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Research paper

Formulation of mucoadhesive vaginal hydrogels insensitive to dilution with vaginal fluids

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ABSTRACT

The main objective of this work was to design thermosensitive and mucoadhesive vaginal hydrogels able to keep their rheological and mucoadhesive properties after dilution with vaginal fluids. Formulations were composed of pluronic F127 or a mix of two pluronics F127 and F68. Both formulations contained hydroxypropylmethyl cellulose (HPMC) as a mucoadhesive polymer. The determination of gelling temperature (T_{gel}) after dilution with simulated vaginal fluid (SVF) demonstrated that hydrogels were resistant to dilution and T_{gel} values were close to 30 °C. *Ex vivo* mucoadhesion experiments conducted on porcine vaginal mucosa founded on the technique of traction of the adhesive/adherent joint allowed the characterization of mucoadhesive properties of hydrogels by measuring work of adhesion (W) and maximum force of detachment (F_{max}). In the case of F127-based hydrogels, W and F_{max} were lowered after dilution with SVF. However, in the case of F127/F68-based hydrogels, W, F_{max} and mucoadhesion profiles were weakly affected by dilution. These differences could be attributed to the higher elasticity of F127/F68/HPMC (22.5/2.5/1% w/w) hydrogel in comparison with F127/HPMC one (20/1% w/w). Indeed, rheological analyses of the formulations showed that both elastic (G′) and viscous moduli (G″) were higher for F127/F68/HPMC (22.5/2.5/1% w/w) than for F127/HPMC hydrogel (20/1% w/w). However, we demonstrated that the higher elasticity of the hydrogel was due to the higher total pluronic concentration and not due to the presence of F68 in the formulation.

1. Introduction

The presence of dense network of blood vessels, the avoidance of the first-pass effect and the relatively high permeability to a wide range of drugs such as peptides and proteins [1–3] have made the vagina as an excellent route of drug delivery for both systemic and local applications [4–7]. The vaginal route offers a favorable alternative to the parenteral route for some drugs such as bromocriptine [8,9], propranolol [10], oxytocin [11,12] and hormones [13–15]. Furthermore, vaginal administration of hydrogels can exhibit a local action in the case of vaginal microbicides. These hydrogels containing the active drug can be self-administered by women before intercourse. They can form a barrier against pathogen entry. It has been demonstrated that the vaginal administration of gels allowed the prevention of infection with viruses such as the human immunodeficiency virus (HIV-1) and other sexually transmitted infections [16–18].

However, despite all these advantages, vaginal gels will be diluted with vaginal fluids after their *in vivo* administration resulting in a modification of rheological and mucoadhesive properties [19,20]. The residence time in the genitourinary tract of these formulations is hence shortened owing to the self-cleansing action of the vagina and the dilution with vaginal fluids. The efficacy of the formulations could be limited making a frequent dosing regimen necessary. In this context, it is believed that vaginal therapy can be significantly improved by increasing the residence time of the formulation in the vagina. This can be achieved by the development of mucoadhesive gels able to keep their rheological and mucoadhesive behaviours even after dilution with vaginal fluids. The adhesion of pharmaceutical formulations to the mucosal tissue offers the possibility of creating an intimate and prolonged contact to the vagina and to improve patient compliance by reducing the frequency of administration.

Particularly, vaginal hydrogels based on block copolymers are receiving a great deal of interest as vaginal delivery systems [6,21,22]. Among block copolymer-based hydrogels, pharmaceuti-

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cal formulations composed of (ethylene oxide)$_a$(propylene oxide)$_b$(ethylene oxide)$_a$ block copolymers known under the generic name of poloxamers and the trade name of pluronics, present both thermosensitive and mucoadhesive properties, which make them very interesting for the development of vaginal delivery devices. By contrast, most of previous research works have developed pluronic-based hydrogels intended for vaginal drug delivery without considering the effect of dilution with vaginal fluids on the variation of gelling temperature, rheological and mucoadhesive properties of pluronic-based hydrogels.

The main objective of this work was to formulate a vaginal delivery device based on thermosensitive and mucoadhesive pluronic hydrogels and to investigate the effect of dilution with vaginal fluids on gelling temperature, rheological behaviours and mucoadhesive properties. In the work to be presented here, formulations were composed of pluronic F127 (poloxamer P407, *a*: 97, *b*: 69) or a mix of two pluronics F127 and F68 (poloxamer P188, *a*: 80, *b*: 27). Gelling temperature can be modulated by changing F127 concentration or by varying the proportion between the two pluronics F127 and F68. Both formulations contained hydroxypropylmethyl cellulose (HPMC) as a mucoadhesive polymer.

2. Experimental

2.1. Materials

Pluronics F127 and F68 (European Pharmacopea 6th Edition 6.7) of pharmaceutical grade were a gift from BASF. According to the supplier, the weight average molecular weights (M_w) were about 13,500 g mol^{-1} (9840–14,600 g mol^{-1}) and 8400 g mol^{-1} (7680–9510 g mol^{-1}) for pluronic F127 and F68, respectively. Hydroxylpropylmethyl cellulose (HPMC) (Methocel® K4M series MM87091702 K) was a gift from Colorcon. All other reagents were supplied by Sigma–Aldrich (Saint-Quentin Fallavier, France) and were of analytical grade.

2.2. Methods

2.2.1. Preparation of citrate buffer

One of the exigencies of the vaginal formulations is the pH. The pH of vaginal hydrogels has to be in the range of 4–5. For this purpose, we make choice to use citrate buffer prior to distilled water in the aim to prevent pH variation of the formulations. Citrate buffer solution 5 mM at pH 4.5 was prepared by dissolving citric acid monohydrate (42 mg) and trisodium citrate dihydrate (59 mg) in about 900 mL of distilled water. The volume of the solution was then completed to 1 L by distilled water.

2.2.2. Preparation of simulated vaginal fluid (SVF)

Vaginal fluid is the resultant of fluids that comes from several sources such as uterus, cervix and sometimes menstrual secretions and sperm [19,23]. Noteworthy, due to the limited quantity of human vaginal fluid and its rapid degradation once collected from its source, researchers have developed a simulated vaginal fluid (SVF) [23]. SVF was prepared as previously described [23]. To 900 mL of distilled water contained in a beaker, NaCl (3.51 g), KOH (1.4 g), $Ca(OH)_2$ (0.22 g), bovine serum albumin (0.018 g), lactic acid (2.00 g), acetic acid (1.00 g), glycerol (0.16 g), urea (0.4 g) and glucose (5.00 g) were added and stirred mechanically until complete dissolution. The pH of the mixture was then adjusted to 4.5 using HCl, and the final volume was adjusted to 1 L.

2.2.3. Preparation of hydrogels

Hydrogels were prepared by weight according to the so-called "cold method", using a mixer equipped with a turbine adapted to the mixing of viscous preparations (Rayneri-turbotest, Rayneri, France) [24–26].

- For the preparation of pluronic-based hydrogels, the pluronic powders were gradually added under agitation (1000 rpm) at 4 °C to a liquid phase, which consisted on citrate buffer. For formulations containing F127 alone, the final concentration of pluronic in the hydrogel was varied as 15%, 16%, 17% and 20% w/w. For the preparation of hydrogels composed of F127 and F68 mixture, the final concentration of pluronic was kept constant (20% w/w), while the proportion of F127/F68 in the formulation was varied as 0/20%, 10/10%, 17/3%, 18/2%, 19/1% and 20/0% w/w. The different preparations were denominated by two numbers indicating the w/w percentage of pluronic F127 and pluronic F68, respectively.
- For the preparation of formulations composed of F127/HPMC and F127/F68/HPMC, HPMC powder was gradually added under agitation (1000 rpm) at 4 °C to a liquid phase, which consisted on citrate buffer. After complete dissolution of HPMC, the pluronic powders were gradually added to this phase under the same conditions (agitation speed: 1000 rpm at 4 °C). Preparations composed of F127/HPMC were denominated by two numbers indicating the % w/w of F127 and HPMC, respectively. Preparations composed of F127/F68/HPMC were denominated by three numbers indicating the w/w percentage of pluronic F127, F68 and HPMC, respectively.
- HPMC at a concentration of 1% w/w was used as a control. The formulation was prepared by progressive solubilization of HPMC powder in citrate buffer solution at 4 °C.

In all cases, after complete dissolution of pluronic and/or HPMC powders, each formulation was equilibrated 48 h at 4 °C to eliminate foam and air bubbles.

2.2.4. Preparation of diluted hydrogels

Generally, current topical vaginal products are applied in volumes in the range of 2–5 mL. The volume of ambient fluid present in the vagina (vaginal fluid transudate and mucus) is approximately 0.5–0.75 mL [23]. To simulate the dilution of formulations that might occur after application, we mixed 2 mL of each formulation with 0.25, 0.5 and 0.75 mL of SVF or citrate buffer and tested the influence of the dilution on thermogelling, rheological and mucoadhesive properties of pluronic-based hydrogels.

2.2.5. Rheological evaluation of hydrogels

All rheological measurements were carried out on a CSL 100 controlled stress rheometer (Carri-Med, Rhéo Champlan, France) [24,25]. The geometry was a stainless steel cone/plate (diameter 40 mm, angle 2° and gap 54 μm), which provided a homogeneous shear of the sample. The cone was equipped with a solvent trap to limit evaporation during measurement. Thanks to Pelletier diodes which were placed in the lower plate, it was possible to perform temperature sweeps from 0 to 80 °C with a precision of 0.1 °C. Oscillatory (or dynamic) experiments were carried out. A sinusoidal shear was applied to the sample, where the stress $\tau(t)$ and the strain $\gamma(t)$ were defined as follows:

$$\tau(t) = \tau_0 \cos(\omega\, t) \quad (1)$$

$$\gamma(t) = \gamma_0 \cos(\omega t - \delta) \quad (2)$$

τ_0 and γ_0 are, respectively, the maximal amplitudes of the stress and strain, $\omega = 2\pi N$, with N the frequency and ω the shear pulsation and δ is the phase angle stress/strain.

From the phase angle, one could define various dynamic viscoelastic quantities and especially the elastic (or storage) modulus G' (Eq. (3)) and the viscous (or loss) modulus G'' (Eq. (4))

$$G' = \frac{\tau_0}{\gamma_0}\cos\delta \quad (3)$$

$$G'' = \frac{\tau_0}{\gamma_0}\sin\delta \quad (4)$$

The higher the G' value, the more pronounced the elastic character and conversely, the higher G'', the more pronounced the viscous properties. The elastic modulus is a measure of the energy stored and recovered per cycle of deformation and reflects the solid-like component of elastic behaviour.

The oscillatory experiments were used mainly in order to determine the sol–gel transition temperature, by measuring the temperature for which G' underwent a critical variation, and to characterize the gel texture beyond the gel point, by recording the G' variations as a function of the shear frequency N. These latter experiments were carried out under a stress value which belonged to the viscoelastic linear regime, where G' and G'' remained invariant and were the sample did not undergo irreversible structural modifications.

All rheological results are the means of $n = 3$ experiments.

2.2.6. Micro-DSC

The measurements of Micro-DSC were carried out with a calorimeter Micro-DSC III Setaram in the aim to determine the critical micellization temperature (CMT). The cells used to deposit the sample and the reference (distilled water) were the type batch (1 mL). Two empty cells with the caps were weighed after complete drying, and the joints were chosen to obtain the same mass (±0.2 mg). The sample and the reference should be introduced to the cells at room temperature and weighed for the identical mass (±0.3 mg). After their insertions into the oven at room temperature, the temperature of the oven was reduced until 5 °C with 1 °C/min, and then we waited one hour at this constant temperature to equilibrate the thermal flow. The scan of temperature was performed at 0.1 °C/min until 70 °C. After this, the oven should stay one hour at 70 °C to balance the thermal flow before the beginning of fusion with −0.1 °C/min to 5 °C.

The analysis of the enthalpograms led to the determination of the CMT according to the previously described and well-known method [27].

2.2.7. Mucoadhesion experiments

2.2.7.1. Animals. The ethical and practical problems of using human tissues to study the mucoadhesion of therapeutic agents on genital mucosa have led to the development of a variety of model systems, including *ex vivo* animal tissues. Among the larger experimental animals, the pig has the advantage of being remarkably similar to human in terms of anatomy, physiology, metabolism and histology. Furthermore, many research works have reported that excellent correlation was found between human and porcine vaginal tissues [28,29].

Experiments were carried out on female pigs (INRA Jouy en Josas, France) weighing between 60 and 63 kg in average. The animals were fasted for 24 h, but had free access to tap water. All experiments on animals adhered to the Communities Commission Directive (DE/86/609/CEE) and were performed in conformance with the French Ministry of Agriculture Permission No. 78-16.

Pigs were sacrificed by intravenous injection (20 mL) of overdosed sodium phenobarbital (Dolethal, Vetoquinol Laboratory, Lure, France), and the vaginal mucosa was taken over a 10 cm length. The mucosa was placed in SVF and stored at −20 °C immediately after sacrifice of the animal and kept at this temperature until use. It has been shown that porcine mucosa can be frozen during storage without affecting the mucus layer (for review see [29]). Samples were defrosted before experiments at ambient temperature in the presence of SVF.

2.2.7.2. Mucoadhesion experimental procedure. The experimental procedure used for determining the mucoadhesion of hydrogels has been derived from a previously published method [24,25]. Briefly, a hydrogel layer (10 mm in height) was placed in contact with a fragment of pig vaginal mucosa under thermostated conditions, thus forming an adhesive joint between the two surfaces. On one hand, a mucosal fragment was placed at the surface of a metallic plot. The external wall of the vaginal mucosa was secured on the muscular side with cyanoacrylate glue on a 14 mm in diameter metallic support. Finally, this metallic plot supporting the vaginal mucosal fragment was fasten to the mobile traverse of a texture analyzer (TAXT 2, Rhéo Distributor, Champlan, France).

On the other hand, a hollow plastic support (10 mm in depth) was filled with a preset amount of 3 g of the hydrogel to be studied and was used to maintain the thermogelified preparation during the experiment. Further, this support was immobilized in the texture analyzer apparatus and placed in such a position that a perfect contact could be created between the surface of the hydrogel and the mucous membrane. The whole system was then heated to 37 °C thanks to a Pelletier diode, which ensured the gelation of the system.

After a preset contact time (2 min) under an initial contact strength (0.5 N), which was determined during preliminary experiments, the two surfaces were separated at a constant rate of displacement. The strength was recorded as a function of the displacement, which allowed to determine the maximal detachment force (F_{max}), the work of adhesion (W) and other mucoadhesion parameters: deformation to failure (C), deformation to peak (A) and deformation peak to failure (B). These parameters were calculated from the force–elongation curve [30,31] and considered as indicators of the mucoadhesive potential of the samples.

Finally, HPMC solution at a concentration of 1% w/w was used as a control.

All mucoadhesion results are the mean of $n = 6$ experiments.

2.2.8. Statistical analysis

The results obtained were statistically analysed by using Mann–Whitney's t-test with a 95% confidence level (po0:05).

3. Results and discussion

3.1. Effect of dilution of hydrogels with SVF on gelling temperature and re-formulation of hydrogels

Usually, the gelling temperatures of vaginal hydrogels are considered to be suitable if they were lower than human vaginal temperature (37.2 °C). If gelling temperature is higher than 37.2 °C, hydrogels will lose their gelling properties resulting in a leakage of the formulations from the vagina. In the present work, two strategies have been investigated to modulate gelling temperature. The first one consisted on the variation of F127 concentration in the formulations (15%, 16%, 17% and 20% w/w) and the second one consisted on the variation of the proportion of F127 and F68 in the formulations (0/20%, 10/10%, 17/3%, 18/2%, 19/1%, 20/0% w/w).

The thermosensitive properties of hydrogels were evaluated by sol/gel transition temperature. The curve obtained with the different preparations is presented in Fig. 1. This curve is typical for pluronic systems because the elastic modulus, G' was very low at solution stage but increased drastically when gelling temperature is reached. As can be seen from the results in Table 1, gelling temperature is monotonically decreased when the concentration of F127 was increased from 15% to 20% w/w or when the proportion of F68 in the hydrogel is decreased from 20% to 0% w/w. The two formulations which gelled in the desired temperature range were F127 (16% w/w) and F127/F68 (18/2% w/w).

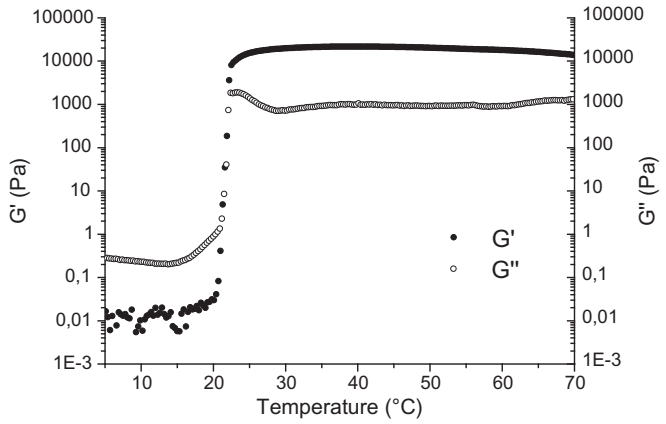

Fig. 1. Typical profile of the variations of the elastic (G') and viscous (G'') moduli, as a function of temperature. Experiments performed with a pluronic hydrogel of F127 at a concentration of 20% w/w.

Table 1
Gelling temperature, T_{gel} (±standard deviation, $n = 3$) and elastic G' and viscous modulus G'' (±standard deviation, $n = 3$) ($N = 1$ Hz, $T = 37$ °C) for different pluronic-based formulations.

	Formulation (% w/w)	T_{gel} (°C)	G' (10^3 Pa)	G'' (Pa)	δ (°)
Effect of F127 concentration (% w/w)	15	67 ± 1	0	0	88.8
	16	28 ± 1	11.0 ± 0.2	2.2 ± 0.1	11.5
	17	26 ± 1	12.4 ± 1.6	1.5 ± 0.2	6.9
	20	22 ± 1	22.8 ± 2.8	0.5 ± 0.1	1.3
Effect of F127/F68 proportion (% w/w)	0/20	59 ± 2	0	0	81.2
	10/10	54 ± 3	0	0	85.9
	17/3	35 ± 1	3.7 ± 0.4	1.6 ± 0.1	23.0
	18/2	30 ± 1	15.4 ± 0.2	1.5 ± 0.1	5.7
	19/1	25 ± 1	17.0 ± 0.7	1.0 ± 0.2	3.4
	20/0	22 ± 1	22.8 ± 2.8	0.5 ± 0.1	1.3

The two previously selected formulations F127 (16% w/w) and F127/F68 (18/2% w/w) were diluted with SVF according to protocol described in Section 2.2.4. However, as shown in Fig. 2, both formulations showed no gelation ($T_{gel} > 60$ °C) after their dilution. Dilution of F127 and F127/F68 formulations (16% w/w and 18/2% w/w, respectively) with 0.75 mL of SVF resulted in a decrease in the total concentration of pluronics and consequently, a dramatic increase in gelling temperature. The two formulations of F127 at 16% and F127/F68 at 18/2% w/w were thus re-formulated in such a way they remain in the form of gel even after dilution with SVF. The calculation of pluronic concentrations after re-formulation considering dilution with SVF were 20% and 22.5/2.5% w/w for F127 and F127/F68, respectively. The two re-formulated hydrogels were prepared and diluted according to the same protocol. As expected, gelling temperatures of these formulations after dilution with SVF were about 30 °C (Fig. 2).

Finally, HPMC as mucoadhesive polymer has been incorporated into hydrogels at a concentration of 1% w/w. After incorporation of HPMC, final formulations have been progressively diluted with SVF, and gelling temperatures were determined after each dilution. As reported in Table 2, progressive dilution of formulations resulted in a progressive increase in gelling temperature. About 10 °C of difference was observed before and after dilution of 2 mL of hydrogel with 0.75 mL of SVF. For both formulations, the final gelling temperatures were around 30 °C.

It is noteworthy that the variation of gelling temperature is not only due to the decrease in copolymer concentration after the dilution of the formulations, but also caused by a modification of the physicochemical properties of the hydrogels. As exposed in Fig. 3 and Table 2, gelling temperature is lower when the formulations were diluted with SVF than with citrate buffer. These results can be explained by the presence of co-solutes, e.g., ions, proteins, electrolytes in the SVF medium, which could interact with pluronics [32]. As demonstrated by previous works [33], in the case of pluronics, the gelation and micellization processes are strongly connected. The decrease in gelling temperature results from the decrease in the critical micelle concentration (CMC) or a decrease in the critical micelle temperature (CMT).

This was confirmed by Micro-DSC experiments (Fig. 3 and Table 2). For the same composition of the hydrogel, the CMT was slightly lower when the formulation was diluted with SVF than with citrate buffer. The decrease in the CMT when adding salt to the copolymer solution results from the rearrangement of water molecules, which are bound to the propylene oxide or the ethylene oxide residues. Water content in the micellar core decreases and the micellization is favored resulting in a significant decrease in the CMT, and in turn, a decrease in gelling temperature (Fig. 3 and Table 2).

3.2. Effect of dilution with SVF on viscoelastic properties of hydrogels

The viscoelastic properties of the selected formulations F127/HPMC (20/1% w/w) and F127/F68/HPMC (22.5/2.5/1% w/w) at 37 °C (above their gelling temperature) were then compared. The frequency sweep was varied from 0.01 to 10 Hz under a constant shear stress (60 Pa). It was checked that under the selected frequency range and a stress of 60 Pa, all the investigated samples remained in the linear domain and underwent reversible structural modification.

As expected for physical hydrogels, the elastic modulus, G' slightly increased with the frequency whatever the formulation

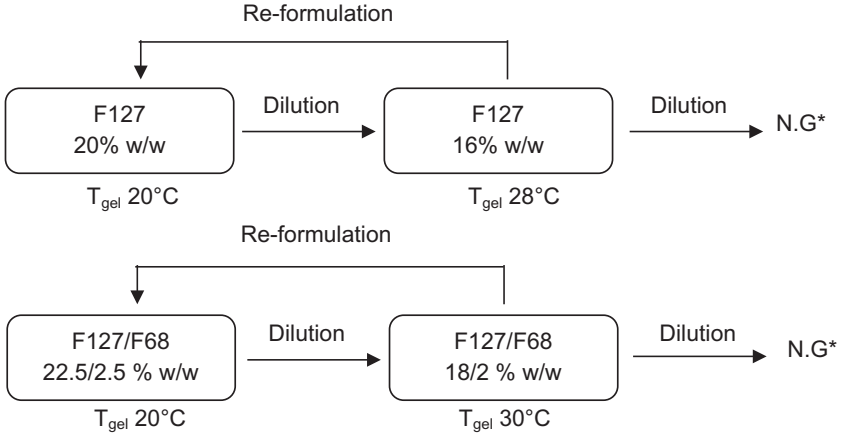

Fig. 2. Initial formulations and iterative formulation process. *NG; no gelation.

Table 2
Effect of progressive dilution of F127/HPMC and F127/F68/HPMC formulations with SVF[a] and water volume on the variation of rheological parameters of. T_{gel} (±standard deviation, $n = 3$), elastic modulus G' and viscous modulus G'' ($N = 1$ Hz, $T = 37$ °C). $n = 3$.

	Formulation (% w/w)	Volume of Dilution (mL)	CMT[b] μDSC (°C)	T_{gel} (°C)	G' (10^3 Pa)	G'' (10^3 Pa)	δ (°)
SVF[a]	F127/HPMC (20/1)	0	12.47	21 ± 1	24.0 ± 1.3	1.0 ± 0.1	2.3
		0.25	14.08	23 ± 1	18.5 ± 2.6	1.2 ± 0.1	3.7
		0.5	14.97	26 ± 1	9.9 ± 1.4	1.7 ± 0.2	9.7
		0.75	15.88	30 ± 1	2.9 ± 1.0	2.0 ± 0.1	34.5
	F127/F68/HPMC (22.5/2.5/1)	0	8.86	19 ± 1	37.0 ± 3.7	1.1 ± 0.1	1.7
		0.25	11.40	23 ± 1	23.4 ± 0.4	0.6 ± 0.1	1.4
		0.5	12.27	26 ± 2	17.8 ± 0.1	1.5 ± 0.1	4.8
		0.75	13.55	32 ± 1	9.6 ± 1.7	1.4 ± 0.1	45.8
Citrate buffer	F127/HPMC (20/1)	0	12.47	21 ± 1	24.0 ± 1.3	1.0 ± 0.1	2.3
		0.25	14.27	26 ± 1	13.8 ± 0.1	1.8 ± 0.1	7.6
		0.5	15.25	33 ± 1	4.8 ± 0.5	1.7 ± 0.1	19.7
		0.75	16.27	NG[b]	–	–	
	F127/F68/HPMC (22.5/2.5/1)	0	8.86	19 ± 1	37.0 ± 3.7	1.1 ± 0 .09	1.7
		0.25	11.44	25 ± 1	22.5 ± 2.5	1.0 ± 0.1	2.5
		0.5	12.51	30 ± 1	15.0 ± 0.1	1.5 ± 0.1	5.7
		0.75	14.18	35 ± 1	3.2 ± 1.9	1.5 ± 0.2	25.2

[a] SVF: simulated vaginal fluid. (b) NG: no gelation.
[b] CMT: critical micelle temperature.

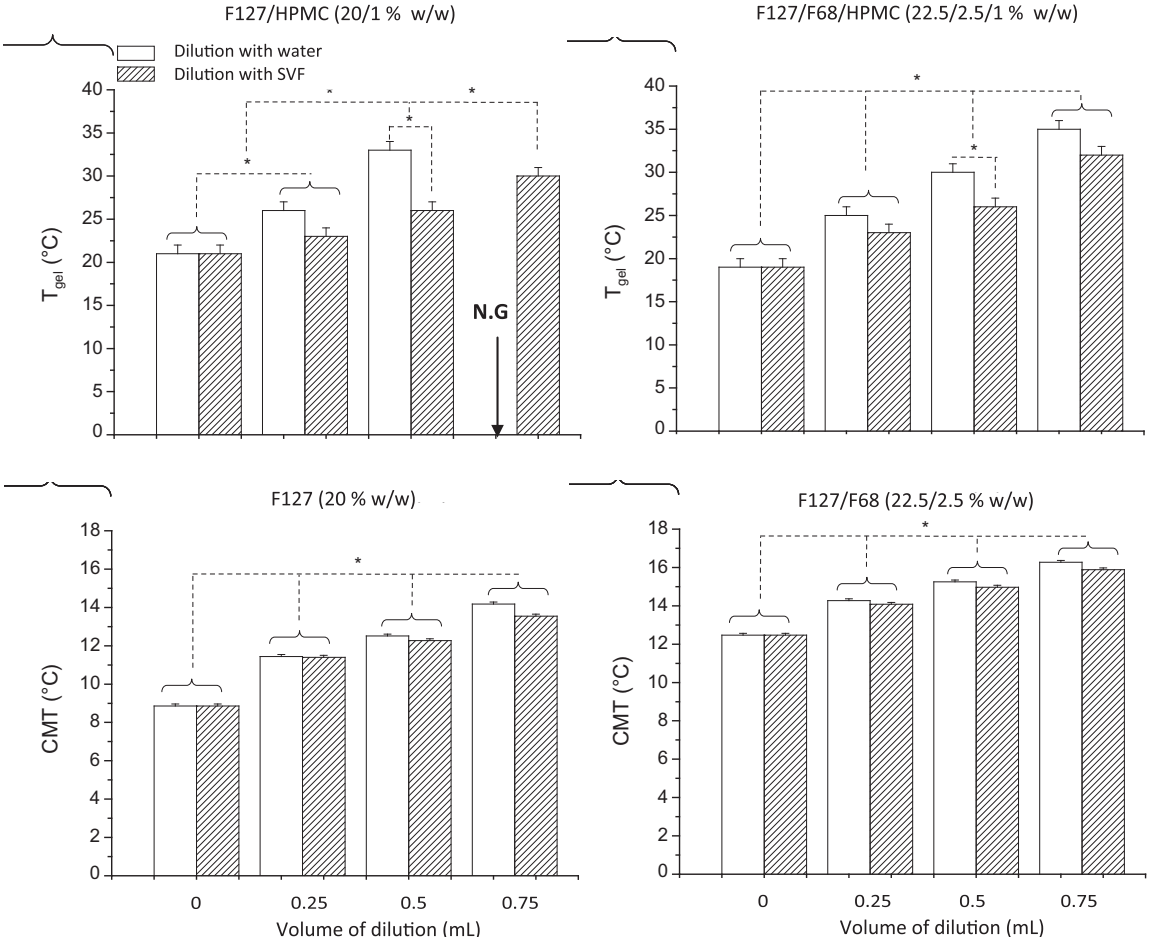

Fig. 3. Effect of dilution with water and SVF on variation of T_{gel} and CMT* for F127 and F127/F68-based hydrogels. Dilutions were conducted by mixing 2 mL of formulations with 0.75 mL of water or SVF. $n = 3$. NG: no gelation. *CMT: critical micelle temperature. (*) represents significant difference ($P < 0.05$).

used (Fig. 4). More precisely, oscillatory measurements by varying the frequency showed that the elastic modulus of the hydrogels was higher than the viscous modulus, G'' over almost all the frequency range ($G' > G''$ and $0° < \delta < 45°$). For the reminder, the elasticity of a hydrogel is higher when the elastic modulus increased and when viscous modulus and phase angle stress/strain were decreased.

From the results presented in Table 1, when the concentration of F127 was increased, the elasticity of the hydrogel increased as shown by an increase in G' from 11 to 22.8 kPa when the percent-

age of F127 was increased from 16% to 20% w/w. Evidence that G' and G'' were near zero when gelling temperature was higher than 37 °C. In that case, hydrogels conserved their viscous character at 37 °C. This was shown by a phase angle stress/strain (δ) values higher than 45° (Table 1).

Concerning the rheological evaluation of re-formulated hydrogels F127/HPMC (20/1% w/w) and F127/F68 (22.5/2.5/1% w/w), as we can see from Fig. 4, both elastic and viscous moduli were higher for F127/F68-based hydrogel at a concentration of 22.5/2.5% w/w than the modulus of hydrogel based on F127 alone at a concentration of 20% w/w. It is worth to note that the elastic behaviour is not due to the presence of F68 in the formulation, because as we can see from the results in Table 1, for the same total concentration of pluronic, the addition of F68 to hydrogels resulted in a decrease in elastic modulus. This parameter decreased from 22.8 to 3.7 kPa when the percentage of F68 in the formulation was increased from 0% to 3% w/w (Table 1). One could conclude that the elasticity of the hydrogel composed of F127/F68/HPMC (22.5/2.5/1% w/w) was higher than the one composed of F127/HPMC (20/1% w/w), because the total amount of pluronic is higher in the first case.

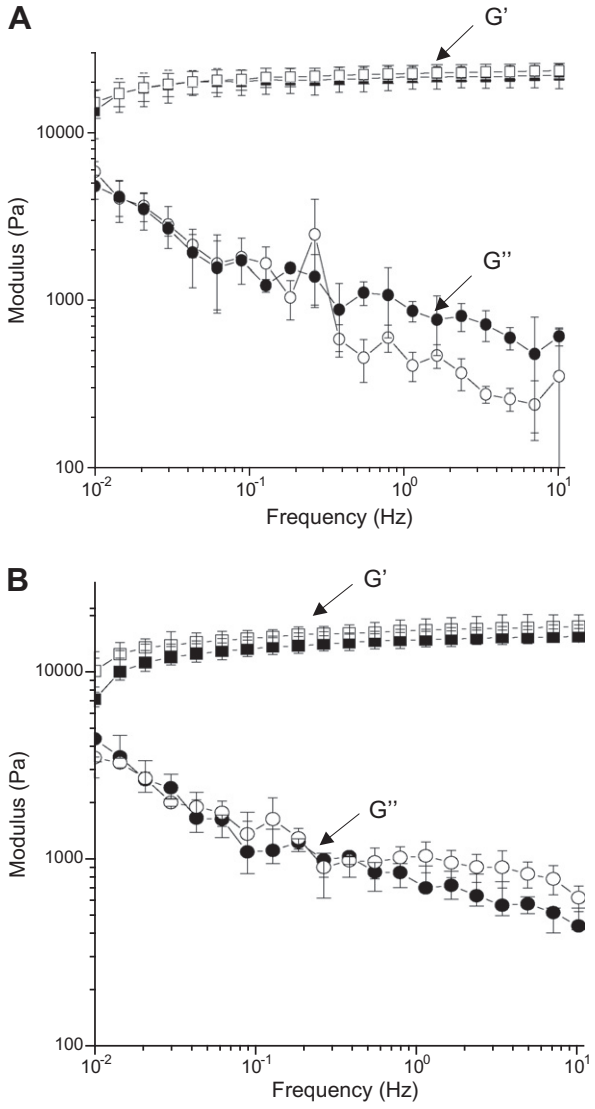

Fig. 4. Variations of the elastic (G') and viscous moduli (G'') as a function of the shear frequency, N (τ_0 = 60 Pa, T = 37 °C) for F127 (20% w/w) (A) and F127/F68 (22.5/2.5% w/w) (B), before (dark symbols) and after (open symbols) addition of HPMC at a concentration of 1% w/w.

Dilution of F127/HPMC (20/1% w/w) and F127/F68 (22.5/2.5/1% w/w) hydrogels with SVF or citrate buffer resulted in a decrease in the elasticity of the hydrogels. As we can see from Table 2, whatever the formulation, when hydrogels were diluted, elastic modulus was decreased. This could be attributed to the decrease in total pluronic concentration in the formulations. It is noteworthy that the variation of the elasticity of the hydrogels is not affected only by pluronic concentration but also by medium composition. As exposed in Fig. 3 and Table 2, formulations diluted with SVF had an elastic modulus higher than the one obtained for the formulations diluted with citrate buffer.

The decrease in the elasticity of the hydrogels was compensated by a gain in the viscosity modulus. It is expected that the gain in the viscosity modulus will result in an increase in the spreading of the hydrogel over the vaginal mucosa after its *in vivo* administration.

3.3. Effect of dilution on hydrogels' mucoadhesion

After the *in vivo* administration of mucoadhesive hydrogels, the first step in mucoadhesion process is the creation of intimate contact between the dosage form and the mucosa. The intimate contact is believed to be a result of wetting and spreading of the hydrogel, which increases the area of contact. The interpenetrated chains can then interact, resulting in entanglements and weak chemical bonds (electrostatic attraction, hydrophobic interactions, van der Waals' forces and hydrogen bonds).

Mucoadhesion parameters can be evaluated by several *in vitro* and *in vivo* methods [4]. The majority of the methods were based on an *in vivo*-like situation, usually measuring the contact time or the force required to separate the formulation from the tissue [34–36]. In the detachment-force method, when conducting tensile adhesion tests, the formulation is brought into contact with a biological substrate and the force or the work that is required to break the adhesive bond is measured [30,37]. Interestingly, more information about mucoadhesion parameters can be accumulated using this method, because it not only provides a value for the work of adhesion or the maximal force of detachment, but also gives other deformation parameters (Fig. 5). In the present work, tests on pig vaginal mucous membrane, founded on the technique of traction of the adhesive/adherent joint, made it possible to characterize the bioadhesive properties of the hydrogels by measuring mucoadhesion parameters of the formulation before and after dilution with SVF. Typical force versus elongation graph is presented in Fig. 5. This curve led to characterize the bioadhesive properties of the hydrogels by measuring deformation to peak (A), deformation peak to failure (B), deformation to failure (C), maximal force of detachment F_{max} (D) and work of adhesion W (E).

Deformation to peak (A), deformation peak to failure (B) and deformation to failure (C) gave insight into the elasticity of the adhesion. Materials exhibiting large deformation peak, deformation peak to failure and deformation to failure produce adhesive interactions which are more compliant and less susceptible to disrupt from outside forces. Maximal force of detachment F_{max} (D) corresponds to the force necessary to the detachment of the hydrogel from vaginal mucosa, while work of adhesion W (E) is the work done in separating the two surfaces and is the area between the positive portion of the tensile curve and the zero load line.

From the results in Fig. 6, whatever the formulation, mucoadhesive properties after dilution with SVF were decreased as exemplified by a significant decrease in F_{max} and W, because dilution with vaginal fluids resulted in a decrease in polymer concentration. At low concentrations, there are not enough chains available for interaction with mucus gel layer. Furthermore, the lubricant effect of the immersion fluids makes difficult the contact between mucosa and semisolid formulation, and in turn, affects the mucoadhesive

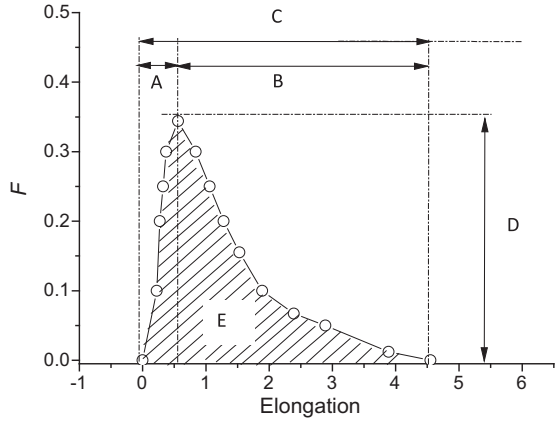

Fig. 5. Typical force versus elongation curve for pluronic-based hydrogels. The analysis of the curve led to the determination of the following mucoadhesion parameters: maximal force of detachment F_{max} (D), work of adhesion W (E) and other mucoadhesion parameters: deformation to failure (C), deformation to peak (A) and deformation peak to failure (B).

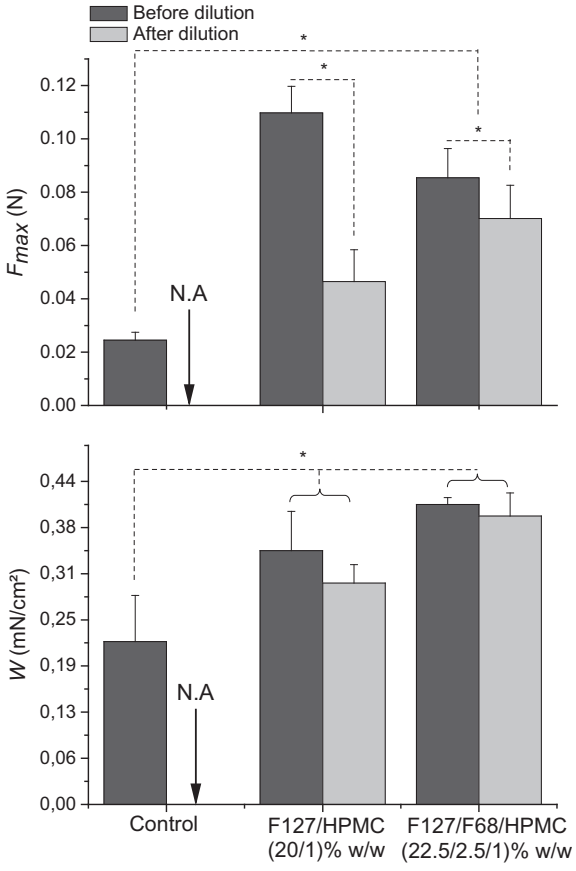

Fig. 6. Effect of dilution with SVF on variation of F_{max} (Higher panel) and W (Lower panel) for F127HPMC (20/1% w/w) and for F127/F68/HPMC (22.5/2.5/1% w/w) hydrogels. Dilutions were conducted by mixing 2 mL of formulations with 0.75 mL of SVF. Control consists on a solution of HPMC at a concentration of 1% w/w. $n = 6$. NA: no adhesion. (∗) represents significant difference ($P < 0.05$).

potential of the hydrogels. From a practical point of view, it is noteworthy that the much higher mucoadhesive potential of formulations without dilution with SVF should be taken into account particularly when considering products for women with poor or scarce vaginal lubrication, as for example, during menopause.

We noted that before dilution, F_{max} was higher for pluronic F127-based hydrogel, while work of adhesion was higher for

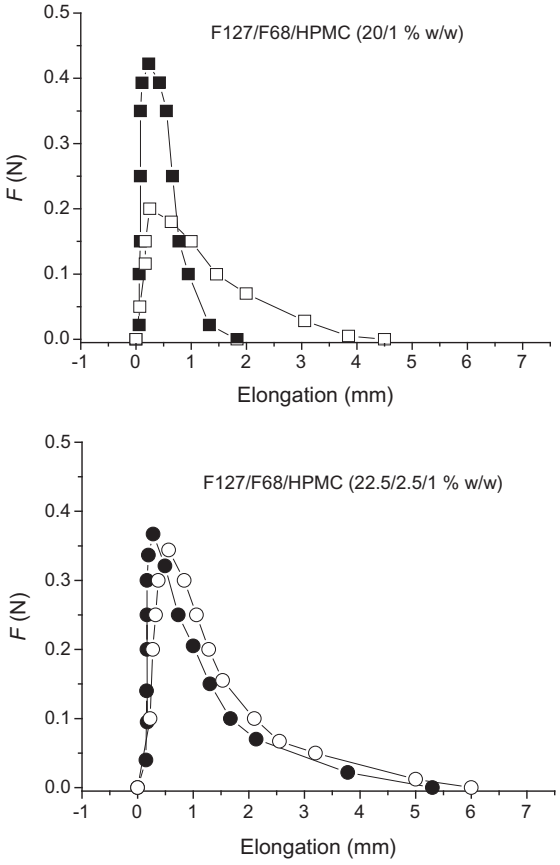

Fig. 7. Force–elongation curves for F127/F68/HPMC hydrogels before (■ and ●) and after dilution with SVF (□ and ○). Dilutions were conducted by mixing 2 mL of formulations with 0.75 mL of SVF.

hydrogels composed of a mix of two pluronics in comparison with F127-based hydrogels. However, after dilution, the two types of formulations F127/HPMC and F127/F68/HPMC responded differently. Indeed, in the case of F127-based hydrogels, F_{max}, W and mucoadhesion profiles were affected by dilution with SVF. However, in the case of F127/F68-based hydrogels, F_{max}, W and mucoadhesion profile were not significantly affected by dilution (Fig. 6).

As exposed in Fig. 7, the general shape of the tensile experiments is different depending on the formulation used. In the case of F127/F68 mixture, the shape of the curve is similar before and after dilution with SVF. However, F127 hydrogel tensile curve exhibited significant difference after dilution. For both formulations the parameters A, B and C (deformation to peak, deformation peak to failure and deformation to failure, respectively) were similar before dilution with SVF (Fig. 7). However, after dilution, hydrogels based on F127 exhibited a brittle fracture (small value of A) while, in the case of F127/F68 mixture, all parameters A, B and C were larger than the one reported for F127 hydrogels after dilution.

These results can be due to the difference between the elasticity of the two types of hydrogels. Indeed, we demonstrated that elasticity of F127/F68-based hydrogel was higher than the one composed of F127. The higher elasticity of F127/F68 hydrogels will increase the possibilities of micelle interpenetration with mucus. Furthermore, F127 (20% w/w) and F127/F68 (22.5/2.5% w/w) contained 32 mM and 42 mM of hydroxyl groups, respectively. It is well known that polymers possessing hydrophilic functional groups such as hydroxyls are susceptible to interact more favorably with mucus gel layer. Consequently, mucoadhesion was

stronger in the case of F127/F68-based hydrogels due to numerous hydrogen bounds generated by hydroxyl groups.

In conclusion, mucoadhesion process of pluronic hydrogels was affected by (i) the rheological properties of the hydrogels, (ii) polymer concentration and (iii) its chemical composition.

4. Conclusions

In this work, we developed pluronic hydrogels able to keep their thermosensitive and mucoadhesive properties after their dilution with vaginal fluids. The formulated hydrogels were composed of F127 (20% w/w) or a mix of two pluronics F127 and F68 (22.5/2.5% w/w). Both formulations contained HPMC as mucoadhesive polymer at a concentration of 1% w/w. Interestingly, little variation of mucoadhesion parameters were observed in the case of hydrogels composed of F127/F68 mixtures. These differences were attributed to the higher elasticity of the hydrogel composed of F127/F68 mixture and to the stronger interaction of hydroxyl groups with mucous gel layer. The combination of all results showed that hydrogels composed of F127/F68 exhibited interesting rheological and mucoadhesive properties, making them promising formulations for the vaginal administration of drugs.

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