetic acid 1.00 g (*Fischer Scientific*, United States of America), glycerol 0.16 g (*Acofarma*, Spain), urea 0.4 g (*VWR Prolabo*, France) and glucose 5.00 g (*VWR Prolabo*, France) were added to 1 L of milliQ water and stirred mechanically until complete dissolution (Owen and Katz, 1999). The pH of the mixture was then adjusted to 4.5 using hydrochloric acid (HCl), and the final volume was adjusted to 1 L. All other chemicals and reagents were of analytic grade or equivalent. For the modified VFS ($VFS_m$) a 1.5% (w/w) concentration of porcine gastric mucin type II (*Sigma*, Germany) was used (das Neves et al., 2013).

### 2.3. Organoleptic Characteristics

The organoleptic characteristics studied were: general aspect, homogeneity, colour, odour, feel to touch.

### 2.4. pH and Buffering Capacity

For the pH determination, the probe (for viscous products, InLab Viscous, *Mettler Toledo*, USA) was directly immersed on the formulation (Seven Compact, *Mettler Toledo*, USA). The pH-buffering capacity was accomplished by titration with NaOH 1 N added in increments of 20 μL to the dispersion of formulations in Normal Saline - NS (NaCl 0.9%) or VFS until pH ≥ 9 (Cunha et al., 2014). The relevant and absolute buffering capacity were determined using the best fit linear model - *Curve Expert Version 1.4* (Copyright 2013, Daniel G. Hyams). The absolute buffering capacity was calculated and defined as the amount of sodium hydroxide needed to change by one unit the initial pH value. The relevant pH-buffering capacity was calculated as the amount of NaOH required to reach a pH value of 5 (higher than the normal vaginal

pH of reproductive aged women) (Garg et al., 2001a, b; Haineault, 2003). For products which had initial pH higher than 5, the reverse buffering capacity was performed by adding 20 µL of HCl 1 N until pH was lower than 3.

### 2.5. Osmolality

The osmolality was determined in triplicate using a freezing point osmometer (Osmomat 3000, *Gonotec*, Germany), as previously described (Adriaens and Remon, 2008; Cunha et al., 2014), on a 50 µg aliquot. The standardization was performed using three standards: distilled water (zero point), NaCl 300 mOsm/Kg and NaCl 850 mOsm/Kg, commercially available from the equipment manufacturer. Furthermore, osmolality was determined in a mixture with vaginal fluid simulants. An amount of each product corresponding to the daily dose (measured with the proprietary applicator) was diluted in 0.75 mL of VFS or $VFS_m$. This procedure was established to estimate the osmolality of the product when put in contact with vaginal fluid simulants, since 0.75 mL is the estimated mean volume of fluid present in the vagina at any moment (Aka-Any-Grah et al., 2010a; Baloglu et al., 2009; Owen and Katz, 1999).

### 2.6. Texture: Firmness and Adhesiveness

Texture analysis included adhesiveness (N.mm) and firmness (N) determinations using a texturometer (TAXT Plus, *Stable Micro Systems*, United Kingdom). These two parameters were determined in the same run, using a cylindrical probe with a diameter of 10 mm (P10) (Almeida and Bahia, 2006; Jones et al., 1997a) in compression mode and «return to start test». The maximum positive force (N) to penetrate the formulation for 5 mm was registered and corresponds to the formulation firmness (pre-test speed, test speed and post-speed: 3 mm/s). Force measurements required to detach the probe from the formulation during the returning movement allowed for the calculation of the work of adhesion, which is herein described as adhesiveness. Measurements were performed, in triplicate, at room temperature, complying with the laboratorial and equipment manufacturer protocol.

### 2.7. Bioadhesion

Formulation adhesiveness to the biological substrate (porcine vaginal tissue) was accessed using the texturometer (TAXT Plus, *Stable Micro Systems*, United Kingdom). The method employed consisted on a mechanical approach to bioadhesion since it is based on the evaluation of tensile strength of the interfacial layer formed between the formulation and the vaginal epithelium (Caramella et al., 2015). The vaginal epithelia was excised from porcine vaginal tubes (obtained from approximately 6 months' year old animals, kindly conceded from a local slaughterhouse). The vaginal tubes were cut longitudinally, washed with Hank's Balanced Salt Solution (HBSS) pH 4.2, wrapped in aluminium foil, and preserved in an air tight bag at − 20 °C. For the experiment, vaginas were thawed at room temperature in HBSS. The epithelium samples were fixed using a mucoadhesion rig 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 ± 0.5 °C in an oven. The tissue was hydrated with 50 µL of $VFS_m$, since mucin is the protein most likely to be responsible for bioadhesion. A double-sided adhesive tape was used to attach a small piece of cellulose acetate membrane to the probe, where 30 mg of formulation were adsorbed (the formulation was weighted directly on the probe). Cellulose acetate membrane without formulation was used as control. The software was used in adhesive mode. The pre-test speed was 0.5 mm/s with a trigger force of 0.02942 N to allow for sensitive detection of the tissue. Post-test speed was 0.1 mm/s (Dobaria et al., 2009). The contact/hold time was 3 min, and the force applied was 2.5 N (Bonferoni et al., 2006). The force of detachment was recorded as well as the graphical negative area, representative of the work of adhesion (N.mm) necessary to unbind the two surfaces. One-way ANOVA statistical test with multiple comparisons was applied to denote differences ($p < 0.05$) between the control and formulations (GraphPad Prism 6.0).

### 2.8. Viscosity

Viscosity was assessed using a cone-plate rheometer (Brookfield DV-3 T, *Brookfield*, USA). Viscosity measurements were performed at room temperature (25 °C) and at vaginal physiologic temperature (37 °C), using plain formulations and after diluting in VFS. Cone spindles used were CPA-52Z and CPA-40Z (*Brookfield*, USA), both required a 0.5 g or 0.5 mL sample, and had 3° and 8°, and 1.2 cm and 2.4 cm, cone angle and radius, respectively. To assess the thixotropic behaviour of formulations, a range of test speeds between 5 and 200 RPM was established (torque 10–100%). Tests were performed in triplicate during 1 min, and the formulation was left to rest for 1 min between measurements (Prista et al., 1996). Formulations' dilution to physiologic assemblies were performed as described on the Osmolality section.

Furthermore, to study rheological modifications following application, an *ex vivo* model for administration was developed using porcine vaginas. For this purpose a daily dose of all the formulations was placed, with the help of the proprietary applicator, onto an excised vagina obtained from a local slaughterhouse. The organ was isolated from the upper part of the remaining reproductive system, placed on a tray, and 0.75 mL of VFS were inserted into the vagina. Then, the trays were placed in an incubator at 37 °C with mild agitation (50 rpm). After 3 h the trays were removed and the vaginas were opened longitudinally for observation and collection of the formulation which was further assessed concerning viscosity.

## 3. Results and Discussion

### 3.1. Organoleptic Characteristics

Organoleptic characteristics were determined taking into account both users' perspective and formulation suitability for vaginal administration. Thus, evaluated parameters comprised colour, odour, texture and homogeneity. As previously described, women prefer vaginal products presented as semisolids, odourless and colourless, either being gels, creams or ointments (Hardy et al., 1998c; Palmeira-de-Oliveira et al., 2015a). Also, women would rather privilege natural origins for drugs/excipients, and application by means of an applicator (Palmeira-de-Oliveira et al., 2014). In terms of pharmaceutical dosage form the majority of the studied products are labelled as vaginal creams, except for Blissel® and Replens® that are presented as gels. These gels are both colourless. None of the formulations showed strong odours. Also, all of them had a soft texture and were homogeneous, being in accordance with female users expected preferences (Palmeira-de-Oliveira et al., 2015a). All products, except Colpotrophine®, are marketed with disposable applicators, in order to allow for a more comfortable application. These characteristics are also in accordance with women's expectations reported in previous studies for determination of the ideal organoleptic characteristics for microbicides using personal interviews (Hardy et al., 1998b) and focus groups (van den Berg et al., 2013). Regarding the clinicians' perspectives, it has been published that both general practitioners and gynaecologists believe that vaginal products for self-administration are valuable, but consider that more pharmaceutical counselling should be provided in view of increasing therapies' compliance and efficacy (Sihvo et al., 2000). Likewise, in recent years, clinicians and patients' preferences were studied on the different oral and vaginal therapeutic options for vaginal fungal infections, concluding that given the therapeutic efficacy and equivalence of the individual antifungal agents as well as route of administration, treatment selection should be driven by the patient's personal preferences (Sobel,

**Table 2**
pH and osmolality studies of vaginal products included in this study. S.D. = standard deviation (n = 3). * represents statistically different from the respective dilution media; [a] represents statistically different between dilutions with VFS and the undiluted formulation; [•] represents statistically different between dilutions with $VFS_m$ and the undiluted formulation (two-way ANOVA, $p < 0.05$).

| | Direct pH | Diluted pH (VFS) | Direct osmolality | Diluted osmolality (VFS) | Diluted osmolality ($VFS_m$) |
|---|---|---|---|---|---|
| | pH ± S.D. | pH ± S.D. | Osmolality ± S.D. (mOsmol/kg) | Osmolality ± S.D. (mOsmol/kg) | Osmolality ± S.D. (mOsmol/kg) |
| Gino-Canesten® | 5.89 ± 0.07 | 4.22 ± 0.01 | 144 ± 3 | 165 ± 2* | 163 ± 1* |
| Sertopic® | 2.71 ± 0.01 | 4.21 ± 0.01 | 300 ± 2 | 272 ± 7* | 284 ± 1* |
| Dermofix® | 2.70 ± 0.02 | 4.19 ± 0.03 | 248 ± 4 | 228 ± 2 | 240 ± 2* |
| Gyno-pevaryl® | 2.74 ± 0.03 | 4.07 ± 0.01 | 340 ± 8 | 264 ± 3* [a] | 266 ± 2* [•] |
| Lomexin® | 3.57 ± 0.01 | 4.02 ± 0.05* | 1446 ± 20 | 1210 ± 16* [a] | 1253 ± 18* [•] |
| Gino Travogen® | 3.56 ± 0.04 | 4.16 ± 0.00 | 43 ± 2 | 75 ± 1* | 57 ± 1* |
| Dalacin V® | 3.54 ± 0.01 | 4.19 ± 0.01 | 1681 ± 10 | 1223 ± 8* [a] | 1288 ± 21* [•] |
| Ovestin® | 3.92 ± 0.01 | 3.95 ± 0.03* | 3332 ± 60 | 938 ± 7* [a] | 1061 ± 27* [•] |
| Blissel® | 4.79 ± 0.05 | 4.32 ± 0.02* | 1537 ± 6 | 920 ± 12* [a] | 1042 ± 18* [•] |
| Colpotrophine® | 7.26 ± 0.04 | 6.76 ± 0.04* | 1723 ± 20 | 396 ± 4* [a] | 472 ± 47* [•] |
| Universal placebo | 4.51 ± 0.04 | 4.19 ± 0.02 | 339 ± 2 | 306 ± 2* | 324 ± 5* |
| Replens® | 2.81 ± 0.02 | 4.12 ± 0.00* | 2350 ± 81 | 1859 ± 22* | 1903 ± 17* |
| Dilution media | | 4.21 ± 0.01 | | 212 ± 1 | 254 ± 1 |

2013).

### 3.2. pH and Buffering Capacity

The pH of the formulations is an important parameter since it must be compatible with the vaginal pH which is normally 3.5–4.5, but may vary according to specific vaginal conditions (hormonal stimulation, menstrual cycle phase, presence or absence of infections) (das Neves et al., 2014a, b). However, as the pH values of vaginal products do not allow by their own for a complete prediction of their safety, it is more relevant to assess the ability of these formulations to actually change the physiological pH once administered, by other words the pH-buffering capacity. This parameter, especially when determined in VFS, contributes to a better understanding of what will happen *in vivo* regarding pH changes after the formulation is applied in the vagina (Cunha et al., 2014).

Table 2 shows the results of pH determinations for the vaginal formulations enrolled in this study. Antifungals in general had low pH, except for Gino-Canesten® that had a pH of 5.89. This fact might be due to poor solubility and stability of clotrimazole in acidic solutions/formulations (Bachhav and Patravale, 2009), since it is as weak base with a pKa of 6.9 (Hashem et al., 2011). Additionally, it should be considered that fungal infections, which are mainly caused by *Candida* spp., tend not to affect the normal vaginal pH (Sobel, 2007). On the other hand, bacterial infections, the most common being vaginal bacteriosis (VB), are characterized by pH increase to 5.00–6.00. Dalacin-V® has a pH in the lower limit of the physiologic range (3.54 ± 0.01), which can contribute to reduce the high vaginal pH value present in VB. Since antimicrobial products are intended to an occasional administration (vaginal infections are isolated conditions and usually do not require prolonged therapies) it may be acceptable that they can have pH values out of the physiological range. On the other hand, products as Ovestin® and Blissel®, that are intended to be used for prolonged therapies, should exhibit pH values compatible with the normal vaginal pH for safety issues. In fact, we confirmed that both presented pH values within those considered as physiological (3.92 ± 0.01 and 4.79 ± 0.05, respectively). Since these two products are prescribed for chronic application this characteristic may help the formulation to have an adequate impact in the vaginal milieu. Moreover, vaginal atrophy in menopause is characterized by vaginal irritation and discomfort, so pH changes due to products application (either acidic or basic) are expected to increase those symptoms. Colpotrophine® has a manufacturer specification for vulvar application other than the vaginal, so its pH is not expected to be comprised within the vaginal range (pH = 7.26 ± 0.04), but fitting the pH range recommended for skin applications: 4.0 to 7.0 (Lambers et al., 2006). As shown in Table 2, after the dilution in VFS for the pH-buffering capacity assay, formulations acquired the simulant pH (4.21), except for Lomexin®, Ovestin®, Blissel®, Colpotrophine® and Replens®, which resulted in pH in statistically different from the control (one-way ANOVA $p < 0.05$). On the other hand, dilution with the NS control, did not affect markedly the formulations pH (data not shown).

To evaluate formulations pH-buffering capacities two different endpoints were considered: the relevant buffering capacity (RBC) and the absolute buffering capacity (ABC). While the relevant buffering capacity represents the ability of a formulation to overcome a pH of 5, meaning that it has left the physiologic interval for the vaginal environment, the absolute buffering capacity represents the ability for a formulation to change 1 pH point from its natural pH (Cunha et al., 2014). Fig. 1A represents the relevant buffering capacities for all products included in this study. Gino-Canesten®, Colpotrophine® and the NS control are represented in white, since they had initial pH higher than 5 and were titrated with HCl 1 N instead of NaOH 1 N, until pH ≤ 3.00. These calcutations were performed equaly to the other formulations. Although, these two formulations had pH above the physiologic limit, they had low buffering capacities (both RBC and ABC), which means they can easily reach the normal vaginal pH interval.

As expected all products showed higher buffering capacity after being mixed with VFS due to the intrinsic effect of this fluid (lighter grey control, Fig. 1A and B), when comparing with the capacity of the normal saline solution.

Universal placebo has revealed little buffering capacity even on VFS (similar to the results obtained for this control), probably meaning that its application will not modify the vaginal pH. Dalacin V®, despite having a pH value compatible with the vaginal physiological pH, did not show a favourable RBC on VFS. Although it would be important to retain a low pH in the presence of VB (its main therapeutic indication). Blissel®, unlike Ovestin® labelled for the same therapeutic purpose, had a high RBC, meaning that it is able to maintain the physiological pH in less acidic conditions.

The ABC values for Gino-Canesten®, Sertopic®, Ovestin®, Colpotrophine® and Universal placebo were not statistically different from the control on the NS assay. As for the assays in VFS no differences from the control were found for Sertopic®, Gino Travogen®, Dalacin V®, Ovestin® and Universal Placebo. Overall, buffering capacity is probably related to the presence of acidic polymers, as seen for Blissel® (Cunha et al., 2014). Also, formulations which had pH out of the vaginal physiologic range (Gino-Canesten®, Sertopic®, Dermofix®, Gyno-pevaryl® and Colpotrophine®), had relatively low capacity to maintain their own pH, which means that by mixing with acidic vaginal fluids they could change to a value closer to the physiological range although

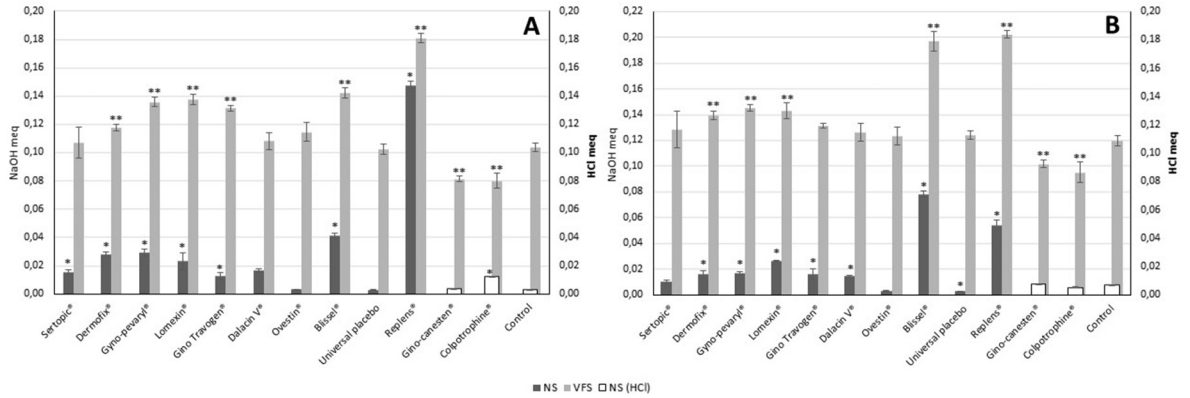


**Fig. 1.** (A) Relevant and (B) Absolute pH-buffering capacity expressed as NaOH meq for the vaginal products included in this study. For Gino-Canesten®, Colpotrophine® and Control in NS, the addition was made with HCl, since their pH were higher than 5. Results correspond to the mean of 3 determinations. NS = Normal saline; VFS = vaginal fluid simulant; NS (HCl) = normal saline tritrated with HCl. * represents statistically different from the NS control and ** represents statistically different from the VFS control (one way-ANOVA, $p < 0.05$).

not being expected to correct the abnormal vaginal pH associated with the target problem. It is well established that deviations from the normal vaginal pH in the healthy adult (3.5–4.5) are considered as potentially deleterious for the vaginal epithelium (das Neves et al., 2014a, b). Classically, the acidic pH in the vaginal environment is believed to contribute to the normal physiology, to favour microbiota, and to promote a balanced immune response. Vaginal products should be compatible with vaginal pH and, ideally, maintain it or even help in its reestablishment (e.g., in cases of bacterial vaginosis or post-menopausal vaginal atrophy) (Wu et al., 2007). Even if the consequences of the administration of vaginal formulations presenting undesirable pH is not readily assessable, it is well known that increased vaginal pH is associated with the presence or favours bacterial vaginosis, trichomoniosis or mixed infections (Sobel, 1997). Outcomes of low pH are even less understood, but animal data suggest that values of three or less are generally unacceptable for human use (Kaminsky and Willigan, 1982). Furthermore, apart from being pH compatible, vaginal products should allow the maintenance of the vaginal acidic environment and oppose pH-raising/decreasing events. Indeed, the use of acid-buffering gels has been proposed for the reestablishment of pH in cases of infection (Garg et al., 2001a, b; Simoes et al., 2006) or menopausal atrophy (Sinha and Ewies, 2013). A study of pH and buffering capacity of diverse marketed vaginal lubricants has already been performed within our workgroup (Cunha et al., 2014) and we concluded that most of the lubricants presented pH and/or osmolality values outside the ranges recommended by the WHO (World Health Organization). This later study made clear the need for further characterization in order to fully understand the potential hazard profile of the vaginal products.

### 3.3. Osmolality

Osmolality was determined directly on the formulations, but also after dilution on the correspondent volume of fluid in amounts equal to those expected to be present in the vagina (0.75 mL), using normal VFS and the $VFS_m$ (containing mucin). The osmolality of the control VFS (212 mOsmol/Kg) was in accordance to previous results found in the literature (Lai et al., 2008). Although being slightly hyposmolal (considering isosmolal around 300 mOsmol/Kg), this value is similar to the physiological one (260–290 mOsmol/Kg) (*Use and procurement of additional lubricants for male and female condoms: WHO/UNFPA/FHI360 Advisory note*, 2012). However, it is expected that a broader interval of osmolality for vaginal administration will be well tolerated, as for ocular delivery, formulations between 260 and 480 mOsmol/Kg, results in no irritation, although the osmolality of the lacrimal fluid normally ranges between 310 and 350 mOsm/Kg (Gad, 2007).

Osmolality measurements on plain formulations revealed that Lomexin®, Dalacin V®, Ovestin®, Blissel® and Colpotrophine® had higher osmolality than the higher limit recommended by the WHO for lubricants (< 1200 mOsmol/kg) (*Use and procurement of additional lubricants for male and female condoms: WHO/UNFPA/FHI360 Advisory note*, 2012). On the other hand, Gino-Canesten®, Sertopic®, Dermofix®, Gyno-pevaryl®, Gino Travogen® and Universal placebo are in accordance to the recommended value, 380 mOsmol/kg. The high osmolalities might be due to the presence of high levels of glycerin and/or propylene glycol in the formulations' composition. Indeed, the WHO recommends that glycerin and propylene glycol concentrations should not exceed 9.9% (w/w) and 8.3% (w/w), respectively (Cunha et al., 2014). However, after mixing on both normal VFS and modified VFS, the final osmolalities were in accordance to this criteria.

For the VFS mixtures, all osmolalities were affected by the dilution, being statistically different from the control (media itself), two-way ANOVA, $p < 0.05$; except for Dermofix®, which already had an osmolality similar to the media. In respect to the mixtures on $VFS_m$ only Sertopic®, Dermofix® and Gyno-pevaryl® were statistically affected by the dilution (two-way ANOVA, $p < 0.05$). When comparing VFS diluted with undiluted formulations' osmolalities, there is a statistically difference in almost all formulations except for Gino-Canesten®, Sertopic®, Dermofix®, Gino Travogen® and Universal Placebo. This behaviour was already expected, since they were mixed with a fluid with similar tonicity (VFS = 212 mOsmol/kg), not affecting the final dilution. While comparing undiluted formulations with the diluted ones in $VFS_m$ there were few differences. The resulting osmolality was only statistically different for Gyno-pevaryl®, Lomexin®, Dalacin V®, Ovestin®, Blissel® and Colpotrophine®. These results show that, while there are few statistical differences between diluted and undiluted formulations whatever the dilution media is, it might be of special interest to consider the evaluation of osmolality in a physiological dilution. This dilution clearly indicates the potential irritation that might be associated with the product, which is not deducted when measurements are made directly on the formulation. So, for future determinations a complete osmolality assessment should not only comprise the direct measurement but also the dilution in the VFS, the most used by research groups. The osmolality of the resulting dilution has the capacity to early predict the *in vivo* formulation behaviour, representing an initial strategy for safety foresee.

WHO in collaboration with the United Nations Population Fund (UNFPA) and Family Health International (FHI360), recently issued an "Advisory Note" on the technical requirements of vaginal lubricants, namely when used in addition to condoms. Osmolality has been highlighted, and specific recommendations have been proposed: values of 380 mOsmol/kg or lower are desirable (hypo and isosmolal), but values as high as 1200 mOsmol/kg have been considered acceptable on an interim basis (*Use and procurement of additional lubricants for male and female condoms: WHO/UNFPA/FHI360 Advisory note*, 2012). Available

pre-clinical and clinical data support that hyperosmolal vaginal products may be related to safety issues (Adriaens and Remon, 2008), as well as detrimental effects on sperm motility, viability and chromatin quality (Agarwal et al., 2008; Kutteh et al., 1996). Vaginal products' safety goes beyond chemical toxicity to include physical parameters such as osmolality (Dezzutti et al., 2012). If a formulation is excessively hyperosmotic there is the potential to cause irritation leading to an inflammatory response. Each dissolved ingredient in a topical formulation contributes to the final product osmolality, so the effect of the overall composition of the formulation must be considered in addition to the impact of each individual ingredient. In general, the loss of cell viability is possibly correlated to the gels' osmolality; the higher the solute concentration, the greater the dilution that is needed to maintain viability (Cunha et al., 2014; Dezzutti et al., 2012).

On the other hand, hypotonic formulations can conduct to increased fluid absorption, leading to higher permeation rates of drugs and nanoparticles through the vaginal epithelial surface (Ensign et al., 2013). Hypotonic formulations toxic effects (especially irritant) in vaginal administration have not been largely investigated. Ensign et al. hypothesized the administration of hypotonic solutions and found that hypotonic formulations markedly increased the rate at which small molecule drugs and muco-inert nanoparticles reached the vaginal epithelial *in vivo* in mice. Furthermore, using a mouse model of vaginal genital herpes (HSV-2) infection, these researchers found that hypotonic delivery of free drug led to improved immediate protection, however, diminishing longer-term protection.

### 3.4. Firmness and Adhesiveness

Textural characteristics of vaginal formulations are important not only in view of effectivity, but also in what concerns to patient compliance (Mahan et al., 2014). Conventional semisolids for vaginal administration are reported to suffer from relatively low patient acceptability and poor vaginal retention and so, the development of new vaginal semisolids requires a fundamental understanding of their rheological and textural properties within the vaginal cavity (Andrews et al., 2009). Several works have reported the use of firmness and adhesiveness determinations in view of texture characterization of vaginal products (Almeida and Bahia, 2006; Jones et al., 1997a, b). Also, the measurement of the work of syringeability has been applied for a vaginal applicator model in order to determine expelling capacity of applicators (Andrews et al., 2009).

Firmness and adhesiveness were determined as physic-mechanical characteristics of the formulations and were studied in view of a correlation between these two parameters. However, as shown by Table 3, it is not evident that the higher the adhesiveness the lower/higher the firmness. In fact, these two characteristics do not have any correlation neither on the Spearman non-parametric test ($r = 0.2657$) nor on the Pearson parametric test ($r = 0.0939$), with CI 95%. Higher adhesiveness conducted to low firmness, such as the case of Universal placebo and even Blissel®, but not statistically supported. These two formulations are the only ones with a polymeric composition and this behaviour may be due to this type of excipients (hydroxyethylcellulose and a combination of polycarbophil/carbomer, respectively). Antifungals had almost the same textural behaviour: medium adhesiveness (0.400–0.600 N.mm, approximately) and medium to high firmness (0.070–0.300 N, approximately). Dalacin V®, the only antibacterial product enrolled in the study, presented similar results to the antifungals. This might mean that this texture is adequate to short-term therapies, and also easy to filling-in the applicator and posterior administration. Actually, several excipients are common to this group of formulations (antimicrobials), such as, liquid paraffin, cetyl palmitate, propylene glycol and cetostearyl alcohol, although not being known their quantitative composition. Topical oestrogens had different textures among them. While Blissel® showed to be averagely adhesive, it is the less firm of all formulations, meaning that it could have a good

**Table 3**
Mechanical (adhesiveness (N.mm) and firmness(N)) and bioadhesive parameters (work of adhesion (N.mm), peak force-adhesiveness (N) and debounding distance (mm)) determined for the products in study. S.D. = Standard deviation (n = 3). * Represents statistically different from the control (one-way ANOVA, $p < 0.05$).

| Formulations | Adhesiveness (N.mm) ± S.D. | Firmness (N) ± S.D. | Work of adhesion (N.mm) ± S.D. | Peak force-adhesiveness (N) ± S.D. | Debounding distance (mm) ± S.D. |
|---|---|---|---|---|---|
| Gino-Canesten® | 0.531 ± 0.050 | 0.206 ± 0.007 | 0.022 ± 0.001 | 0.033 ± 0.015 | 2.160 ± 0.044 |
| Sertopic® | 0.401 ± 0.071 | 0.123 ± 0.010 | 0.019 ± 0.006 | 0.020 ± 0.000 | 2.337 ± 0.452 |
| Dermofix® | 0.438 ± 0.070 | 0.131 ± 0.008 | 0.028 ± 0.008 | 0.020 ± 0.000 | 3.167 ± 1.024 |
| Gyno-pevaryl® | 0.500 ± 0.032 | 0.073 ± 0.001 | 0.061 ± 0.008* | 0.060 ± 0.020 | 2.613 ± 0.431 |
| Lomexin® | 0.563 ± 0.041 | 0.260 ± 0.021 | 0.070 ± 0.023* | 0.117 ± 0.050 | 2.547 ± 0.573 |
| Gino Travogen® | 0.407 ± 0.047 | 0.134 ± 0.007 | 0.032 ± 0.008 | 0.037 ± 0.006 | 2.280 ± 0.137 |
| Dalacin V® | 0.364 ± 0.009 | 0.124 ± 0.006 | 0.020 ± 0.004 | 0.033 ± 0.006 | 2.070 ± 0.140 |
| Ovestin® | 0.827 ± 0.055 | 0.204 ± 0.009 | 0.021 ± 0.003 | 0.030 ± 0.017 | 2.027 ± 0.387 |
| Blissel® | 0.638 ± 0.022 | 0.077 ± 0.002 | 0.041 ± 0.001* | 0.057 ± 0.029 | 2.087 ± 0.137 |
| Colpotrophine® | 0.390 ± 0.011 | 0.092 ± 0.002 | 0.026 ± 0.011 | 0.037 ± 0.015 | 2.117 ± 0.156 |
| Universal placebo | 1.198 ± 0.008 | 0.078 ± 0.001 | 0.110 ± 0.021* | 0.140 ± 0.069 | 2.930 ± 0.543 |
| Replens® | 0.181 ± 0.026 | 0.065 ± 0.014 | 0.047 ± 0.002* | 0.070 ± 0.017 | 2.147 ± 0.199 |
| Control | | | 0.015 ± 0.002 | 0.027 ± 0.012 | 2.010 ± 0.111 |

spreadability over the vaginal epithelium. On the other hand, Ovestin® despite being a little more adhesive, it is also more firm and this could lead to lower spreadability over epithelium. Although more adhesive in the mechanic test, Ovestin® was less bioadhesive than Blissel®, when a physiologic feature was endorsed. This might be due to the combination of polycarbophil/carbomer on Blissel®, a recognized derivate highly adhesive acrylic acid (Valenta, 2005).

Formulation characteristics, including viscosity, elasticity, and rheology, are the most important factors in the development and final behaviour of semisolid formulations. Also, temperature and site of application are of extreme importance for formulations spreadability (Garg et al., 2002). To assess the spreadability of a topical or a mucosal semisolid preparation, the important factors to consider include hardness or firmness of the formulation, the rate and time of shear produced upon smearing, and the temperature of the target site. The rate of spreading also depends on the viscosity of the formulation, the rate of evaporation of the solvent, and the rate of increase in viscosity with concentration that results from evaporation (Barry and Grace, 1971).

### 3.5. Bioadhesion

Textural analysis is essential for product characterization. However, studies on formulation mechanical behaviour on a biologic perspective should also be addressed. Bioadhesion represents the ability of a formulation to adhere to a biological surface, in this case, the vaginal epithelium.

The one-way ANOVA ($p < 0.05$) statistics determined that, regarding Work of Adhesion (herein considered as bioadhesion), only Gyno-pevaryl®, Lomexin®, Blissel®, Replens® and Universal placebo were different from the control (performed without any formulation). The Work of Adhesion was compared with the pure textural parameters and it was found a moderate to strong uphill positive linear correlation with Adhesiveness (Pearson, CI95%, $r = 0.6233$). This can represent a valuable information, since it means that bioadhesion could be predicted by adhesiveness, a test that does not require the use of biological surrogates and can easily be performed in earlier stages of product development. These results were obtained with an experimental setup that considers the physiological temperature in order to reflect the formulations behaviour in a more physiological condition.

Mucoadhesion (herein referred as bioadhesion since the vaginal epithelium is not considered as a mucosa) represents an attractive interaction that involves a pharmaceutical dosage form and either secreted mucus or a mucosal/epithelial membrane (Shaikh et al., 2011). Bioadhesive properties allow better contact of the formulation with the vaginal surface and longer residence times. In most cases, bioadhesion is imparted to a formulation by the employment of polymeric excipients. The mechanisms of bioadhesion involve, firstly, a contact stage, hydration, wetting and spreading (which are the most important steps), and subsequently a consolidation stage, that involves the strengthening of polymer–mucin joint, thanks to the inter-penetration of the polymer chains into the mucus layer and the occurrence of polymer–mucin bonding (mainly weak van der Waals and hydrogen bonds or electrostatic interactions) (Caramella et al., 2015; Shaikh et al., 2011). Maximum force of detachment ($F_{max}$) (directly measured) and the work of adhesion ($W_{ad}$) (calculated as the area under the curve force *vs* displacement) were the parameters used to evaluate the bioadhesive potential. The reliability of a tensile method is strictly dependent on the failure in the interfacial (mucin/polymer) region: in particular it is difficult to distinguish where the failure of the bioadhesive joint occurs and if the cohesive nature of the sample (failure within the polymer layer) or the strengthening of the mucus layer (failure within the mucus layer) plays the major role (Caramella et al., 2015).

The natural mild slope of the vaginal canal, in association with its self-cleansing mechanisms (e.g. fluid transudation) and possible mechanical stress (e.g. during penile penetration), contributes to the expulsion of products placed in the vagina. Another important issue impacting the bioadhesion phenomenon is related to the variability of the vaginal fluid with the menstrual cycle and hygiene practices (e.g. douching). Vaginal fluid can undergo either quantitative or qualitative changes, such as pH, mucin content and rheology. These factors influence the interaction of bioadhesive formulations with mucin, namely by changing the conformation and properties of the network formed by mucin within the vaginal fluid (das Neves et al., 2011).

Bioadhesive dosage forms or delivery systems can contribute to prolonged *in situ* residence, resulting in advantageous features such as fewer applications needed, reduced vaginal leakage, and intimate contact between drugs and the epithelial tissue. Different dosage forms have been formulated as bioadhesive like tablets, suppositories, creams, and gels (das Neves et al., 2014a). Indeed, one of the first enthusiastic reports on a specific bioadhesive vaginal gel dates back to the 90s by Robinson and Bologna (Robinson and Bologna, 1994). The bioadhesive properties of the proposed gel, currently commercialized as Replens® (Lil' Drug Store Products, Inc.), were attributed to the inclusion of an acrylate polymer, polycarbophil (1–3%). Since then, these polymers have been used as classical bioadhesive and gelling agents for the formulation of various commercially available vaginal gels (das Neves and Bahia, 2006). After that, Garg et al. proposed a new mucoadhesive gel, ACIDFORM which was shown to present enhanced *in vitro* mucoadhesive properties when compared to various commercial gels (Garg et al., 2001a, b). It is composed by acidic substances (lactic acid, citric acid and potassium bitartrate), a preservative (benzoic acid), gelling agents (alginic acid and xanthan gum), a humectant (glycerin), sodium hydroxide and water (Bayer and Jensen, 2014).

The common strategy for increasing bioadhesion of vaginal dosage forms, especially among research published papers, has been to use well known bioadhesive polymers such as polyacrylates, chitosans, cellulose derivatives, hyaluronic acid and derivatives, pectin, starch, and several natural gums, among others (Valenta, 2005). Acidic polymers such as polyacrylates present the additional feature of contributing for the acidic pH-buffering of the vaginal milieu within the desirable normal range, and thus potentially contributing to a healthy vagina (Milani et al., 2000). Regarding chitosan, its intrinsic ability to interact with intercellular tight junctions and to inhibit proteolytic enzymes provides additional mechanisms for promoting the vaginal absorption and peptide/protein protection from degradation, respectively (Sandri et al., 2004). In recent years, thiolated polymers have also been tested for designing vaginal dosage forms with improved bioadhesive performance when compared to their non-thiolated counterparts (Cevher et al., 2008). Even if substantial success has been achieved, much of the rationale behind the choice of bioadhesive polymers for vaginal formulation derives from studies intended to evaluate these excipients for use in other mucosal routes (Grabovac et al., 2005). Also, a formulation to deliver controlled doses of progesterone based on cyclomethicone was prepared as a silicone-water emulsion, with great potential to be bioadhesive, even after dilution on VFS (Campaña-Seoane et al., 2014). The bioadhesive potential of polymers and derived dosage forms is also dependent on the specificities of the mucosal environment, and its evaluation should take this into account. For instances, *in vitro* experimental settings relevant to the vaginal physiology, namely pH values, have been shown to significantly influence the bioadhesive performance of vaginal semisolid formulations (das Neves et al., 2008a, b). This need for mimicking the vaginal environment led to the development of different specific *in vitro/ex vivo* experimental protocols for evaluating the bioadhesive potential of vaginal dosage forms (Caramella et al., 2015). Proposed techniques generally involve measuring the forces involved in the detachment of a formulation from a model synthetic or natural mucosa. Alongside, imaging techniques have been used to evaluate bioadhesion *in vivo* (Chatterton et al., 2004). Several methodologies have been applied to determine bioadhesion (Caramella et al., 2015). However, the one herein described gave reproducible results, skipping their major difficulty when working with

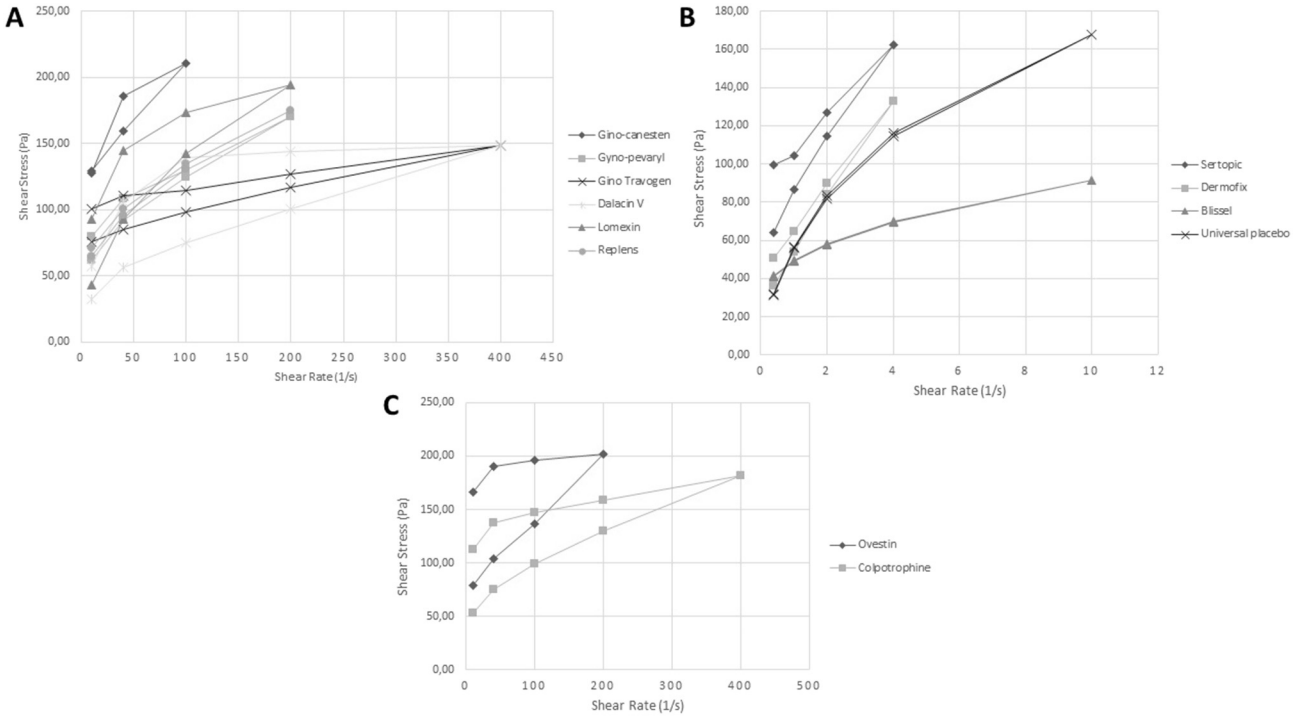


**Fig. 2.** Viscosity expressed as Shear Rate (Pa) demonstrating thixotropic behaviour at 25 ºC for (A) antimicrobials and Replens® gel; (B) topical oestrogens; and, (C) low viscosity formulations. (Results represent the mean of 3 independent determinations).

biological surrogates. Furthermore, our work reflects the usage of a standardized equipment and method. Finally, the assembly of this methodology conducted to the establishment of a correlation between parameters determined in the same equipment (adhesiveness and bioadhesion).

### 3.6. Viscosity

Viscosity, i.e. rheological properties are of great interest given the effect these may have on drug release properties and passive outflow between epithelial surfaces. Moreover, rheological properties are of primary interest because they have been shown to largely govern the ease of application and dispersion of semisolids (Andrews et al., 2009), which will obviously influence the ability of the formulation to coat the vaginal cavity therefore providing efficacy.

Thixotropic profiles were outlined at room temperature (25 °C) directly over the formulations in study (Fig. 2). Sertopic®, Dermofix®, Blissel® and Universal placebo had lower viscosities, and consequently lower shear rates were necessary to obtain an acceptable torque.

Concerning Fig. 2A it is clear that all antimicrobials have a thixotropic textural behaviour (non-Newtonian - pseudo plastic), which is not so marked on the Replens® formulation. Sertopic® and Dermofix®, although having lower viscosities show also this time-dependent behaviour. Thixotropic materials become more fluid as shear rates decreases, short after an increasing shear rate testing. On the contrary, Blissel® and Universal placebo showed not to be affected by time-dependent viscosity determinations (Fig. 2B).

Viscosities were also determined after mixture with the VFS, at 25 °C, 37 °C and considering the administration in an *ex vivo* model (Fig. 3). These dilutions and temperature adaptations can better mimic the rheology adopted after application by the vaginal products. In general, viscosity was clearly lower when compared to the plain formulations. Also, the thixotropic phenomenon for most of formulations was less marked (data not shown). Differences were statistically significant (two-way ANOVA, Multiple Comparisons, $p < 0.05$) for all dilutions at 37 °C compared with dilutions at 25 °C except for Dermofix® and Blissel®. And for all dilutions at 25 °C compared with undiluted formulations measured at the same temperature. Viscosity was highly dependent on temperature, (das Neves et al., 2008a, b; Gad, 2007) as expected. However, the variation observed, was not proportional nor similar among all formulations. Each formulation had its own behaviour, driven by their composition. This could mean that measurements directly made on formulations at room temperature do not represent the viscosity acquired after administration. Furthermore, measurements upon dilution with VFS at room temperature (25 °C) would still be significantly different from viscosities obtained at physiological temperature. Considering the test using the *ex vivo* porcine vagina, it was showed that also this model could be valuable, not only because it mimics the *in vivo* administration, but also it brought different results from the dilution at 37 °C. For almost all formulations the viscosity was higher in this test, except for Gino-canesten® and Sertopic®, when comparing to the formulations under dilution at 37 °C. In fact, this was an unforeseen result, since the effect of rotation movement was expected to decrease the overall viscosity of the sample with the VFS and the vaginal environment. However, these results are quite satisfying in what concerns to comfort and leakages issues. The fact that the formulations can adopt, after administration, a higher viscosity, could circumvent these problems. Furthermore, these results could highlight the need to establish the model herein presented, to better predict the formulations' rheology after administration.

Aka-Any-Grah et al., have also reported major differences among formulations developed with hydroxypropylmethyl cellulose (HPMC) and pluronics F127/F68, especially on the gelling temperature (before and after dilution in VFS), rheological properties and even *ex vivo* adhesion (Aka-Any-Grah et al., 2010b). This data suggests that approximations to vaginal physiological conditions are determinant to foresee *in vivo* rheology of the formulations after administration (Chang et al., 2002). Viscosity can dictate the ability of the formulation to disperse *in vivo*, as well as the residence time in the genitourinary tract of these formulations, hence it is decreased owing to the self-cleansing action of the vagina and the dilution with vaginal fluids, and even environmental temperature (Aka-Any-Grah et al., 2010b).

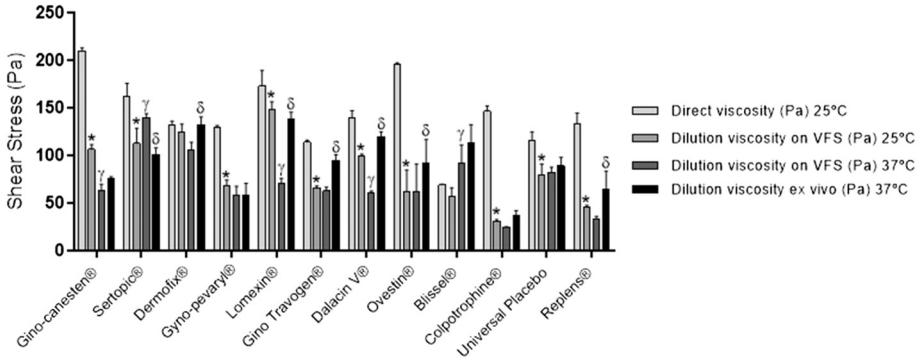


**Fig. 3.** Viscosity/Shear rate (Pa) comparisons for direct measurements and diluted measurements at temperatures of 25 °C and 37 °C. Results correspond to the mean of 3 determinations. * represents statistically different from direct viscosity; $^{\gamma}$ represents statistically different from the dilution at 25 °C and $\sigma$ represents statistically different from the dilution at 37 °C (two way-ANOVA, $p < 0.05$, Tukey's multiple comparisons test).

Katz et al. disclosed the rheological properties of Advantage-S® and Replens® at body and room temperature over a range of physiologically relevant shear rates. It was found that their rheological behaviour was different among temperatures and miscibility with vaginal fluid simulant was also affected (Owen et al., 2001).

Lai et al. (2008) studied the effect of small dilutions (10–30%) in vaginal fluid and semen simulants on KY Jelly®, Replens® and Carraguard®, using a cone-plate rheometer, and data was fitted to power-law, Carreau, or Herschel-Bulkley models. Rheological parameters from these fits were input to models of coating flow due squeezing, and the simulated area coated output from these models was used to compare the responses of the different formulations to the two diluents for varying degrees of dilution. There were differences in the responses of the three materials to dilution; even small dilutions altered the rank order of vaginal coating rates compared to the undiluted formulations (Lai et al., 2008).

Later on, Henderson et al. (2007) used an optical imaging technique to compare human intravaginal coating distributions of Conceptrol® and Advantage®. It was concluded that the results were consistent with those predicted through mechanistic coating theory, using gel rheological data as input (Henderson et al., 2007). Furthermore, in 2008, Mauck et al. studied the vaginal distribution of Replens® and KY Jelly® *in vivo* in women. Time, ambulation, parity and body mass index were factors considered for vaginal spreading. Imaging was achieved by magnetic resonance imaging, gamma scintigraphy and with a fiberoptic probe. Results showed that the initial application of the gel resulted in two thirds of maximum coverage possible, both in linear extent along the vaginal axis and in surface area covered. Over the next 45 min, spreading increased to about three quarters of the maximum possible. Ambulation generally increased linear spreading. Effects of parity and body mass index were similar on most measures of gel spreading, with nulligravid women tending toward greater spread than parous women and women of high body mass, usually showing somewhat greater spread than women of normal weight. Differences between the two gels were not seen when all conditions of application were considered together (Mauck et al., 2008).

Recently, Katz et al. described the fundamental principles of mass transport, highlighting the diffusion and convection of drugs in the vaginal environment. Several mathematical predictive models can be used to this, although having some variability when compared to *in vivo* behaviour. These models can illustrate drug concentration distribution (pharmacokinetics) and effectiveness (pharmacodynamics). Modelling can be used to compare vaginal drug distributions after different gel dosage regimens, comparing the effect of vaginal fluid and the consequences of changes in gel viscosity due to aging. It could also be helpful in comparing drug distribution after the application of different dosage forms. Ultimately, the modelling approach is used to compare vaginal drug distribution across species with differing vaginal dimensions (Katz et al., 2015).

Anwar et al. have investigated the interplay between vaginal tissue elasticity and the yield-stress of non-Newtonian fluids during a microbicide deployment. Yield stress is the applied stress one must exceed in order to make a structured fluid flow, being the “force” implied on the formulation at the beginning of a viscosity determination. Within this research work this group has developed a mathematical model of tissue deformation driven by spreading of microbicidal gels based on thin film lubrication approximation and demonstrated the effect of tissue elasticity and fluid yield-stress on the spreading dynamics. It was concluded that both elasticity of tissue and yield-stress rheology of gel are strong determinants for the coating behaviour (Anwar et al., 2015).

In another study, Kieweg et al. performed experimental and numerical studies on microbicidal gel deployment under constant squeezing force and concluded that squeezing force, gel consistency, shear-thinning behaviour and yield stress are strong determinants of the coating performance of gels (Kieweg et al., 2004; Kieweg and Katz, 2006, 2007). Szeri et al. developed a mathematical model involving wall elasticity to demonstrate the effect of compliant vaginal wall on the deployment of new formulations (Szeri et al., 2008).

Vaginal gels should be highly elastic, even after dilution, as these properties govern drug release and leakage. Furthermore, pseudoplasticity would offer stress-induced viscosity depression and hence ensure ease of application (Yu et al., 2011). This is difficult to achieve using single polymer gels. Therefore, gels offering greater clinical promise may be achieved through the combination of mucoadhesive and gel structuring polymers within a binary or higher polymer platform (Jones et al., 2009; Perioli et al., 2008).

Omar et al. in 2014 proposed an universal vaginal applicator able to homogenously distribute the formulations over the entire vaginal and cervical epithelia. The internal distribution was investigated using pelvic magnetic resonance imaging (MRI) in a group of women which used six different vaginal gels and creams. Comparisons were made against the conventional applicators. The universal applicator showed to have good potential to reach uniform coverage of vagina and cervix, and enhance women protection against sexually transmitted diseases (Omar et al., 2014).

Despite of comfort and administration issues, semisolid formulations distribution is directly related to therapeutic efficacy. The use of a gel with a low viscosity would facilitate spreading and hence contact with the vaginal epithelium. However, a low viscosity gel would be expected to have a limited residence time due to the inability of the gel to resist dilution from vaginal fluids and semen, except if it can exhibit a trigger gelling behaviour (Caramella et al., 2015; das Neves et al., 2014a). Moreover, a low viscosity gel would be unable to “absorb” *in vivo* stresses without causing destruction of polymer gel entanglements and thus would be expected to leak rapidly. Conversely, a highly elastic gel would offer greater resistance to dilution and to *in vivo* stresses; however, application and intravaginal spreading would be limited. Therefore, optimal clinical performance may only be achieved when the elastic-viscous balance is carefully controlled (Yu et al., 2011). The performance of a vaginal gel can be evaluated with respect to a number of properties, including spreadability, coating and retention by using *in*

*vitro*, *ex vivo* and *in vivo* methodologies (Mahalingam et al., 2010).

## 4. Conclusions

In view of designing new formulations for vaginal administration driven by safety and efficacy rationals; biological criteria should be addressed from the early steps of development to accelerate the whole process. The present study showed that a great number of commercial therapeutic vaginal formulations currently used did not present ideal technological characteristics when tested under a physiological perspective. Furthermore, pH buffering capacity, osmolality and viscosity determined using these methodological adaptations were considered focal points to be addressed during products' development. While great effort has been made in the development of innovative vaginal gels in the field of microbicides, antimicrobials and oestrogens formulations have not been the focus of attention in the last years. Nevertheless, they represent the most widely and frequently prescribed all over the world for acute and chronic conditions, respectively. Polymer-based strategies could be applied to re-formulate products already marketed in order to overcome problems of leakage and discomfort, and improve efficacy. The adaptation of these formulations and the use of the methodologic adaptations proposed in this work may optimize cost-efficiency of new and renewed formulations development by predicting efficacy and safety profiles at early stages of product development.

## Acknowledgements

This work was supported by FEDER funds through the POCI - COMPETE 2020 - Operational Programme Competitiveness and Internationalisation in Axis I - Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT - Foundation for Science and Technology (Project UID/Multi/00709/2013).

Financial support was also provided by Labfit, HPRD, Lda. and FCT - Foundation for Science and Technology through a PhD fellowship (Grant Reference SFRH/BDE/111544/2015).

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