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Research paper

Performance of an *in vitro* mucoadhesion testing method for vaginal semisolids: Influence of different testing conditions and instrumental parameters

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Abstract

The purpose of this work was to develop an *in vitro* mucoadhesion testing method for vaginal semisolid formulations. The proposed method was based on the measurement of the force (detachment force, F_{dt}) and the work (work of adhesion, W_{ad}) needed to detach a sample of cow vaginal mucosa from a semisolid formulation, using a commercially available texture analyzer. Several testing conditions and instrumental parameters were tested in order to evaluate the mucoadhesive potential of a model vaginal semisolid formulation (1% Carbopol® 974P gel). Also, mucoadhesive potential of several commercially available vaginal semisolid products was evaluated. Obtained results showed that the method is reproducible even when the same cow mucosa sample is used up to six times. The similarity of the fluid used to bathe the vaginal mucosa to the one naturally occurring in the vagina influenced considerably the performance of the test, advising that simulation of vaginal fluid properties is important when measuring mucoadhesive properties. Also, temperature of experiment was an important fact to be considered, as results showed slight but significant differences between body (37 °C) and room (20 °C) temperature. F_{dt} and W_{ad} increased with increasing instrumental parameters while a plateau region was observable at higher values of probe speed, probe force, and mucosa/sample contact time. Comparison between results for F_{dt} and W_{ad} demonstrated that although both parameters are generally in agreement, W_{ad} seems to be more reliable and reproducible when evaluating mucoadhesion. Evaluation of commercially available formulations confirmed that experimental conditions are important features that can influence significantly the determination of mucoadhesive potential, being the proposed method an interesting and useful tool in the *in vitro* evaluation of vaginal semisolids.

Keywords: Mucoadhesion; Vaginal drug delivery; Texture analyzer; Semisolid formulations; Detachment force; Work of adhesion

1. Introduction

Vaginal drug delivery has been in recent years a prominent field of investigation. Several pharmacologic strategies involving the administration of active substances in the vagina, either with local or systemic effects, have been proposed and even some have already reached the market [1]. However, optimization of vaginal drug delivery systems still needs hard work in order to allow the administration of several drugs that have shown promising results in pre-clinical testing. Indeed, lack of vaginal drug delivery systems that have been optimized or even specifically designed for this route may introduce significant bias into clinical trials, potentially causing poor outcomes or at least a degree of uncertainty in their results [2–4].

Vaginal semisolids, particularly gels, have been used for a long time and are currently receiving a great deal of interest as vaginal drug delivery systems. Conversely, further work still has to be done in order to improve the performance of these systems, allowing excellence of clinical results [5]. Mucoadhesion is widely recognized as an impor-

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tant research field in the optimization of drug delivery systems, as it can directly influence *in loco* drug retention, spreading and bioavailability [6]. The vagina is no exception, being mucoadhesive formulations of particular interest due to anatomic and physiologic particularities of this drug delivery route. Indeed, utilization of various mucoadhesive polymers is common in vaginal drug formulations, with several advantageous features such as prolonged retention, improved drug absorption, controlled release or protection of labile active substances being claimed [7]. Reports of various analytical methods for *in vitro* evaluation of the mucoadhesive potential of vaginal semisolids (mostly of hydrophilic gels) have been quite common, particularly those based on the measurement of tensile force or work necessary to detach a mucoadhesive formulation from a model membrane [8–12]. However, produced data are often influenced by testing conditions and instrumental parameters, making it difficult to interpret or compare results obtained by different research groups. Hence, lack of a universal method to evaluate the mucoadhesive potential of vaginal semisolid formulations and the influence of different experimental conditions on the performance of utilized methods are important points that need to be addressed, as previously reported for drug delivery systems intended to be administered in other mucosal sites, namely the gastrointestinal tract [13–15].

The objective of this work is to develop an *in vitro* tensile mucoadhesion testing method for semisolid formulations and to study the influence of different testing conditions and instrumental parameters on its performance. Also, we evaluated the mucoadhesive potential of several commercially available vaginal semisolid products: five hydrophilic gels (Conceptrol®, Gynol II®, K-Y®, Replens®, and Zidoval®) and two oil-in-water creams (Canesten® Vaginal and Dalacin® V). Mucoadhesive potential of a gel containing 1% (*w*/*w*) *Thymus vulgaris* L. essential oil (TVEO gel) and its placebo formulation (Placebo gel), recently developed in our laboratory, have also been studied.

2. Materials and methods

2.1. Materials

Carbopol® 974P was a kind gift from Noveon, Inc. (Cleveland, OH, USA). Porcine gastric mucin (type II) was purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). All other chemicals were of analytical grade or equivalent. Commercially available vaginal semisolid products were purchased from local retailers in New York, NY, USA and Monterey, CA, USA (Conceptrol®, Gynol II®, and Replens®), Léon, Spain (Zidoval®), and Porto, Portugal (K-Y®, Dalacin® V, and Canesten® Vaginal). TVEO gel and Placebo gel were manufactured as previously reported by our group [16]. Main features of all nine formulations are presented in Table 1, including their pH (mean value of three samples) as determined by direct read-

Table 1
Vaginal semisolid formulations evaluated by the proposed *in vitro* tensile mucoadhesion testing method

Commercial name	Gelling/mucoadhesive agent(s)	Other excipients	Active substance(s)	pH	Intended use(s)	Company
Canesten® Vaginal	–	Benzyl alcohol, cetyl palmitate, cetostearyl alcohol, polysorbate 60, sorbitan monostearate, 2-octyl dodecanol, water	Clotrimazole (1%)	6.0	Vaginal candidosis	Bayer HealthCare
Conceptrol®	Sodium carboxymethylcellulose	Lactic acid, methylparaben, povidone, propylene glycol, sorbic acid, sorbitol solution, water	Nonoxynol-9 (4%)	4.6	Contraceptive	McNeil-PPC, Inc.
Dalacin® V[a]	–	Sorbitan monostearate, polysorbate 60, propylene glycol, stearic acid, cetostearyl alcohol, cetyl palmitate, mineral oil, benzyl alcohol, water	Clindamycin (2%)	4.6	Bacterial vaginosis	Pfizer, Inc.
Gynol II®	Sodium carboxymethylcellulose	Lactic acid, methylparaben, povidone, propylene glycol, sorbic acid, sorbitol solution, water	Nonoxynol-9 (2%)	4.5	Contraceptive	McNeil-PPC, Inc.
K-Y®	Hydroxyethylcellulose	Chlorhexidine gluconate, gluconolactone, glycerin, methylparaben, sodium hydroxide, water	–	4.8	Vaginal moisturizer	Johnson&Johnson
Replens®	Polycarbophil & Carbopol® 974P	Glycerin, hydrogenated palm oil glyceride, methylparaben, mineral oil, sodium hydroxide, sorbic acid, water	–	3.3	Vaginal moisturizer	LDS Consumer products
Zidoval®[b]	Carbopol® 974P	EDTA, methylparaben, propylene glycol, propylparaben, sodium hydroxide, water	Metronidazole (0.75%)	4.1	Bacterial vaginosis	3 M Pharmaceuticals
TVEO gel[c]	Polycarbophil	Propylene glycol, lactic acid, triacetin, hydrochloride acid, triethanolamine, water	*Thymus vulgaris* L. essential oil (1%)	4.2	Vulvovaginal candidosis	–
Placebo gel[c]	Polycarbophil	Propylene glycol, lactic acid, triacetin, hydrochloride acid, triethanolamine, water	–	4.2	Placebo formulation	–

[a] Available in some countries as Cleocin® (Pfizer, Inc.).
[b] Available in some countries as Metrogel Vaginal® (3 M Pharmaceuticals).
[c] Not commercially available.

ing with a pH electrode (Metrohm 605 pHmeter, Metrohm Ltd., Herisau, Swiss).

2.2. Methods

2.2.1. Manufacture of 1% (w/w) Carbopol® 974P gel (CpGel)

Carbopol® 974P has been recognized as a standard vaginal mucoadhesive polymer, presenting several advantageous properties that contribute to its broad use in investigational and commercially available vaginal drug formulations [17–20]. Thus, a 1% (*w*/*w*) Carbopol® 974P gel (CpGel) was utilized as model for vaginal semisolid mucoadhesive formulations. CpGel was prepared by dispersing 1 g of Carbopol® 974P in approximately 90 g of distilled water by continuous mechanical stirring (1500 rpm, 20 min). Enough triethanolamine was added until pH 4.5 ± 0.1 was achieved, and final weight of formulation was completed with distilled water. Afterwards, gel was kept at rest for 24 h at 2–8 °C in order to allow entrapped air removal. CpGel was stored for 7 days at 20 °C before mucoadhesive measurements, in order to achieve its definitive consistency properties. Immediately prior to the mucoadhesive measurement, CpGel was transferred to cylindrical bottles (62 mm diameter) to a fixed height (10 mm), avoiding introduction of air bubbles, and sealed. Then, bottles were kept at 37 °C (body temperature) or at 20 °C (room temperature) for 90 min, according to test temperature, in order to stabilize the temperature of gel samples.

2.2.2. Model mucosa

Several animal species have been used as source of vaginal mucosa for mucoadhesion testing, including sheeps, rats, rabbits and pigs [8,21,22]. Cow vaginal mucosa has also been widely used as a model membrane, demonstrating to possess good characteristics concerning the simulation of human vaginal mucosa properties [20,23]. Thus, cow mucosa (*Bos taurus*) was used as a model mucosa in our study. Samples from several newly sacrificed animals were obtained from a local slaughterhouse. Vaginal mucosa was carefully separated from underlying tissues, washed with normal saline, and cut in smaller pieces of adequate size. After rinsing, samples were frozen (−20 °C) until required.

2.2.3. Immersion fluids

Three fluids were prepared in order to immerse vaginal mucosa prior to the mucoadhesive measurements: a vaginal fluid simulant (VFS), normal saline (NS) and normal saline with pH adjusted to 4.2 (NS 4.2). VFS composition and preparation was similar to the one developed by Owen and Katz [24], added of 1.5% (*w*/*v*) porcine gastric mucin (type II). Final composition of VFS was as follows (quantities in grams per liter): porcine gastric mucin (type II), 15.0; glucose, 5.0; sodium chloride, 3.51; lactic acid, 2.00; potassium hydroxide, 1.40; acetic acid, 1.00; urea, 0.4; calcium hydroxide, 0.222; glycerol, 0.16; bovine serum albumin, 0.018; and water, e.q. to 1 L. The fluid simulant had a pH value of 4.2, within the interval considered normal for the healthy vagina of women in fertile years (pH 4.0–5.0) [25,26]. Incorporation of 1.0–1.5% mucin in the original VFS is regarded to better simulate biochemical, rheological and mucoadhesive properties of the physiological fluid present in the vagina [27–29]. Although we have not used cervical mucins to prepare VFS (due to their unavailability in the market), substitution by porcine gastric mucin seems to be legitimate by the fact that cervical mucins have similar composition and structure to those commonly found in other mucous body fluids [30]. Also, VFS with different pH values (4.2, 5.0, 6.0 and 7.0) were obtained. NS pH was measured as 5.5 and NS 4.2 was obtained by adding a negligible quantity of concentrated hydrochloride acid to NS.

2.2.4. Mucoadhesive measurements

The basic concepts of the mucoadhesion test developed in this work are similar to others reported previously by several research groups [11,21,31–33]. Mucoadhesion was evaluated by means of a tensile test, where the measurement of maximum force (detachment force, F_{dt}) or work (work of adhesion, W_{ad}) required for detach a piece of bovine vaginal mucosa from a sample of CpGel, after an initial period of intimate contact, is indicative of the gel mucoadhesive potential. The forces involved in the detachment process were measured by a TA-XT2*i* texturometer (Stable Micro Systems Ltd., Surrey, UK) in adhesive mode. The mucoadhesion testing apparatus is shown in Fig. 1. Immediately before the mucoadhesive measurements, samples of cow vaginal mucosa were defrosted in a bath of NS (37 °C, 60 min), rinsed, and attached to the instrument probe (cylindrical, 25 mm diameter) by means of rubber bands. Care was taken in order to assure that the mucosal surface covering the lower end of the probe was as flat as possible. Mucosal tissue was then immersed in a fluid (immersion fluid) at a pre-defined temperature (test temperature) for 15 min. Afterwards, vaginal tissue was raised above the bottle containing CpGel sample, previously placed and immobilized in a water bath (20 °C or 37 °C, according to test temperature), and excess of immersion fluid was carefully rinsed with absorbent paper. The probe was then lowered at a known speed (probe speed) until it touched the gel surface. Subsequently, intimate contact between mucosal tissue and gel sample was assured by means of a constant downward force applied by the probe on the sample surface (probe force). After a pre-determined time period (mucosa/sample contact time), probe was brought to its initial position above CpGel sample, at a known speed (probe speed), and F_{dt} and W_{ad} were measured automatically by the software provided with the texturometer. These two parameters were considered as indicators of the mucoadhesive potential of the samples (mucoadhesive parameters).

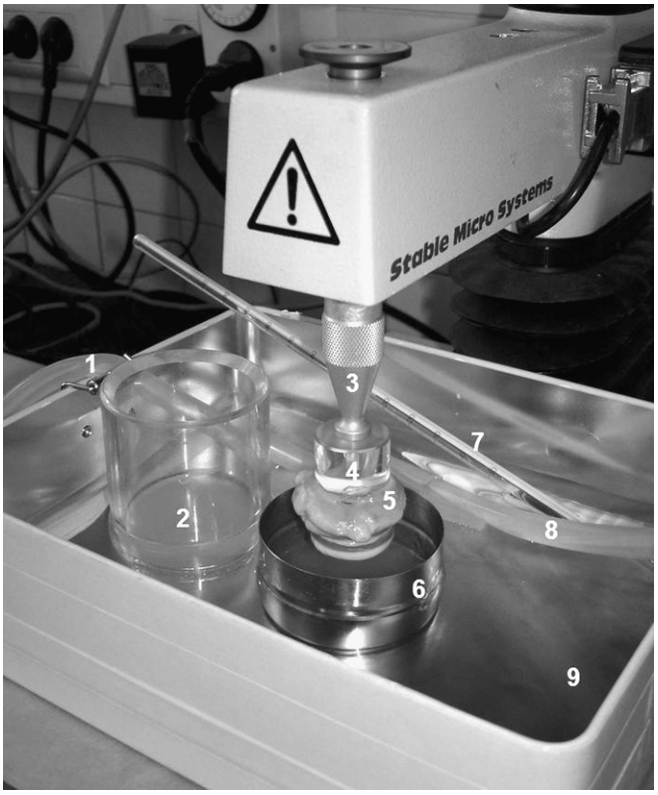

Fig. 1. Mucoadhesion testing apparatus. 1 – water in; 2 – immersion fluid; 3 – probe holder; 4 – probe; 5 – mucosa; 6 – sample bottle; 7 – thermometer; 8 – water out; 9 – water bath.

Influence of different testing conditions (immersion fluid, VFS pH, and test temperature) and instrumental parameters (probe speed, probe force, and mucosa/sample contact time) on the obtained F_{dt} and W_{ad} values was tested, according to Table 2. Unless otherwise stated, mucoadhesive measurements were performed according to pre-established values indicated in the same table, which were chosen according to previous reports [11,21,32,34]. In order to assess the possibility of using the same mucosa sample for multiple determinations, variation of F_{dt} and W_{ad} was evaluated after a series of five washes with NS of the mucosal tissue (one after each mucoadhesive experiment). Also, mucoadhesion variability between mucosa samples obtained from different animals was assessed. At least six replicate measurements were performed for each set of testing conditions and instrumental parameters.

Table 2
Pre-established testing conditions and instrumental parameters, and their variations, used in the mucoadhesive measurements

Testing conditions and instrumental parameters	Pre-established values	Tested variations
Immersion fluid	VFS	NS 4.2, NS, No fluid
VFS pH	4.2	5.0, 6.0, 7.0
Test temperature (°C)	37	20
Probe speed (mm s^{-1})	1.0	0.5, 2.5, 5.0, 10.0
Probe force (N)	0.20	0.05, 0.10, 0.15, 0.25
Mucosa/sample contact time (s)	60	30, 150, 300, 600

2.2.5. Statistical analysis

Statistical analysis of the results was performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to investigate the influence of a series of five washes, mucosal samples from different animals, different immersion fluids, or different VFS pH values on the mean values of F_{dt} and W_{ad}. Also, differences between mucoadhesive parameters of commercially available vaginal products were assessed utilizing one-way analysis of variance. Post hoc comparisons of the means of individual groups were performed according to Tukey's HSD test. Influence of test temperature (20 °C or 37 °C) on the mean values of F_{dt} and W_{ad} was statistically evaluated using Student's t test. In all cases, $p < 0.05$ was accepted to denote significance.

3. Results and discussion

3.1. Influence of different testing conditions

The inherent heterogeneity of biologic tissues, such as cow mucosa, influences results obtained in this type of experiment. In this way, reproducibility of the assay should always be tested in order to assure that when different samples obtained for assorted animals allow obtaining similar results that only depend on the mucoadhesive properties of studied formulations [35]. Results of F_{dt} and W_{ad} using mucosa samples from different animals are shown in Fig. 2. Values of F_{dt} presented some variability among tissue samples coming from different animals, namely for #5. This last group of samples showed to be significantly different ($p < 0.05$) when compared to samples coming from other animals. As for W_{ad}, all five groups of mucosa samples demonstrated to be homogeneous, presenting no significant differences among them ($p > 0.05$). Overall coefficient of

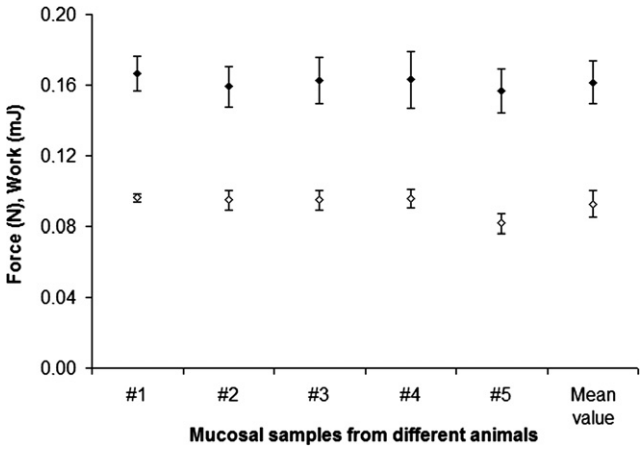

Fig. 2. Influence of mucosa samples obtained from different animals on F_{dt} (open symbols) and W_{ad} (closed symbols) ($n = 6$; bars represent SD). Mean value group includes all the results obtained for mucosa samples obtained from different animals ($n = 30$; bars represent SD).

variance (CV) was also slightly higher for F_{dt} than for W_{ad} (7.9% vs. 7.6%). These results showed a coherent homogeneity of the mean results of mucoadhesive potential obtained for mucosal tissues coming from different animals, particularly when W_{ad} was considered as indicator of the mucoadhesive potential, with standard deviations comparable to those previously obtained by several research groups [11,21,28]. Thus, proposed methodology seems to be appropriate in order to assure that different experimental values result from different mucoadhesive potentials of tested formulations, not due to intrinsic heterogenic characteristics of cow vaginal mucosa.

Difficulty of obtaining enough cow vaginal mucosa, time requested to previously prepare this tissue and its correct attachment to the instrument's probe are disadvantages of the proposed method. Fig. 3 presents the results of F_{dt} and W_{ad} obtained when the same vaginal mucosa sample was submitted to a series of five washes with NS between measurements. Both mucoadhesion parameters showed no significant differences ($p > 0.05$) before and after washings. Analysis of the graphic plot indicates no trend for increasing or decreasing of mucoadhesion parameters, although W_{ad} was less variable than F_{dt} (CV, 8.8% vs. 12.1%), in agreement with the results previously obtained for mucosa samples coming from different animals (Fig. 2). Obtained results suggest that the same portion of mucosa can be used, at least, up to six determinations, making the assay less time consuming and requesting fewer biologic samples. Conversely, it seems advisable that results obtained with the same mucosa sample should be evaluated for each tested formulation, as some of them may induce important alterations to vaginal tissues (e.g., degeneration of epithelial cells resulting in intracellular content leakage) that may influence mucoadhesion results. Thus, although advantageous this proceeding should be pre-validated particularly when considering different types of formulations.

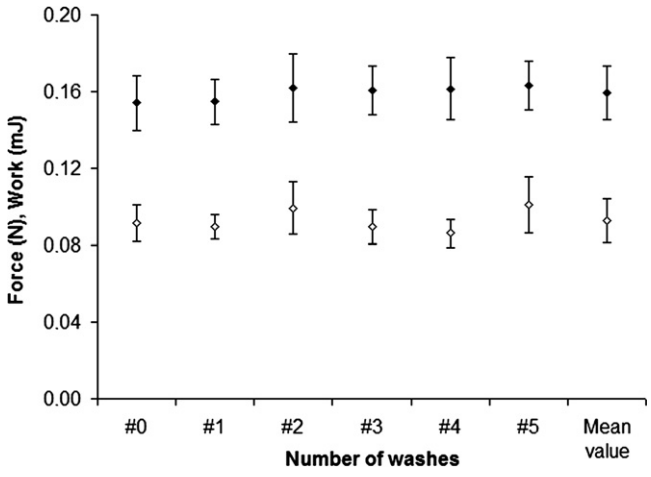

Fig. 3. Influence of a series of five washes of the cow mucosa on F_{dt} (open symbols) and W_{ad} (closed symbols) ($n = 6$; bars represent SD). Mean value group includes all the results obtained during the series of washes ($n = 36$; bars represent SD).

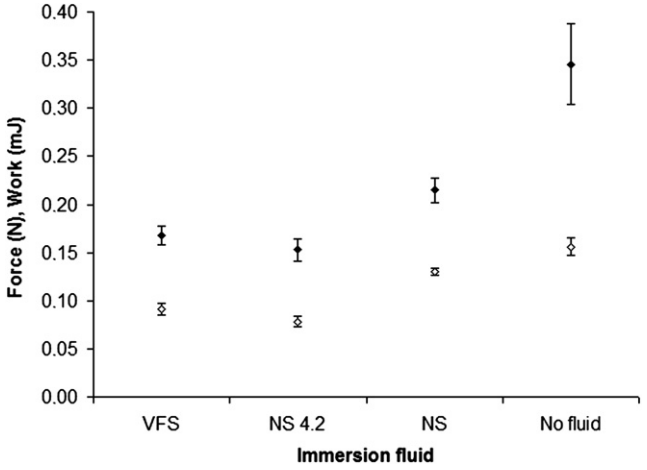

Fig. 4. Influence of different immersion fluids on F_{dt} (open symbols) and W_{ad} (closed symbols) ($n = 6$; bars represent SD). VFS – vaginal fluid simulant; NS 4.2 – normal saline with adjusted pH to 4.2; NS – normal saline (pH 5.5); No fluid – no immersion fluid.

Influence of different immersion fluids, used to bathe mucosal samples, on the performance of the mucoadhesive test is shown in Fig. 4, indicating that there are significant differences among fluid choice. Only fluids with pH 4.2 (VFS and NS 4.2) presented similar results for F_{dt} and W_{ad}. This fact seems to support the importance that mimicking the vaginal fluid or conditions encountered *in vivo* may have in the final results and their interpretation. It is also noteworthy verifying that, when no immersion fluid was utilized, mucoadhesion parameters were noticeably increased; this increment was only moderate for NS (pH 5.5) when compared to VFS and NS 4.2. The much higher mucoadhesive potential of CpGel when no immersion fluid was used should be taken into account particularly when considering products for women with poor or scarce vaginal lubrication, as for example, during menopause [36]. This enhanced mucoadhesive potential can probably be explained by water movement between surfaces with different degrees of hydration [37]. Rapid diffusion from a highly hydrated system (CpGel) and a substantially less hydrated surface ("dry" mucosa) induces the movement of polymeric chains of Carbopol® in the same way water does, augmenting the proximity with the mucosa and the possibility of interpenetration to occur with mucin chains present in the mucosa surface. It is also possible to explain these results with the lubricant effect of the immersion fluids, which difficult contact between mucosa and semisolid formulation, thus the mucoadhesive potential. Indeed, this is an expected function of human vaginal fluid, namely because of the presence of low concentration of mucins in its composition [25,38]. Results appear to reveal that pH was the main characteristic of the different immersion fluids which most influenced mucoadhesive potential of CpGel. This may be explained by high pH-sensitiveness presented by Carbopol® 974P. However, similar results obtained for VFS and NS 4.2 should be interpreted with caution, as other formulations may interact differently with

constituents of VFS, particularly mucins. Thus, use of a fluid similar to the one naturally occurring in the vagina would be more prudent in order to simulate *in vivo* conditions.

Changes to normal pH values of vaginal environment (4.0–5.0) are quite common, particularly when local saprophyte flora, as for example, *Lactobacilli* populations, is disturbed (e.g., due to bacterial vaginosis or use of antibiotics), during estrogen levels' decrease (e.g., due to menopause), or during sexual intercourse (semen, alkaline in nature, originates a pH rising) [39–41]. These phenomena induce an increase in pH, usually above 5.5. Also, several new approaches to vaginal drug administration include the utilization of pH sensitive polymers in the design of delivery systems, which can influence their behavior at different pH values [42,43]. Thus, it may be important to characterize the mucoadhesive potential of vaginal formulations at diverse pH conditions, mainly when different pH values are expected to be found *in vivo*. Fig. 5 presents the mean values for F_{dt} and W_{ad} when using VFS with different pH values (4.2, 5.0, 6.0 and 7.0) as immersion fluid. Analysis of the graphic plot indicates a decreasing for both mucoadhesion parameters trend with increasing values of pH. In the case of F_{dt}, results obtained at pH 4.2 only differed significantly from those at 7.0; as for W_{ad}, there were significant differences between results obtained at 4.2 and those at 6.0 and 7.0. These results confirm that altered pH values of VFS influence mucoadhesive potential of polyacrylic acid-based gels, corroborating previously reports by Lee and Chien [8]. This may probably be explained by the quotient between ionized/non-ionized carboxylic groups of mucin and polyacrylic acid chains. Indeed, it is known that this ratio influences the interpenetration ability of these polymeric molecules, being commonly accepted as an important feature that contributes to mucoadhesion [44,45]. Our results seem to evidence that rising pH values of VFS from 4.2 to 7.0 augmented electrostatic repulsion between polymeric chains due to the increment of its positive charges. Excessive distancing of mucin and polyacrylate chains originates a loose structure that limits the establishment of adhesive bonds, and ultimately mucoadhesion.

Still concerning *in vivo* emulation, the importance of experimental temperature was assessed in this study. Results of the influence of test temperature on the performance of the mucoadhesion test indicate that there were no significant differences between both temperatures, when considering F_{dt} (0.08 ± 0.01 N at 20 °C, vs. 0.09 ± 0.01 N at 37 °C; $n = 6$ and $n = 27$, respectively). Conversely, W_{ad} obtained at 20 °C and 37 °C showed to be significantly different (0.13 ± 0.01 mJ and 0.16 ± 0.01 mJ, respectively). Although environmental temperature (20–25 °C) is frequently used in mucoadhesive studies [12,20,23], the slight variations observed in this study may be important, and have been explained by conformational alterations in the chain structures of mucin and polyacrylates, as well as by thermodynamic changes, that influence the establishment of adhesive bonds [45]. Also, several other vaginal semi-solid formulations being developed are known to be highly influenced by temperature, particularly those containing thermosensitive polymers [11,43]. In these cases, utilization of physiologic temperature may assume the utmost importance in the determination of the mucoadhesive potential.

3.2. Influence of different instrumental parameters

Importance of studying the parameters related to the instrument used to measure F_{dt} and W_{ad} has been exposed by results obtained for different values of probe speed, probe force and mucosa/sample contact time. Fig. 6 presents the results of F_{dt} and W_{ad} for different speeds of the testing probe. It can be noticed an increase of the mean values of both mucoadhesion parameters with increasing probe speed. For smaller speeds of the probe (1.0 mm s^{-1} or less) results showed higher variability, as revealed by higher CV. On the other hand, for higher speeds of probe results of mucoadhesion parameters tend to achieve a pla-

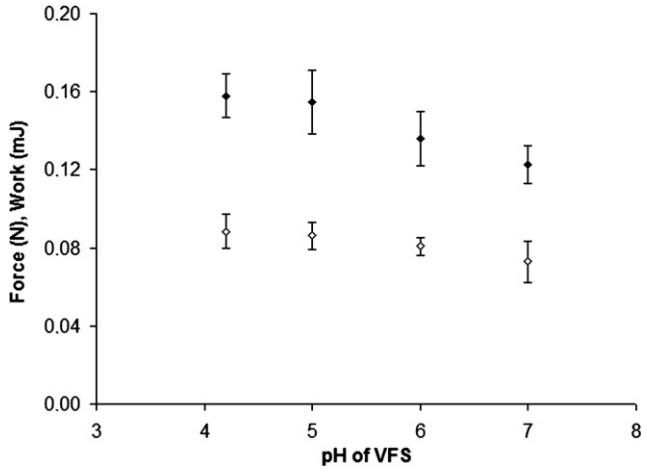

Fig. 5. Influence of the pH of VFS on F_{dt} (open symbols) and W_{ad} (closed symbols) ($n = 6$; bars represent SD).

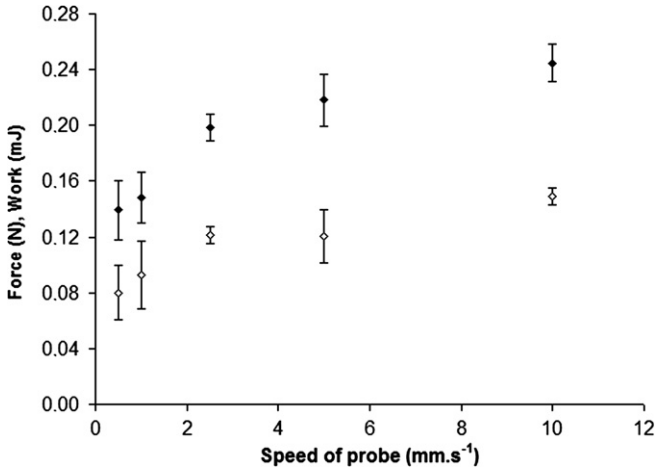

Fig. 6. Influence of speed of probe on F_{dt} (open symbols) and W_{ad} (closed symbols) ($n = 6$; bars represent SD).

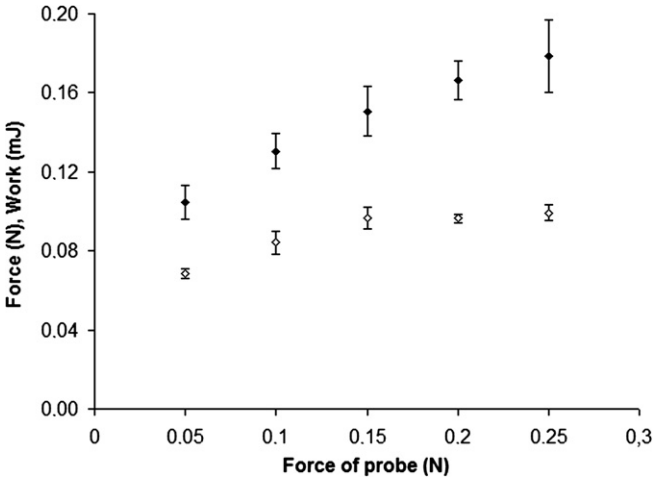

Fig. 7. Influence of force of probe on F_{dt} (open symbols) and W_{ad} (closed symbols) ($n = 6$; bars represent SD).

teau. Influence of the force applied on the surface of CpGel samples by the probe on the performance of the mucoadhesive test is presented in Fig. 7. The range of forces tested is in accordance with those that will probably be applied to semisolid formulations after being placed in the vaginal canal [46]. Results seem to indicate a direct relationship between this instrumental parameter and mucoadhesive parameters. At higher probe forces mucoadhesive results tend to reach a plateau. This appears to be most pronounced for F_{dt}, with maximum values being achieved for probe forces starting at 0.15 N. It was also noticed that for higher forces (higher than 0.20) the ability of CpGel samples to hold penetration of the probe started to weaken, with full penetration being observed for forces superior to 0.25 N. Fig. 8 presents the variation of F_{dt} and W_{ad} with time of contact between mucosal tissue and gel sample. Experimental data indicates that there is a direct relationship between both mucoadhesive parameters and mucosa/sample contact time. As in the case of the influence

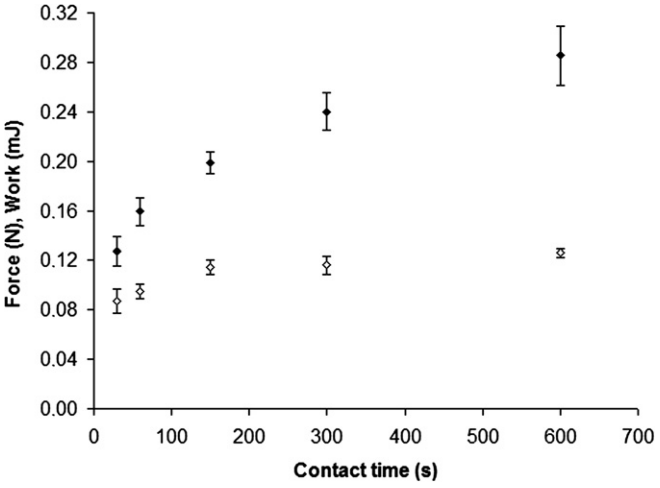

Fig. 8. Influence of the mucosa/sample contact time on F_{dt} (open symbols) and W_{ad} (closed symbols) ($n = 6$; bars represent SD).

of probe force and probe speed on mucoadhesive potential, it seems that a plateau is achieved for higher times of contact, being this particularly obvious for F_{dt} when mucosa/sample contact time exceeds 150 s. These results appear to support the principles of the interpenetration theory as revealed by mucin/polymer proximity and time dependency of mucoadhesive potential [6]. According to this theory, it is possible to say that the probe force applied to the mucosa/sample interface promotes the proximity between these two surfaces, facilitating their interpenetration, while mucosa/sample contact time enhances the probability of adhesive bond formation.

Results of mucoadhesion parameters obtained with different probe speeds, probe forces and mucosa/sample contact times were adjusted to three simple mathematical models (linear, logarithmic and exponential), as presented in Table 3. Generally, it can be stated that there is a better adjustment of results of W_{ad} than those of F_{dt} to all models. Also, experimental data seem to be better correlated with instrumental parameters when using both non-linear mathematical models. These results emphasize the idea of a plateau region for mucoadhesion parameters at higher values of probe speed, probe force and mucosa/sample contact time.

According to obtained results, it seems more appropriate to choose intermediate values of probe speed, probe force and mucosa/sample contact time, within the ones experimented. When using lower values of these instrumental parameters the obtained mucoadhesion parameters are closer to the texturometer detection limit, originating more variability among experimental readings. On the other hand, experimental data obtained at higher values of the studied instrumental parameters appear to achieve a maximum plateau, being this fact also evidenced by better adjustment to the logarithmic and the exponential models (Table 3). Also, higher values may lead to experimental bias, such as penetration of gel samples for augmented probe force or water loss from the mucoadhesive site due to prolonged mucosa/sample contact time.

3.3. Comparison between F_{dt} and W_{ad} as mucoadhesion parameters

Data obtained for both studied mucoadhesion indicators seem to globally support that W_{ad} is more reliable (better model fitting) and reproducible (less variability between results) than F_{dt} in the evaluation of adhesive potential of semisolid formulations to the vaginal mucosa. Although both data are in agreement and several studies consider only F_{dt} as an indicator of mucoadhesive potential [22,28], we think that W_{ad} may be regarded as a better mucoadhesion indicator. This position has also been previously supported by other researchers [47,48], since W_{ad} provides wider evaluation of the detachment phenomena, representing the sum of all established adhesive bonds, not only the maximum force of detachment which corresponds to F_{dt} evaluation. Even so, when possible, both

Table 3
Comparison of three simple mathematical models for evaluation of the relation between mucoadhesive parameters (F_{dt} and W_{ad}), and probe speed, probe force and mucosa/sample contact time

		Mathematical models							
		Linear model ($M = ax + b$)			Logarithmic model ($M = a\ln x + b$)			Exponential model ($M = ax^b$)	
		a	b	r^2	a	b	r^2	a	b	r^2
Probe speed	F_{dt}	0.0064	0.0885	0.8498	0.0219	0.0948	0.9483	0.0935	0.1994	0.9543
	W_{ad}	0.0107	0.1492	0.8441	0.0371	0.1591	0.9775	0.157	0.2008	0.9718
Probe force	F_{dt}	0.147	0.067	0.8233	0.0196	0.129	0.9486	0.1426	0.2354	0.9408
	W_{ad}	0.3679	0.0909	0.9788	0.0461	0.24	0.9917	0.2842	0.335	0.9995
Mucosa/sample contact time	F_{dt}	0.00006	0.094	0.7609	0.013	0.0436	0.9597	0.058	0.1238	0.9526
	W_{ad}	0.0003	0.1432	0.9125	0.0521	-0.0545	0.9918	0.0522	0.2668	0.998

M – mucoadhesive parameter (F_{dt} or W_{ad}); x – instrumental parameter (probe speed, probe force, or mucosa/sample contact time); a, b – model's variables.

Table 4
Testing conditions and instrumental parameters utilized in the evaluation of the mucoadhesive potential of vaginal semisolid products

	Experimental set #1	Experimental set #2
Immersion fluid	VFS	VFS
Test temperature (°C)	37	37
Probe speed (mm s^{-1})	1.0	5.0
Probe force (N)	0.2	0.2
Mucosa/sample contact time (s)	60	150

indicators should be considered in order to better assess the mucoadhesive potential of vaginal semisolids.

3.4. Mucoadhesive potential of several vaginal semisolid products

After the analysis of the results obtained for CpGel, two experimental sets of testing conditions and instrumental parameters (Table 4) were used to evaluate the mucoadhesive potential of the vaginal semisolid products. Obtained results of F_{dt} and W_{ad} are presented in Figs. 9 and 10, respectively. Generally, cellulose derivatives-based gels exhibited significantly higher mucoadhesive potential, followed by both oil-in-water creams. Indeed, excellent mucoadhesive properties of cellulose derivatives are well recognized, with the particular advantage of being relatively pH-independent [49,50]. While creams do not possess polymeric mucoadhesive constituents in their formulation other kind of mucoadhesive interaction may potentially be observed (e.g., hydrophobic bonds), due to their physical–chemical nature, explaining the performance of these products. Polyacrylic acid-based gels presented, surprisingly, the lowest mucoadhesive potential of all products. Although polyacrylic acid derivatives are recognized as

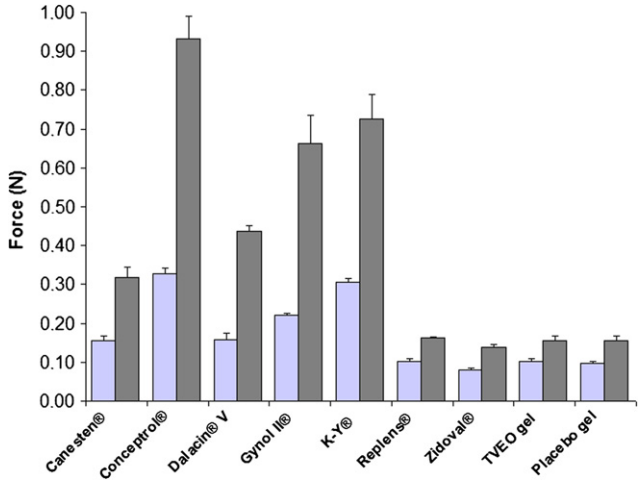

Fig. 9. Mucoadhesive potential of several vaginal semisolid products. Light gray and dark gray bars represent the mean value of F_{dt} determined using experimental set #1 and experimental set #2, respectively ($n = 6$; bars represent SD).

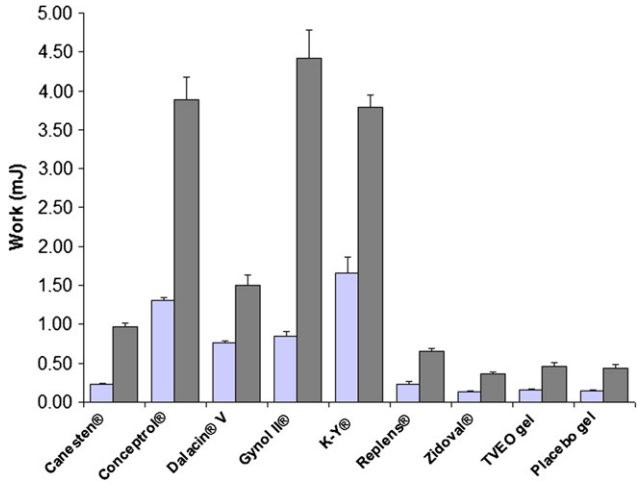

Fig. 10. Mucoadhesive potential of several vaginal semisolid products. Light gray and dark gray bars represent the mean value of W_{ad} determined using experimental set #1 and experimental set #2, respectively ($n = 6$; bars represent SD).

good mucoadhesive polymers [51], these results may probably be explained by the low concentrations commonly utilized in vaginal gels and the pH of these formulations. Also, unexpected low mucoadhesive potential of polyacrylic acid-based gels emphasizes the necessity of using and characterizing methods specifically designed for the evaluation of mucoadhesive potential of vaginal semisolids.

While both experimental sets revealed similar trends in the mucoadhesive potential of tested products, several significant differences could be found, highlighting the importance that experimental conditions have in the final results of this type of methods. For example, experimental set #1 did not find significant differences between Dalacin® V and Gynol II® (when considering W_{ad}), while experimental set #2 did. The opposite was observed when comparing F_{dt} obtained for Replens® and Zidoval®: experimental set #1 was able to distinguish these two formulations while experimental set #2 was not. Another interesting situation was the comparison between both experimental sets for Gynol II® and K-Y®. Obtained results not only showed that the mucoadhesive potential of these two gels, when considering W_{ad}, was significantly different for experimental sets #1 and #2 but also that their relative rank was reversed. Also, F_{dt} and W_{ad} presented differences in the ability of distinguishing tested products. The most significant case was the comparison between Gynol II® and K-Y®. When considering experimental set #2, F_{dt} was not significantly different for these two gels, while W_{ad} presented significant differences.

Additionally, obtained results for commercial products were compared with a number of their rheological and textural parameters previously obtained by our research group [16,52] in order to verify the hypothesis reported by several investigators that bioadhesive properties of semisolid formulations, namely polyacrylic acid derivatives [32,33,53] or cellulose derivatives [34] based gels, are correlated with their flow and textural properties. However, no correlation was observed between mucoadhesive and rheological or textural properties, which is understandable considering that these relationships may only be suitable to explain the behavior of simpler systems (two or three components) based in the same type of gelling/mucoadhesive agents. In the case of tested commercial products, multi-ingredient formulations and different types of polymeric nature or even physical arrangement (e.g., colloid or emulsion) can influence in different ways their mucoadhesive, rheological and textural properties.

4. Conclusion

Inexistence of a standardized method to evaluate the mucoadhesive potential of vaginal semisolids limits comparison of results obtained by different investigators. Our results indicate that variations in experimental conditions can significantly influence mucoadhesive measurements, thus emphasizing the importance of using *in vitro* mucoadhesion testing methods that have been previously characterized and that consider the specificities of the mucosal administration site, in this case the vaginal cavity. Also, variability of these *in vitro* methods recommends that researchers be precautious about the interpretation of their own results. The proposed methodology hereby described appears to be a useful tool when evaluating the mucoadhesive potential of commercial or investigational vaginal semisolid products.

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