

## Screening of mucoadhesive vaginal gel formulations

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Rational design of vaginal drug delivery formulations requires special attention to vehicle properties that optimize vaginal coating and retention. The aim of the present work was to perform a screening of mucoadhesive vaginal gels formulated with carbomer or carrageenan in binary combination with a second polymer (carbomer, guar or xanthan gum). The gels were characterised using *in vitro* adhesion, spreadability and leakage potential studies, as well as rheological measurements (stress and frequency sweep tests) and the effect of dilution with simulated vaginal fluid (SVF) on spreadability. Results were analysed using analysis of variance and multiple factor analysis. The combination of polymers enhanced adhesion of both primary gelling agents, carbomer and carrageenan. From the rheological point of view all formulations presented a similar behaviour, prevalently elastic and characterised by loss tangent values well below 1. No correlation between rheological and adhesion behaviour was found. Carbomer and carrageenan gels containing the highest percentage of xanthan gum displayed good *in vitro* mucoadhesion and spreadability, minimal leakage potential and high resistance to dilution. The positive results obtained with carrageenan-xanthan gum-based gels can encourage the use of natural biocompatible adjuvants in the composition of vaginal products, a formulation field that is currently under the synthetic domain.

**Uniterms:** Mucoadhesive vaginal gels/screening. Xanthan gum/use/vaginal products. Mucoadhesion. Vaginal products/rheology. Vaginal products/spreadability. Natural products/use/vaginal gels.

O planejamento racional de formulações para a liberação vaginal de fármacos requer atenção especial às propriedades do veículo, que otimizem o revestimento e a retenção vaginal. O objetivo do presente trabalho foi realizar uma triagem de géis vaginais mucoadesivos formulados com carbomero ou carragenina em combinação binária com um segundo polímero (carbomero, goma guá ou xantana). Os géis foram caracterizados usando estudos *in vitro* de aderência, espalhabilidade e potencial de vazamento, bem como medições reológicas (testes de varredura de tensão e frequência) e o efeito de diluição com fluido vaginal simulado (SVF) na espalhabilidade. Os resultados foram analisados utilizando a análise de variância e de fator múltiplo. A combinação de polímeros reforçou a adesão de ambos os agentes gelificantes primários, carbomero e carragenina. Do ponto de vista reológico todas as formulações apresentaram comportamento semelhante, predominantemente elástico e caracterizado por valores de tangente de perda bem abaixo de 1. Não se encontrou correlação entre as medições reológicas e o comportamento de adesão. Os géis de carbomero e carragenina contendo o maior percentual de goma xantana apresentaram melhor mucoadesão e espalhabilidade, menor potencial de vazamento e maior resistência à diluição *in vitro*. Os resultados positivos obtidos com géis de carragenina-goma xantana podem incentivar o uso de adjuvantes biocompatíveis naturais na composição dos produtos vaginais, um campo de formulação atualmente sob o domínio de produtos sintéticos.

**Unitermos:** Géis vaginais mucoadesivos/triagem. Goma xantana/uso/produtos vaginais. Mucoadesão. Produtos vaginais/reologia. Produtos vaginais/espalhabilidade. Produtos naturais/uso/géis vaginais.

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## INTRODUCTION

The vagina has been traditionally used as a drug delivery site for local treatments. Due to the “vaginal first-pass effect”, the vaginal route of administration can also be used for targeted drug delivery to the uterus with minimal systemic adverse effects (Bernkop-Schnürch, Hornof, 2003). The large surface area and rich blood supply, as well as its many advantages as administration route, make the vagina an excellent drug delivery site for systemic treatments also. The advantages of vaginal administration include the avoidance of hepatic first-pass metabolism, reduction in the incidence and severity of gastrointestinal side effects, decrease in hepatic side effects of drugs such as steroids, and overcoming pain and tissue damage observed with parenteral routes (das Neves, Bahia, 2006; Valenta, 2005). Also, high permeability for low molecular weight drugs (Hussain, Ahsan, 2005) and higher residence times than other absorption sites, such as intestinal mucosa (Andrews, Laverty, Jones, 2009), have been reported as benefits offered by this route of administration. On the other hand, drug delivery via the vagina presents some drawbacks that should be considered in product formulation design: changes of vaginal characteristics with menstrual cycle and in post-menopause - particularly concerning pH and fluid volume (das Neves, Bahia, 2006) - considerable variability in rate and extent of absorption of drugs by changes in the thickness of the vaginal epithelium, and risk of local irritation (Hussain, Ahsan, 2005).

According to das Neves and Bahia (2006), ideal vaginal drug delivery systems should be easy to use, discreet, painless, safe for continuous administration and widely available, allow self-administration, provide high bioavailability, have minimal interference with body functioning and daily life, as well as low cost. These systems should also present minimal leakage and long local residence after administration (Liu *et al.*, 2009), and ideally, distribute uniformly throughout the vaginal cavity (Hussain, Ahsan, 2005).

Gels show many of the above-mentioned characteristics for an ideal vaginal drug delivery system and are among women's preferred vaginal dosage forms (Hardy *et al.*, 1998). Another attractive feature of gels is that they can generally be manufactured by simple and easily scalable processes. In particular, bioadhesive gels show one important advantage over conventional gels: they allow longer residence times in the vagina, which is important for reducing the frequency of administration and allowing controlled drug delivery.

Several polymers with bioadhesive properties

have been reported in the literature (Andrews, Laverty, Jones, 2009; Morales, McConville, 2011; Valenta, 2005), including: polyacrylates (polycarbophil, carbomer, thiolated polyacrylates), cellulosic derivatives (sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose) and other polysaccharides (chitosan, hyaluronic acid, gums, carrageenan). The following polymer combinations have been studied for mucoadhesive drug delivery systems: Carbopol® 974P and hydroxyethylcellulose (Garga *et al.*, 2010), poloxamer 407, kappa carrageenan and Carbopol® 934P-NF (Liu *et al.*, 2009), and dispersible microcrystalline cellulose and xanthan gum (D'Crux *et al.*, 2003) in vaginal gels, Carbopol® 940 and hydroxypropylmethylcellulose in vaginal tablets (Perioli *et al.*, 2011), Pemulen™ TR-1 and Synperonic PE/L 101 in topical gel-emulsions (Szucs *et al.*, 2008), Polyox and Gelcarin® GP-812 NF in film dressings (Boateng, Pawar, Tetteh, 2013). Most of the limited number of commercially available vaginal semisolid products are based on cellulosic and polyacrylic polymers (das Neves, Amaral, Bahia, 2008; Hussain, Ahsan, 2005; Valenta, 2005). In this sense, further research into the application of biomaterials such as carrageenan and other biodegradable polymers for bioadhesive vaginal gels is necessary.

Formulation of vaginal drug delivery systems needs to consider physiological characteristics such as pH. During the active genital age, the normal pH value of the vagina is between 4 and 5.5 (Bernkop-Schnürch, Hornof, 2003; das Neves, Bahia, 2006), deviating to lower or higher values depending on the stage of the menstrual cycle and sexual activity. Depending on excipients choice, pH may mainly influence gel adhesion to the vaginal mucosa. Rational design of vaginal drug delivery systems also requires special attention to vehicle properties that optimise vaginal coating and retention (Valenta, 2005). In this context, adhesion and spreadability studies are useful for assessing gels' residence time and their ability to spread in simulated vaginal conditions. According to das Neves and Bahia (2006), rheological properties are important for the critical functions of spreading and retention of gels over the vaginal surface. Gels present non-Newtonian flow behaviour, and as Owen, Peters and Katz (2000) discussed, such rheological behaviour has a substantial impact on the mechanics of gel spreading and retention. Considering that gels are viscoelastic materials, dynamic rheological tests are well suited for studying their rheological characteristics (Rao, 1999). Oscillation tests are useful to characterise gel texture and determine the conditions under which it does not undergo irreversible structural modifications (Aka-Any-Grah *et al.*, 2010).

It should also be noted that vaginal gels are diluted with vaginal fluids after their *in vivo* administration, leading to changes in their rheological and mucoadhesive properties (Aka-Any-Grah *et al.*, 2010). These changes should be taken into account in formulation design. Daily production of vaginal fluid is approximately 6 mL/day, with 0.5–0.75 mL continually present in the vagina (Bernkop-Schnürch, Hornof, 2003), and decreases with increasing age (Valenta, 2005). Spreadability is an important property of topical preparations from patient compliance point of view (Bachhav, Patravale, 2009), responsible for correct dosage transfer to the target site, ease of application and extrudability from the package (Garg *et al.*, 2002). This property could provide useful information for gel development when studied on diluted samples. In this sense, assays performed on diluted samples, here referred to as the leakage test, are used to explore gels leakage potential from the vagina and could provide relevant information for the design of formulations.

The aim of this work was to perform a screening of mucoadhesive vaginal gel formulations based on carbomer or carrageenan in binary combination with a second polymer, by means of adhesion, spreading, leaking and rheological measurements, in order to select the most promising formulations.

## MATERIAL AND METHODS

### Material

The selected mucoadhesive agents were Carbopol® 980 (*Cb*) (Lubrizol, USA), Gelcarin® GP-812 NF (*Cg*) (FMC BioPolymer, USA), Pemulen™ TR-1 (*C*) (Lubrizol, USA), guar gum (*G*) (Chopra Gums, India) and xanthan gum (*X*) (Weifang Ouchem, China). Other excipients used were propylene glycol, methylparaben, sorbic acid, FD&C blue n°1 colorant, sodium hydroxide or hydrochloride acid and deionised water. The composition of the formulated gels is shown in Table I. A commercially available hydroxyethylcellulose vaginal gel was purchased in a pharmacy in Montevideo (Uruguay).

Carbopol® and Pemulen™ belong to the group of polyacrylic acid derivatives. Both Carbopol® 980 and Pemulen™ TR-1 are covalently cross-linked polyacrylic acids, while Pemulen™ TR-1 is hydrophobically modified by the incorporation of long-chain alkyl (C10-C30) acrylates (Lochhead, Rulison, 1994). Gelcarin® 812 NF is a moderate potassium salt kappa carrageenan, an anionic polysaccharide of red seaweed origin. Carrageenans have been identified as inhibitors of herpes simplex virus

(Vo *et al.*, 2011) and extremely potent infection inhibitors of a broad range of genital human papillomaviruses (HPVs), providing protection against HPV transmission (Campo *et al.*, 2009). Guar and xanthan gums are also polysaccharides. The first is non-ionic and commercially isolated from the seeds of several leguminous plants, while the latter, is an anionic polysaccharide commercially obtained by bacterial fermentation (Goddard, Gruber, 1999). Therefore, the selected mucoadhesive agents comprised synthetic polymers and biomaterials, to be used in combinations that have not been previously reported in the literature. Carbopol® 980 (designed to perform similarly to the well-known Carbopol®940, but obtained from a toxicologically preferred polymerisation solvent) and Pemulen™ TR-1 (generally, used as polymeric emulsifier) appeared to be interesting synthetic agents. The already described promising properties of carrageenans as excipients for vaginal use made Gelcarin® GP-812 NF a worthy biocompatible candidate. Guar and xanthan gums, widely used in food products, were proposed to enhance the mucoadhesion of the formulations and reduce syneresis, which is a common defect of kappa carrageenan gels.

### Methods

#### Preparation of gels

Gels were prepared using Carbopol® 980 or Gelcarin® GP-812 NF in binary combination with either Pemulen™ TR-1, guar gum or xanthan gum, as a secondary mucoadhesive agent. Each pair of polymers was dispersed by mechanical stirring (Cole-Parmer Instrument Co., Servodyne 50003-45, USA) in hot water (70 °C ± 5 °C) in the presence of propylene glycol, preservatives and colorant. Acid or basic solution was added to make the final pH 5. Gel final weight was completed with water. Also, two gels formulated with only each one of the primary mucoadhesive agents (Carbopol® 980 or Gelcarin® GP-812 NF) were prepared following the same procedure.

In the present study, a commercial vaginal gel was used as a reference product (*Ref*). This gel contains hydroxyethylcellulose as the gelling/mucoadhesive agent and has already been reported to show *in vitro* mucoadhesive properties (das Neves, Amaral, Bahia, 2008).

#### Characterisation of gels

All tests were performed in duplicate and average results are presented. Bioadhesive devices exist in various physical forms and biological substrates widely

**TABLE I** – Composition of the formulated mucoadhesive vaginal gels

Sample	Polymer 1: Cb 0.4 %			Sample	Polymer 1: Cg 0.5 %		
	Polymer 2 (%)				Polymer 2 (%)		
	C	G	X		C	G	X
Cb	-	-	-	Cg	-	-	-
CbC0.5	0.5	-	-	CgC0.5	0.5	-	-
CbG0.5	-	0.5	-	CgG1	-	1.0	-
CbG1	-	1.0	-	CgG1.5	-	1.5	-
CbG2	-	2.0	-	CgX1	-	-	1.0
CbX1	-	-	1.0	CgX1.5	-	-	1.5
CbX1.5	-	-	1.5				

Other components: propylene glycol 5 %, methylparaben 0.2 %, sorbic acid 0.1 %, FD&C blue n° 1 0.001 %, sodium hydroxide 10 % solution or hydrochloric acid 2 % solution to make the final pH 5 and deionised water up to 100 % (500g).

vary in nature. Thus, there is no standard test for the evaluation of mucoadhesion (Wise, 2000). The same statement applies to spreadability and leakage tests. For this reason, adhesion, spreadability and leakage tests, as well as dynamic rheological measurements, were used to characterise the gels.

#### Adhesion test

This test was based on a method proposed by Bachhav and Patravale (2009). Here, 80 mg of sample was placed at the centre of an agar plate (1.5% w/w agar in pH 4.5 citrate buffer or pH 4.5 simulated vaginal fluid, SVF) and a 50 g weight was placed over the sample for 1 min. The agar plate was attached to an USP disintegration test apparatus and moved up and down in buffer or SVF at  $37\text{ °C} \pm 1\text{ °C}$ . The plate remained immersed in the solution during the whole test. The residence time of gels on the plate (adhesion time) was visually determined. SVF was prepared as reported by Aka-Any-Grah *et al.* (2010).

#### Spreadability test

This test was based on a method also proposed by Bachhav and Patravale (2009). Briefly, 1 g of sample at  $37\text{ °C}$  was loaded on a glass plate placed over squared paper (in millimetres). A second glass plate was placed over the sample and a weight of 100 g was allowed to rest on the upper glass plate for 1 min. The diameter after spreading of the gel was measured.

#### Leakage test

Eighty milligrams of diluted sample (see Preparation of diluted samples) were syringed onto one end of an agar glass slide (1.5% w/w agar in SVF). The agar slide was attached to one of the inner walls

of a transparent chamber, which was maintained at  $37\text{ °C} \pm 1\text{ °C}$  in a water bath. The slide remained in the vertical position at an angle of  $90^\circ$  to the horizontal for 2 hours. The running distance of the gel along the slide was measured against squared paper.

#### Dynamic rheological measurements

Dynamic rheological measurements were performed using a controlled stress Paar Physica MCR 301 rheometer (Anton Parr, Graz, Austria). Rheological data were collected using RheoPlus software version 3.21 (Anton Parr). The viscoelastic properties of the gels were determined using a serrated stainless steel parallel plate sensor geometry of 50 mm of diameter with a gap of 1mm. Before measurements were performed, samples remained between the plates for 5 min for temperature equilibration. Stress sweeps were run following a logarithmic stress increase from 0.1 to 300 or 1200 Pa (depending on the sample) at a constant frequency of 1Hz and a constant temperature of  $37.0\text{ °C} \pm 0.5\text{ °C}$ . Critical shear stress was calculated as the stress that corresponded to the crossover between the elastic or storage modulus ( $G'$ ) and viscous or loss modulus ( $G''$ ). Also, the shear stress that corresponded to the end of the linear viscoelastic regime (LVR) was calculated. The mechanical spectrum of the samples was obtained using frequency sweeps from 1 to 10 Hz at a constant stress within the linear viscoelastic range (0.5 Pa) and a constant temperature of  $37.0 \pm 0.5\text{ °C}$ .  $G'$  and  $G''$  were plotted in logarithmic scale and complex viscosity ( $\eta^*$ ) was determined.

#### Preparation of diluted samples

Gels were diluted in SVF to simulate the natural liquid uptake that takes place after administration of the

semisolid into the vaginal cavity. The dilution took into account the quantity of semisolid pharmaceutical dosage form usually applied by this route of administration, as well as the amount of vaginal fluid normally encountered. Generally, topical vaginal products are applied in volumes in the range of 2-5 mL (Aka-Any-Grah *et al.*, 2010) and, as previously mentioned, the volume of ambient fluid present in the vagina is approximately 0.5-0.75 mL. Thus, the lowest quantity of sample (2 g) was diluted in the highest volume of dilution media (0.75 mL), and left in repose for 24 hours.

### Data analysis

Analysis of variance (ANOVA) followed by Tukey's test and Multiple Factor Analysis (MFA) were used for data analysis. MFA is a factorial technique that enables working with several groups of variables (numerical and/or categorical) evaluated on the same samples. The core of MFA is a principal component analysis applied to the whole set of variables in which each group of variables is weighted, rendering possible the analysis of different points of view by taking them equally into account (De Tairac *et al.*, 2009). In the present work, 11 samples, comprising all of the gels formulated with two polymers, and 14 variables divided into five groups were taken into account to perform MFA. *Ref* was included as a supplementary individual. The variables included in each group were as follows:

Group 1 - stress sweep variables: stress corresponding to the end of the LVR ( $\sigma_{LVE}$ ), critical storage modulus ( $G^*c$ ) and critical stress ( $\sigma_c$ ) in which storage modulus and loss modulus curves cross each other, storage modulus ( $G^*1Pa$ ) and loss modulus ( $G''1Pa$ ) at 1 Pa, and storage modulus ( $G^*10Pa$ ) and loss modulus ( $G''10Pa$ ) at 10 Pa.

Group 2 - frequency sweep variables: storage modulus ( $G^*10\text{ Hz}$ ) and loss modulus ( $G''10\text{ Hz}$ ) at 10 Hz, and storage modulus ( $G^*1\text{ Hz}$ ) and loss modulus ( $G''1\text{ Hz}$ ) at 1 Hz.

Group 3 - adhesion time in min (AdhSVF).

Group 4 - spreading diameter in mm (Spread).

Group 5 - running distance in mm (Leak)

All data analyses were performed using R language (R Development Core Team, 2011) and FactoMineR package (Lê, Josse, Husson, 2008).

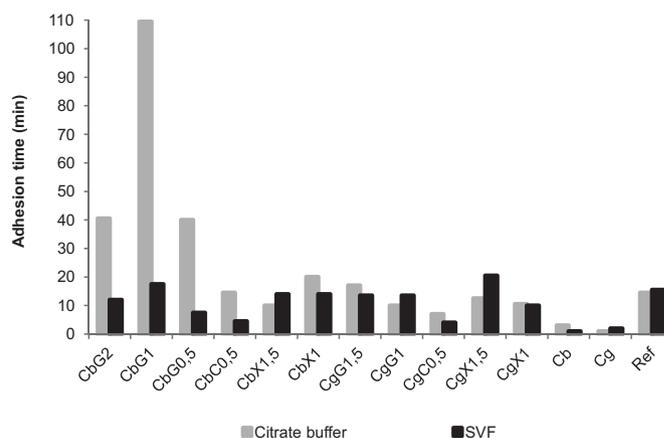
At this point, an initial selection of formulations was done. The effect of simulated vaginal dilution on gel behaviour was evaluated in these pre-selected formulations by means of spreadability measures (see Preparation of diluted samples). ANOVA was used for data analysis.

## RESULTS

### Adhesion, spreadability and leakage

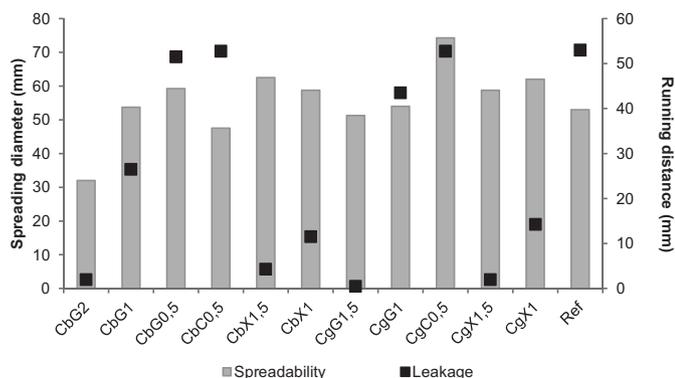
The developed gels showed adhesion times similar to or even higher than *Ref*, which has been reported to show high adhesive potential (das Neves, Amaral, Bahia, 2008). All of the samples formulated using polymer combinations showed longer adhesion times than the gels containing only Carbopol® 980 or Gelcarin® GP-812 NF (Figure 1).

The adhesion of *Ref* and Gelcarin® GP-812 NF gels appeared to be independent of the test media used in the assay. No significant differences were found among results obtained in both media, with the exception of *CgXI.5*, which showed a significant increase in SVF adhesion. However, adhesion behaviour of Carbopol® 980 gels was strongly dependent on test medium composition. Results obtained in SVF tests were significantly lower than those obtained in citrate buffer. As pH is the same in both test media (pH = 4.5), differences should have been caused by ionic interactions between carbomers and the high salt content present in SVF.



**FIGURE 1** - Mean adhesion time values of gels in pH 4.5 citrate buffer and simulated vaginal fluid (SVF).

Spreading diameter and running distance of the gels increased as the percentage of secondary polymer in the formulation decreased (Figure 2). Spreading diameter of the developed gels were similar or even higher than that of *Ref* (an easy spreading lubricant gel), with the only exception of *CgG2*, which showed a significantly lower value. Although both parameters increased with secondary polymer concentration, *CgG1.5* and gels containing xanthan gum appeared to have good spreadability, while showing low or even minimal leakage.



**FIGURE 2** - Mean spreading diameter and leakage distance values of gels.

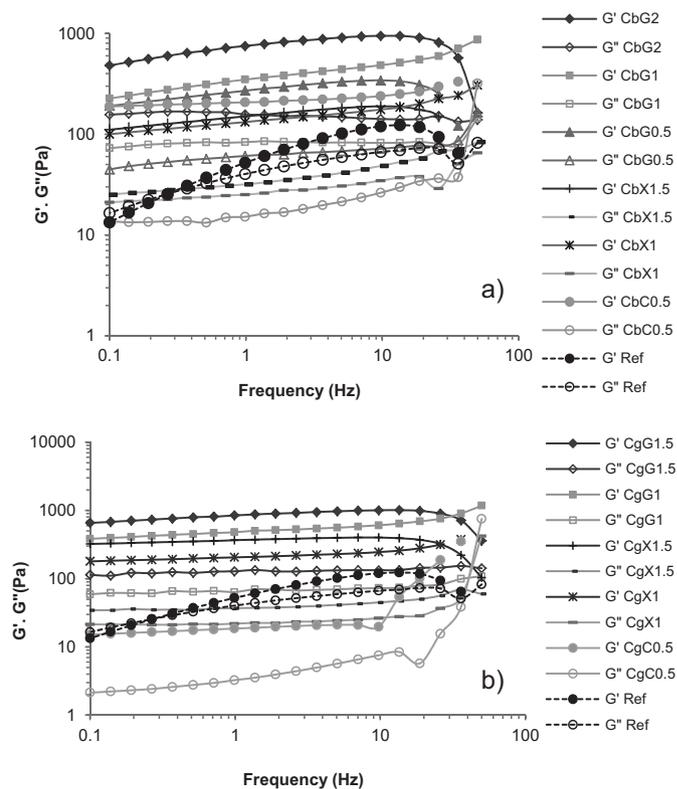
### Rheological measurements

The rheological behaviour of all of the developed formulations was similar and prevalently elastic. Stress sweep tests showed that the LVR of the majority of the gels corresponded to a stress range of up to at least 20 Pa. However, for *CgC0.5* the LVR ended at a very low stress value ( $\sigma_{LVE} = 0.86$  Pa).

Frequency sweep tests showed that the elastic modulus of the gel samples was clearly higher than the viscous modulus ( $G' > G''$  and  $\tan\delta < 1$ ) over almost the entire LVR. As expected, for all of the evaluated formulations,  $G'$  only slightly increased with frequency. Strong gels, after the aqueous dispersion of Carbopol or with moderate to high concentrations of kappa carrageenan in the presence of potassium ions, have been described in the literature (Bonacucina, Martelli, Palmieri, 2004; Tecante, Núñez Santiago, 2012).

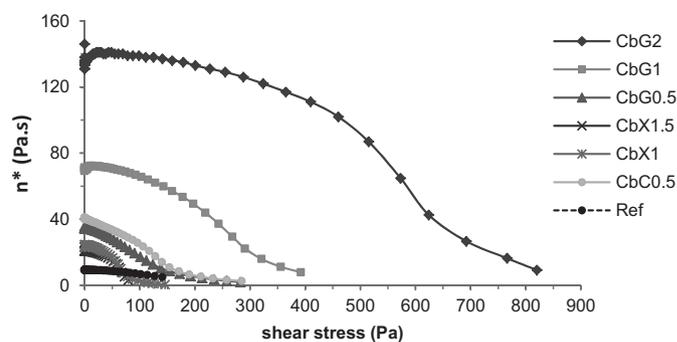
On the contrary, the rheological behaviour of *Ref* showed higher frequency dependence, showing a crossover between  $G'$  and  $G''$  at low frequency (approximately 0.3 Hz). The storage and loss moduli of this sample were very close to each other throughout the entire frequency range (Figure 3), which indicates a weak structure. Weak gels show a higher dependence on frequency for the dynamic moduli, which suggests the existence of relaxation processes, occurring even at short time scales, and a lower difference between moduli values (Rao, 1999). *CgC0.5* was the least elastic and viscous of the developed formulations, being the only gel that showed lower elasticity than *Ref*.

As shown in Figure 4,  $\eta^*$  of Carbopol® 980 gels increased in the presence of the secondary polymer according to the sequence:  $G \gg C > X$ . In the case of  $G$ , complex viscosity increased with the increase of gum concentration in the formulation. All Carbopol® 980 gels showed significantly higher viscosity than *Ref*.



**FIGURE 3** - Frequency sweep of gels: a) Carbopol® 980 formulations; b) Gelcarin® GP-812 NF formulations.

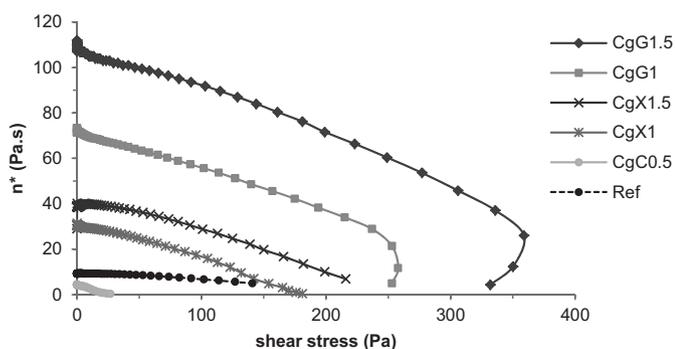
In the case of Gelcarin® GP-812 NF gels,  $\eta^*$  increased according to the following order:  $G > X > C$  (Figure 5). Regarding gums, this parameter increased as the percentage of these polymeric excipients increased in the gel.



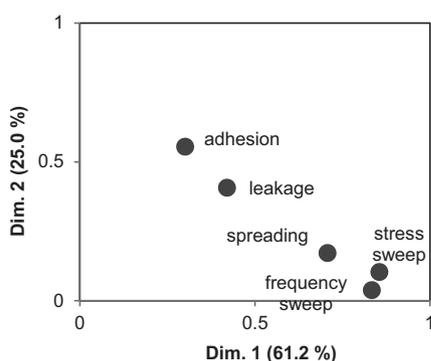
**FIGURE 4** - Complex viscosity ( $\eta^*$ ) of Carbopol® 980 gels as a function of shear stress.

### Multiple Factor Analysis

The first two dimensions of the MFA explained 86% of the variance of the experimental data. The groups of variables are plotted in Figure 6. As observed, spreading diameter and rheological measurements were located close



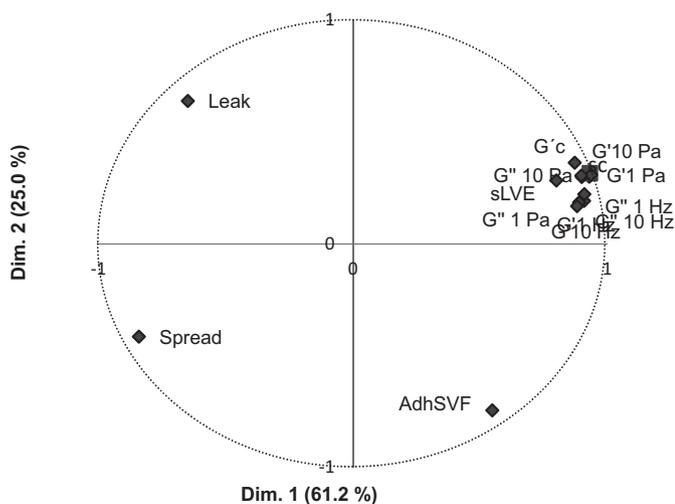
**FIGURE 5** - Complex viscosity ( $n^*$ ) of Gelcarin® GP-812 NF gels as a function of shear stress.



**FIGURE 6** - Representation of the groups of variables in the first two dimensions of the MFA.

to each other on the graph, indicating that they provided similar information.

A representation of the variables in the first two dimensions of the MFA is shown in Figure 7. Oscillatory rheological parameters were positively correlated with the first dimension, while spreading diameter was negatively



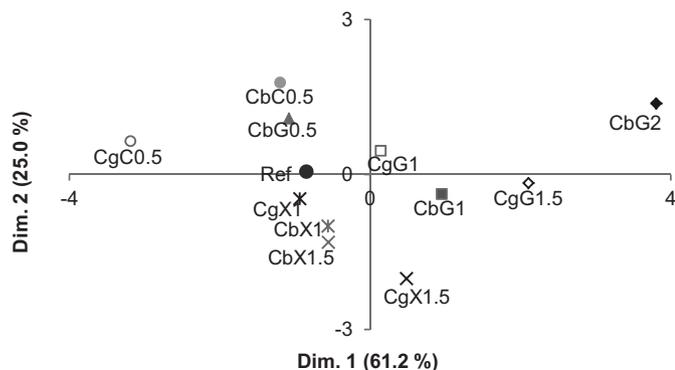
**FIGURE 7** - Representation of the variables in the first and second dimensions of the MFA.

correlated with this dimension. Adhesion time was negatively correlated with dimension 2, while leakage running distance was negatively correlated with dimension 1 and positively correlated with dimension 2.

Spreadability was negatively correlated with shear and frequency sweep variables, while no correlation between rheological behaviour and the adhesion of gels was found. This lack of correlation is understandable considering that these relationships may possibly only be able to explain the behaviour of systems based on the same type of gelling/mucoadhesive agents, as already pointed out in the literature (das Neves, Amaral, Bahia, 2008). In the present work, binary combinations of structurally different gelling agents were used in each formulation. In a few binary systems, gelation has been reported to take place by the association of two different polymers into conformationally ordered cooperative junction zones that are analogous to those in single-component gels (synergism), as occurs with carrageenan in combination with glucomannans (Rao, 1999) or galactomannans (Goddard, Gruber, 1999). According to the authors, the prevailing explanation is that gluco- and galactomannans bind to the k-carrageenan double helix, interrupting the aggregations of these helices, increasing the number and strength of the junction zones, and causing stronger gels. Among the secondary polymers used in this work, only guar gum belongs to the group of galactomannans. However, in both gels combining Gelcarin® GP-812 NF and this gum, a certain degree of syneresis appeared after a few days. Instead, more stable Gelcarin® GP-812 NF gel structures were produced in combination with xanthan gum, a polymer which has been reported to exhibit synergism with gluco- or galactomannans, similar to k-carrageenan (Goddard, Gruber, 1999).

Inverse correlation between spreadability and viscous modulus can probably be explained considering that spreadability decreased as viscosity increased. The higher the  $G''$  value, the more pronounced the viscous properties of the sample (Aka-Any-Grah *et al.*, 2010). For example, *CbG2* presented the highest  $G''$  values and the smallest spreading diameter, thus it was located at positive values of the first dimension, while *CgC0.5* was at negative values of the first dimension (Figure 8).

MFA revealed other trends as well. Adhesion values of gels including biomaterials were higher than that of *CbC0.5*, a gel prepared only with synthetic polymers. This is in accordance with Basu and Bandyopadhyay (2011) when working with *Linum usitatissimum* L. mucilage, hydroxypropyl methylcellulose and Carbopol. As the percentage of guar gum increased, the gels moved



**FIGURE 8** - Representation of gels in the first and second dimensions of the MFA. Ref was included as supplementary object.

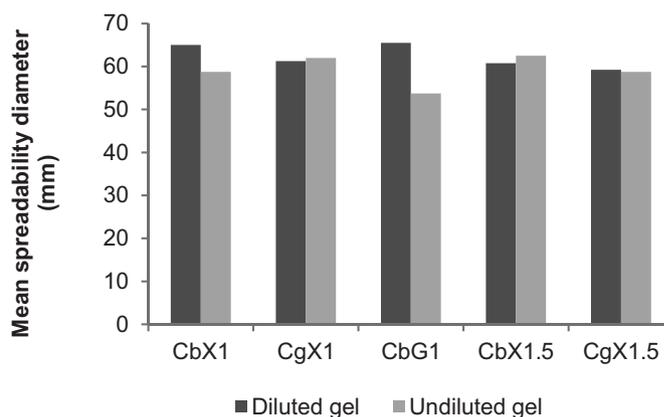
towards higher values of the first dimension of the MFA (Figure 7), which corresponded to a decrease in Spread, and an increase in  $\sigma_{LVE}$ ,  $\sigma_c$ ,  $G'$  and  $G''$ . Moreover, it was observed that at similar percentages of guar gum, this secondary polymer led to more viscous gels (higher  $G''$  values) when combined with carbomer than when combined with carrageenan. This trend was not observed with the other gum.

In the case of xanthan gum, as its percentage in the formulations increased, gels - and in particular carrageenan-containing samples - moved towards lower values of dimension 2, which corresponded to an increase in AdhSVF. This difference between gums could be explained as the presence of charged groups on polymers enhances adhesion. Xanthan gum is anionic by virtue of carboxylic acid residues on the D-glucuronic acid and the pyruvic acid moiety on the terminal D-mannose (Goddard, Gruber, 1999), while guar gum is a non-ionic polysaccharide. Particularly, carboxyl, hydroxyl, amide, and sulphate groups appear to be able to form strong adhesive bonds with mucin glycoproteins (Zatz, Kusla, 1996) or, as in this work, presumably with the polysaccharide chains present in the agar used to simulate the mucous membrane.

Among Gelcarin® GP-812 NF gels, those formulated with xanthan gum showed the best adhesion, spreadability and leakage results for potential mucosal application, without displaying evidence of syneresis. In the case of Carbopol® 980, those gels formulated with xanthan gum and with an intermediate percentage of guar gum (*CbG1*) were the formulations that presented the best *in vitro* adhesion, spreadability and leakage behaviour. Therefore, *CgX1*, *CgX1.5*, *CbX1*, *CbX1.5* and *CbG1* were selected, as potential mucoadhesive gels in the first instance. These five pre-selected gels were diluted with SVF, as previously described.

## Spreadability after dilution

Spreading diameters are plotted in Figure 9. ANOVA confirmed that only *CbX1* ( $F = 125.000$ ;  $p = 0.008$ ) and *CbG1* ( $F = 169.923$ ;  $p = 0.006$ ) significantly increased their spreadability after dilution. Unlike the decrease in viscosity reported by Chopra et al. (2007) in SVF-diluted Carbopol gels, in the present work, higher spreadability results cannot be attributed to a change in the pH of the formulation. Once more, the ionic strength of SVF should be the cause of the change in carbomer gels' behaviour.



**FIGURE 9** - Spreadability results at 37 °C of preselected gels before and after dilution with SVF.

## DISCUSSION OF RESULTS

High spreadability of gels is desirable during administration to achieve better coating of the vaginal cavity. Besides, high adhesion results are pursued to achieve retention of the pharmaceutical dosage form on the mucous membrane, while minimal leakage is also needed when formulating discreet vaginal gels. Thus, samples exhibiting long spreading diameter and adhesion time, as well as short running distance over the vertical plane, were chosen in the first instance.

From a rheological point of view, the fact that  $G'$  was higher than  $G''$  over almost the entire frequency range and that  $G'$  only slightly increased with the frequency for all samples, suggested that the developed formulations behaved as strong gels, in which molecular rearrangements within the network were reduced over the time scales analysed (Rao, 1999). Gels including guar gum showed higher complex viscosity than those formulated with xanthan gum or Pemulen™ TR-1 as a secondary polymer. Besides, the addition of xanthan gum reduced syneresis in Gelcarin® GP-812 NF gels.

As das Neves and Bahia (2006) have already pointed out, vaginal gels should retain their viscosity after dilution

with fluids present in the vagina. Therefore, under such circumstances, spreadability should not experience significant variation either. This criterion was applied among the preselected formulations.

In conclusion, both Carbopol® 980 and Gelcarin® GP-812 NF gels containing the highest percentage of xanthan gum displayed good *in vitro* mucoadhesion and spreadability, and minimal leakage potential. However, the decrease of *in vitro* adhesion shown by carbomer gels in presence of SVF is less favourable than the adhesion behaviour of carrageenan formulations towards vaginal administration. Also, regarding the effect of vaginal simulated dilution on gel spreadability, Gelcarin® GP-812 NF formulations showed a more resistant behaviour. Nevertheless, both primary polymers have several advantages for vaginal application. Polyacrylates are the most investigated bioadhesive polymers for vaginal applications, while carrageenan is safe, widely available, effective over a wide pH range, retains its properties at high temperatures and is not absorbed by the body, causing no systemic side effects (Valenta, 2005).

## CONCLUSION

The combinations of polymers proposed here enhanced the adhesive behaviour of primary gelling agents (Carbopol® 980 and Gelcarin® GP-812 NF), which are excipients known for their bioadhesive properties. Formulations presenting auspicious characteristics to develop mucoadhesive gels with potential vaginal application were identified. Gels including xanthan gum as secondary mucoadhesive polymer in the highest proportion (*CbX1.5* and *CgX1.5*) exhibited the best results. It is noteworthy that the carrageenan-xanthan gum-based gel presented several positive characteristics. This formulation combined good *in vitro* adhesion and spreadability, minimal leakage, appropriate rheological properties (before and after dilution), and its adhesion potential was not influenced by SVF composition. Another relevant advantage is the previously mentioned ability of this primary polymer to inhibit herpes simplex virus and prevent HPV infection, appearing to be the most promising formulation for the vaginal route of administration. Furthermore, results of the presented work contribute to encourage the use of natural biocompatible adjuvants in the composition of vaginal products, a formulation field that is currently under the synthetic domain.

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