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Research Article

Gels Containing Poloxamer 407 and Sodium Alginate as Vehicles for Vaginal Administration

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Abstract

Sodium alginate and poloxamer 407 were both studied in order to design a hydrogel-based delivery system as a platform for the formulation of vaginal medecines. Sodium alginate is a natural anionic mucoadhesive polymer while poloxamer 407 is a temperature-sensitive polymer. A commercial alginate was characterized. Then, its solutions (0.5-2 % w/w), poloxamer 407 solutions (16-21.5 % w/w) and their mixtures were prepared. Their rheological properties and the influence of vaginal simulated fluid were assessed. This commercial alginate had a mannuronate-to-guluronate ratio of 1.24, a weight-average molecular weight of 300 000 ± 2% g.mol^{-1}, a polydispersity index of 1.8 ± 3%. Alginate solutions displayed a viscous behavior not suitable for a vaginal administration. Poloxamer solutions and their mixtures with alginate were temperature-sensitive, shear-thinning, non-thixotropic and had an elastic behavior suitable for a vaginal administration. Their resistance to dilution with vaginal fluid could prolonge vaginal residence.

Keywords: Formulation, poloxamer 407, rheology, sodium alginate, temperature–sensitive, vaginal fluid, viscoelastic properties.

1. INTRODUCTION

Vagina seems to be a strong route for the prevention and the treatment of genitourinary infections in women. Therefore, researches have been focused on vaginal delivery systems [1–3]. Numerous polymeric matrices have been investigated for the vaginal administration, using different dosage forms [3–5], with emphasis on hydrogel-based delivery systems and particularly thermo-responsive hydrogels. Indeed, thermo-responsive hydrogels can be liquid at storage temperature to facilitate administration as a liquid, whereas the gelation occurs at physiological temperature conferring a high resistance to flow, and prolonging its residence time at the site of administration. Poloxamer 407 is a temperature-sensitive amphiphilic triblock copolymer of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide). It has been widely investigated in the development of temperature-sensitive gels, due to its ability to undergo reversible thermal gelation [6,7]. It is already widely used in the pharmaceutical field and is considered as a safe excipient. Poloxamer 407 is often mixed with a mucoadhesive polymer which may increase its adhesion to the mucus, so its residence time at the administration site [8]. Several mucoadhesive polymers have been studied for this purpose [9]. A great interest was devoted to naturally occurring polysaccharides such as alginate due to their biocompatibility, biodegradability and non-toxicity [10]. Alginate is an anionic linear polysaccharide extracted from different brown seaweed (*Phaeophyceae*), consisting of (1-4)-linked blocks of poly-β-d-mannuronic acid (M) and poly-α-l-guluronic acid (G) residues. Its M/G ratio as well as the arrangement of these residues can vary depending on its source [11]. Both poloxamer 407 and sodium alginate may be combined to obtain a solution with *in situ* gelation capacities [12], may be gelled with calcium chloride to obtain beads or capsules [13,14], gelled by CaEDTA and glucono-δ-lactone to obtain hydrogels [15–17], or gelled both thermally and ionically to obtain a film [18,19]. Both polymers may be mixed together or alginate may be grafted onto poloxamer [20].

The aim of this study was to develop a gel intended for an administration into the vagina by the women themselves. This gel should have optimized flow properties, viscoelastic behavior, been liquid before administration, and should

maintain its *in-situ* gelation at 37 °C in the presence of vaginal fluid.

Poloxamer 407, sodium alginate and their mixtures were prepared by varying their concentrations in order to improve their mechanical properties to withstand vaginal shear forces and dilution with the vaginal fluid. The formulations were first investigated to ensure both thermal gelation with poloxamer 407 and ionic gelation with alginate. Then, they were characterized by rheological studies *(i)* at 25 °C to mimic the administration conditions, and *(ii)* at 37 °C, after dilution with the simulated vaginal fluid (SVF) in order to mimic the body conditions.

2. MATERIALS AND METHODS

2.1. Materials

Poloxamer 407 of pharmaceutical grade (Lutrol® F127) was purchased from BASF (Germany). According to the supplier, its average molecular weight (Mw) was 11774 g.mol^{-1} (9840-14600 g.mol^{-1}). Sodium alginate was provided by Roth (France), lot N° 351172083. Bovine serum albumin and all other reagents were supplied by Sigma-Aldrich (France) and were of analytical grade. Sterile ultrapure water (18.2 MΩ, MilliQ, Millipore, France) was used. The simulated vaginal fluid (SVF) was prepared according to Owen and Katz [21].

2.2. Characterization of sodium alginate powder

The molecular weight of sodium alginate was determined using size-exclusion chromatography (SEC) coupled online with multiangle light scattering (MALLS). The whole line was equipped with a degasser (DGU-20A3, Shimadzu, Japan), a pump (LC10Ai, Shimadzu, Japan) with a flow rate of 0.5 mL min^{-1} (the eluent was 0.1 µm filtered 0.1 mol.L^{-1} $LiNO_3$), an automatic injector (SIL-20A, Shimadzu, Japan), a stainless steel pre-filter (0.45µm), one pre-column (OHPAK SB-G), two analytical size-exclusion columns (OHPAK SB 804 HQ and 806 HQ), and three detectors: *(i)* a multi-angle light scattering (MALLS) detector (Dawn EOS, Wyatt Technology, CA, U.S.A.) fitted with a K5 cell of 50 µL, with a red source (Ga-As 690 nm) of mW and 18 photodiodes (angles distributed around the cell), *(ii)* a differential refractive index (DRI) detector (RID 10A, Shimadzu, Japan), and *(iii)* a differential viscosimeter detector (Viscostar II, Wyatt Technology, CA, U.S.A.). The Astra 6.0.6.13 software package was set to collect and extrapolate data according to the known value of the differential refractive index, dn/dC (dn/dC = 0.14 mL.g^{-1}.) [22].

The sodium alginate M/G ratio was determined by ^{1}H NMR spectroscopy. It was performed in a 5 mm diameter tube at 80 °C, on a Bruker Avance 400 spectrometer at 400 MHz. The chemical shift scale was calibrated on the basis of the solvent (D_2O) peak. The acquisition time was 1 s and the number of scans was 128. The sample was previously partially hydrolyzed, freeze-dried, dissolved in D_2O and freeze-dried to replace exchangeable protons with deuterium, and finally dissolved in D_2O at 10 mg/mL for measurements as described by Jensen et al. (2015).

2.3. Hydrogel preparation

Hydrogels containing 16-21.5 % w/w of poloxamer 407 and/or 0.5-2 % w/w of sodium alginate were prepared by using a mixer equipped with a turbine adapted to the mixing of viscous preparations (Rayneri-turbotest, Rayneri, France) under an agitation of 1000 rpm [8]. Sodium alginate powder was gradually added in ultrapure water at room temperature. The poloxamer powder was progressively added either in ultrapure water or in alginate solution placed in an ice bath. After complete dissolution, each formulation was equilibrated 48 h in a refrigeratpr to eliminate foam and air bubbles and was autoclaved (121 °C, 15 min). The compositions of the different formulations are summarized in **Table 1**.

Table 1: Formulations developed with alginate (A) and poloxamer (P)

Denomination	Alginate (% w/w)	Poloxamer (% w/w)
A0.5	0.5	
A1	1	
A1.5	1.5	
A2	2	
P16		16
P18.5		18.5
P21.5		21.5
P16A0.5	0.5	16
P16A1	1	16
P18.5A0.5	0.5	18.5
P18.5A1	1	18.5
P21.5A0.5	0.5	21.5
P21.5A1	1	21.5

2.4. Hydrogels diluted by simulated vaginal fluid

SVF was prepared according to Owen et Katz [21] by adding 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) to 900 mL of ultrapure water. Their mixture was stirred mechanically until complete dissolution. The pH of the mixture was then adjusted to 4.5 using HCl 1N. The final volume was adjusted with water to 1 L. In this study, to mimic the maximum dilution of gels that was likely to occur after vaginal administration, 5 mL of each formulation were mixed with 0.75 mL of SVF by vortexing. The aim of this test was to assess the influence of the dilution on the rheological properties of the formulations.

2.5. Alginate ionic gelling capacity

The evaluation of ion activated gelation of alginate was performed in the presence of the SVF as it contains calcium in order to access *in situ* ionic gelling possibility. Gelling capacity of the formulations was determined by mixing the 5 g of formulations with 0.75 mL of SVF using tube inversion method according to Ur-Rehman et al [24].

2.6. Rheological characterization

The flow properties, the gelation temperatures and viscoelastic properties of the formulations were accessed by this study, using a rotational rheometer ARG2 (TA instruments, New Castle, USA). The geometry was an aluminum cone/plate (diameter 40 mm, angle 1° and cone truncation 28 µm) equipped with a solvent trap to limit evaporation during measurement. Temperature was controlled by Peltier diodes placed in the lower plate. Experiments were started after a 3-minute equilibration time. Three experimental conditions were performed 25 °C, 37 °C and 37 °C after dilution with SVF.

The viscosity was recorded as a function of shear rate which was increased gradually from 0.01 s^{-1} to 1000 s^{-1} over 3 min, maintain to 1000 s^{-1} for 1 min and gradually decreased from 1000 s^{-1} to 0.01 s^{-1} over 3 min.

For oscillatory measurements, linear viscoelastic regions, where the elastic modulus (G') and the viscous modulus (G'') remained constant with stress were determined first.

Then, oscillatory measurements were performed under a stress value belonging to the linear viscoelastic regime. G' (Eq. 1) describes the elasticity or the energy stored and recovered in the material per deformation cycle, whereas G'' (Eq. 2) is related to the viscous character or the energy dissipated during the cycle. The elastic modulus reflects the solid-like component of elastic behavior. The higher the G' value, the more pronounced the elastic character and conversely, the higher G", the more pronounced the viscous properties.

$$G' = \frac{\tau_0}{\gamma_0} \cos \delta \qquad (1)$$

$$G'' = \frac{\tau_0}{\gamma_0} \sin \delta \qquad (2)$$

τ_0 and γ_0 are respectively the maximal amplitudes of the stress and the strain. δ, the phase angle, varies between 0° and 45° (tan δ < 1, G' > G'') for a rather elastic material and between 45° and 90° (tan δ > 1, G'' > G') for a predominantly viscous material (Eq. 3).

$$tan\ \delta = \frac{G''}{G'} \qquad (3)$$

G' and G'' were measured as a function of temperature with a temperature sweep from 5 °C to 45 °C with a heating rate of 1 °C/min, at a frequency of 1 Hz. Thus, the sol–gel transition temperature (T_{gel}) defined as the crossover of G' and G'' curves was determined. In addition, G' and G'' were measured as a function of frequency with a frequency sweep from 0.01 to 50 Hz to determine the viscoelastic properties.

All rheological tests were realized in triplicates and results were expressed as mean ± standard deviation. TRIOS software was used for data analysis.

3. RESULTS AND DISCUSSION

3.1. Sodium alginate characterization

Sodium alginate physical properties are determined by its mannuronate-to-guluronate ratio (M/G), its sequences and its molecular weight.

Molecular weight

The weight-average molecular weight (M_w), the number-average molecular weight (M_n), the hydrodynamic radius and the intrinsic viscosity of sodium alginate in $LiNO_3$ (0.1 $mol.L^{-1}$ were determined by SEC/MALLS. Then, the polydispersity index, the gyration radius (R_g) and critical chain overlap concentration C* were deduced.

M_w was 300 000 ± 2% $g.mol^{-1}$ and M_n was 165 000 ± 3% $g.mol^{-1}$. The polydispersity index (D) (**Eq. 4**) was 1.8 ± 3% (>1). Polymers with the D value greater than one have monomer units arranged in chains of different length. Such result is more often observed with natural polymers.

$$D = \frac{M_w}{M_n} \qquad (4)$$

Alginate intrinsic viscosity [η] was 1200 ± 0.5% $mL.g^{-1}$. From [η], the Mark-Houwink-Sakurada equation (**Eq. 5**) allowed to determine the "a" exponent which is related to the polymer conformation [25].

$$[\eta] = K.M_v^a \qquad (5)$$

K and a are constant parameters giving information for a defined alginate–solvent pair. Two "a" values were obtained: 0.6 corresponding to linear flexible expanded alginate, and 1 due to the stiffness of some hydrated chains. These results showed the presence of two types of chain conformations one flexible and one rigid.

The hydrodynamic radius R_h of sodium alginate was 36 ± 1% nm determined by SEC/MALLS. From R_h, the radius of giration R_g was estimated according to **Eq. 6** [25] and was about 60 nm.

$$R_g \approx \frac{R_h}{0.6} \qquad (6)$$

R_g was used to calculate the critical chain overlap concentration of sodium alginate C*. C* characterizes the transition between the dilute regime and the semi-dilute unentangled regime. C* was calculated according to **Eq. 7** [25] and was 0.06 % w/w.

$$C^* = \frac{M}{N_A\left(\frac{4}{3}\pi R_g^3\right)} \qquad (7)$$

where M is the mass of the chain and N_A the Avogadro's number. Another method to estimate C* is based on **Eq. 8** [25]**.-**

$$C^* = \frac{1}{[\eta]} \qquad (8)$$

With this equation, the obtained C* was 0.08 % w/w. This latter value is of the same order of magnitude as the previous one, but should be more precise due to the presence of two types of chain conformations in the sample.

SEC/MALLS studies were realized in $LiNO_3$ whose ionic strength was higher than water. In high ionic strength media, the conformation of polyelectrolyte coils such as alginate is more compact than in water, due to the screening of electrostatic interactions. Thus, in our formulation conditions (in water), the alginate chains are expected to be more extended, consequently, C* should be lower than 0.08 % w/w.

Mannuronate-to-guluronate ratio (M/G)

The sodium alginate M/G ratio was determined by ^1H NMR spectroscopy. **Figure 1** shows the ^1H NMR spectrum obtained with this sodium alginate. The regions 1 (4.96-5.18 ppm), 2 (4.57-4.82 ppm), 3 (4.38-4.55 ppm) were integrated to obtain respectively I_1, I_2, I_3. This allowed to calculate the alginate M/G ratio according to **Eq. 9** as described by Jensen et al. (2015).

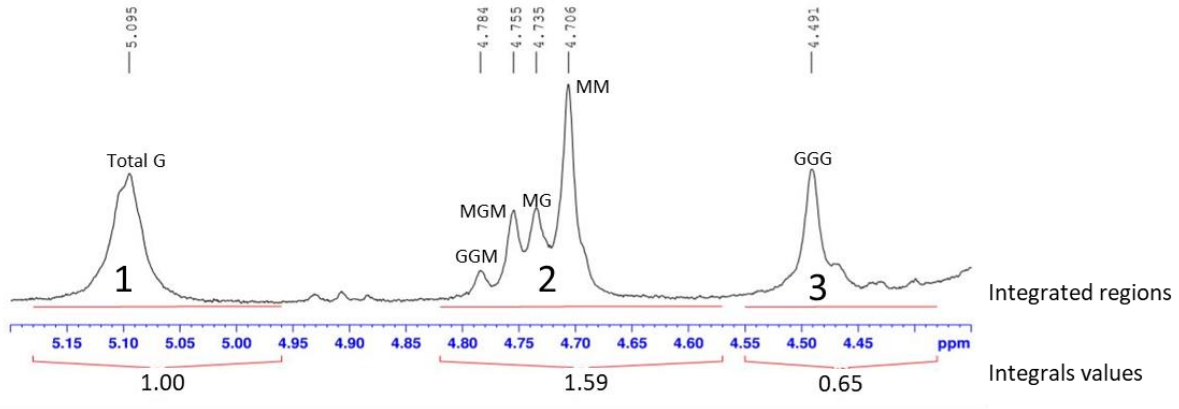

Figure 1. Sodium alginate ^{1}H NMR spectrum.

$$M/G = \frac{I_2+I_3-I_1}{I_1} \qquad (9)$$

The M/G ratio was 1.24 ± 0.04 that corresponded to 55 % of M units and 45 % of G units. This value was similar to the M/G ratio of alginate obtained from the fronds of *Laminaria hyperborean* [26]. It was higher than those of other parts of this algae or other *Laminaria* species [26,27]. Fertah et al. (2014) showed that M/G ratio value of 1.12 allowed the preparation of alginates suitable to form soft and elastic gels.

3.2. Gels formulation and characterization

Different formulations were prepared by varying the concentration of each polymer.

In a first step, formulations containing only sodium alginate were performed. Sodium alginate was used at concentrations ranging from 0.5-2 % w/w, that belonged to the semi-diluted regime as these concentrations were higher than C*. All formulations appeared to be homogeneous to the naked eye after storage and were translucent. However, A1.5, A2 and their corresponding mixtures with poloxamer 407 were so viscous that they were difficult to prepare. Thus, they were not considered for further studies. Poloxamer 407 solutions at concentration ranging from 16 to 21.5 % w/w alone or mixed with alginate at 0.5 and 1 % were studied. The effect of alginate concentration, of the dilution with SVF were assessed.

3.2.1. Rheological behavior at 25 °C and at 37 °C

Sodium alginate formulations

Both A0.5 and A1 were slightly shear-thinning (**Fig. 2A**) at 25 °C. The viscosity of A1 was higher than with A0.5. The formulations also displayed a non-thixotropic behavior showed by the overlay of downward and upward curves of the viscosity *versus* shear rates. The G' and G'' moduli were frequency–dependent at 25 °C (**Fig. 2D**). The viscous modulus of A0.5 and A1 were respectively higher than their elastic moduli and the crossover was observed at high frequency (**Fig. 2D**). At the crossover frequency of A0.5 (10 Hz), G' = G'' = 2.5 Pa, but at the crossover frequency of A1 (about 46 Hz), G' = G'' ≃ 2600 Pa. Alginate solutions had predominant viscous behavior. The rheological behavior of sodium alginate at 37 °C was similar to the one of 25 °C (**Fig. 2B, E** compared to **Fig. 2A, D**). This viscous liquid behavior of alginate formulations when used alone was not suitable for a vaginal administration. Several studies have showed that the mixture of alginate with poloxamer may increase its mechanical properties [17,19].

Poloxamer formulations

All poloxamer formulations displayed a shear-thinning behavior at 25 °C. This behavior was interesting for an easy administration with a device, due to the low flow resistance when applied at high shear conditions. The formulations also displayed a non-thixotropic behavior. As soon as the shear was removed, the viscosity recovered its initial value. The poloxamer 407 formulations were temperature-sensitive. Below the gelation temperature (T_{gel}), G' was lower than G''. Above T_{gel}, G' became higher than G'' (**Fig. 3C**). Around T_{gel}, G' and G'' strongly increased with temperature. The gelation temperatures are gathered in **Table 2**. The one of P21.5 was the lowest (21±1 °C) compared to 25 ± 1 °C for P18.5 and 32 ± 1 °C for P16 (**Table 2**). G' and G'' moduli were also frequency–dependent (**Fig. 3E**). P21.5 had the highest viscoelastic moduli, and thus, lead to the most consistent gels (**Table 2**).

The mixtures of poloxamer 407 with alginate were macroscopically homogeneous and translucent confirming their miscibility. Indeed, the ethylene oxide groups of poloxamer 407 could interact with sodium alginate chains by hydrogen bonds, which contributed to their miscibility in solution [28,29]. Moreover, the miscibility of neutral polymers with polyelectrolytes is also favored by the entropy of the counter ions of the polyelectrolyte.

The mixtures of poloxamer 407 with alginate remained shear-thinning (**Fig 3A**), temperature-sensitive (**Fig. 3C)**. Alginate increased the value of the viscosity of poloxamer formulations. The gelation temperatures of poloxamer formulations alone and equivalent mixtures were similar, showing that alginate did not significantly interfere with the poloxamer 407 gelation. P21.5A1 T_{gel} was 19 ± 1 °C while P21.5 T_{gel} was 21±1 °C. The same trend was observed with P18.5A1 T_{gel} 25 ±1 °C compared to 23 ± 1 °C for P18.5. The administration conditions are 19-20 ° C in Europe while they are 25-30 °C in sub-Saharan Africa. Therefore, P21.5A1 and P18.5A1 might be in gel state when administered at room temperature or may be stored in colder conditions if a liquid state is required for its administration. At temperatures below the gelation temperature, the viscoelastic moduli values of the mixtures were higher than those of the poloxamer formulation alone. Therefore, sodium alginate chains contributed to the elasticity of the mixture thanks to the network they formed. Above the gelation temperature, the viscoelastic moduli of P21.5 and P21.5A1 were not significantly different as well as P18.5 and P18.5A1 suggesting that at high temperatures poloxamer 407 imposed it behavior to the mixtures (**Fig. 3C**). These results are in accordance with those of Grassi et al. (2006) who showed that the properties of pure P407 at 20 % and its corresponding mixtures with alginate 1-2 % were largely different at low temperatures but similar at temperatures above gelation. They also demonstrated that the presence of alginate chains had a favorable influence on the structural process, providing a stabilizing framework for micelles caging and packing. However, once the structural

process was complete, the properties of the systems almost coincided with those of poloxamer 407 alone.

In frequency sweeps, the elastic modulus was higher than the viscous modulus at all the studied frequencies (**Fig. 3E**). As both studied temperatures (25 °C et 37 °C) were above T_{gel}, so the formulations were in gel state. When the frequency increased the elastic modulus remained constant while the viscous modulus slightly decreased.

The mixtures of poloxamer 407 and sodium alginate have improved their mechanical properties.

3.2.2. Rheological behavior at 37 °C after dilution with SVF

These formulations were intended for a vaginal administration. Therefore, some particularity of the vagina (vaginal fluid, quantity of administered gels, vaginal shear rate) should be taken into account.

About the quantity of administered gels, generally, two to five milliliters of topical vaginal products are applied in the vagina [8]. However, a volume of 5 mL could significantly better cover the human vaginal epithelium, immediately following its insertion, within the first 30 min and without sexual intercourse, compared to lower volumes (3 mL) [30]. Therefore, a volume of 5 mL for each gel unit would be interesting to ensure a good epithelial coverage.

About the vaginal fluid, it is composed by fluids that originate from uterus, cervix, and sometimes menstrual secretions and sperm [21]. Unfortunately, the human vaginal fluid quantity is limited, and it degrades rapidly once collected from its source. Thus, Owen et Katz (1999) had developed a simulated vaginal fluid (SVF) in order to test vaginal pharmaceutical forms. Owen and Katz (1999) also showed that the volume of ambient vaginal fluid present at any time in the vagina was approximately 0.5–0.75 mL. Therefore, to mimic the maximum dilution of the formulations that was likely to occur after its application, 5 g of each formulation were mixed with 0.75 mL of SVF by vortexing. To explore the ability of the formulations to withstand vaginal fluid dilution, rheological studies were performed *(i)* at 37 °C without dilution with SVF and *(ii)* at 37°C after dilution with SVF to mimic complete dilution with SVF. Indeed, gels in the vagina should lie between those two extreme conditions.

About the vaginal shear rates, the *in vivo* studies conducted by Owen et al. (2000) showed that shear rates in the vagina are due to movements of the vaginal epithelial surfaces, gravity and capillary flow[31]. They are approximately 0.1 s^{-1} at the time of initial spreading and can increase up to 100 s^{-1} due to coitus. Formulations should withstand these shear forces into the vagina and dilution with the vaginal fluid.

Sodium alginate formulations

Regarding the flow properties after dilution with SVF, A0.5 viscosity was similar to the results at 37 °C while the one of A1 was reduced (**Fig. 2B, C**). After dilution with SVF at 37 °C (**Fig. 2F**), the viscoelastic moduli of A0.5 were not modified but those of A1 were reduced compared to the results at 37 °C (**Fig. 2E**). In these conditions, the crossover frequency of both alginate solutions was 1 Hz, with G' = G'' = 0.1 Pa.

As sodium alginate can form mucoadhesive gels in the presence of divalent cations such as Ca^{2+}, which bind to G units to form an "egg-box" structure, the gelling capacity of alginate solutions at different concentrations was evaluated in the presence of human simulated vaginal fluid, in order to assess the possibility of ionic gelation *in situ*. Indeed, the human vaginal fluid contains 0.003 $mol.L^{-1}$ of calcium ions from calcium hydroxide [21]. As the other components of the vaginal fluid could interfere with the gelation, a calcium hydroxide solution corresponding to its concentration in SVF was also studied as a control. The gelling capacity of A0.5 and A1 in the presence of the maximum volume of vaginal fluid (0.75 mL) was assessed. None of the tested formulations gelled after the addition of SVF. Using the calcium hydroxide solution at the same concentration, volume and nature, simulating the calcium ion content of the SVF, no gelation was observed neither. To the best of our knowledge, there is no previous study reporting sodium alginate gelation induced by vaginal fluid. However, previous studies were performed with simulated tear fluid. Sodium alginate with a high G content (> 65 %) at 0.5-2 % (w/v) could gel instantaneously in the presence of calcium ions simulating their content in simulated tear fluid as well as in simulated tear fluid itself [12,32]. In the present study, both G content of alginate and calcium concentration in SVF were lower, respectively about 45 % and 0.003 mol/L. Thus, they were not sufficient to ensure a gelation. The dilution with SVF reduced the concentration of alginate in the system from 0.5 to 0.4 and from 1 to 0.9 (% w/w), still above C*.

The gelation of the mixtures is only due to the gelification of the poloxamer 407.

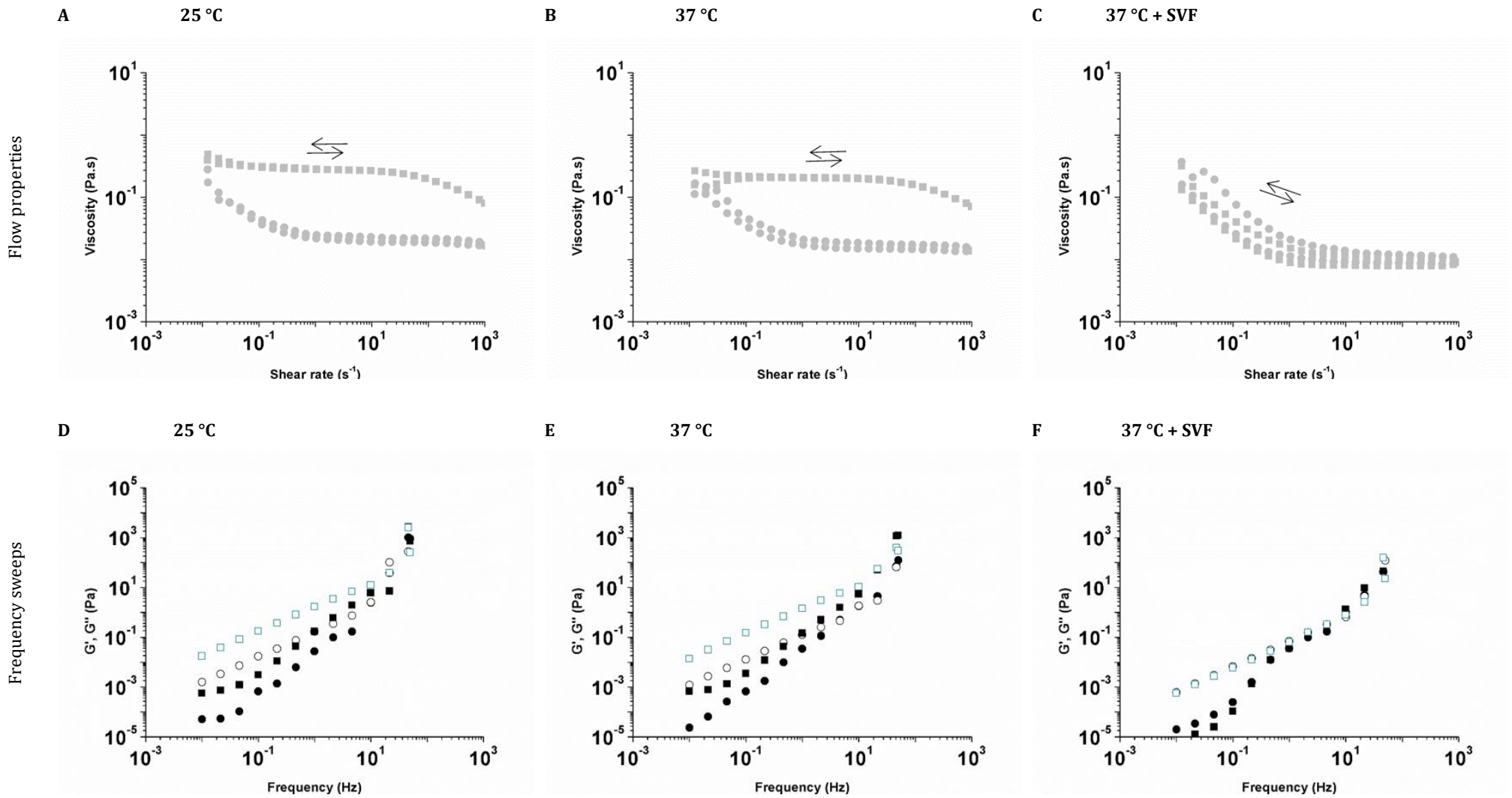

Figure 2. Flow properties (A, B, C), frequency sweeps (D, E, F) at 25 °C (A, D), at 37 °C (B, E) and at 37 °C after dilution with simulated vaginal fluid (C, F) of alginate solutions A0.5 and A1: (●) η for A0.5, (●) G' for A0.5, (○) G'' for A0.5, (■) η for A1, (■) G' for A1, (□) G'' for A1.

Poloxamer formulations

All poloxamer formulations remained shear-thinning and non-thixotropic after dilution with SVF as exemplify by the **Figure 3B** for P21.5. P16 diluted by SVF lost its thermal gelation due to the concentration of poloxamer that became 13.9 %, which was less than the minimal concentration required to obtain a gelation. This diluted system was not characterized. The other poloxamer formulations diluted with SVF had a gelation temperature 4 °C higher than the non-diluted ones (**Table 2**). The dilution of P18.5 by SVF lead to a final poloxamer concentration of 16 % which is the minimal concentration to obtain a gelation. About P21.5, the final poloxamer concentration after dilution with SVF was 18.7 %. Therefore, P18.5 and P21.5 appeared to be optimal poloxamer concentrations.

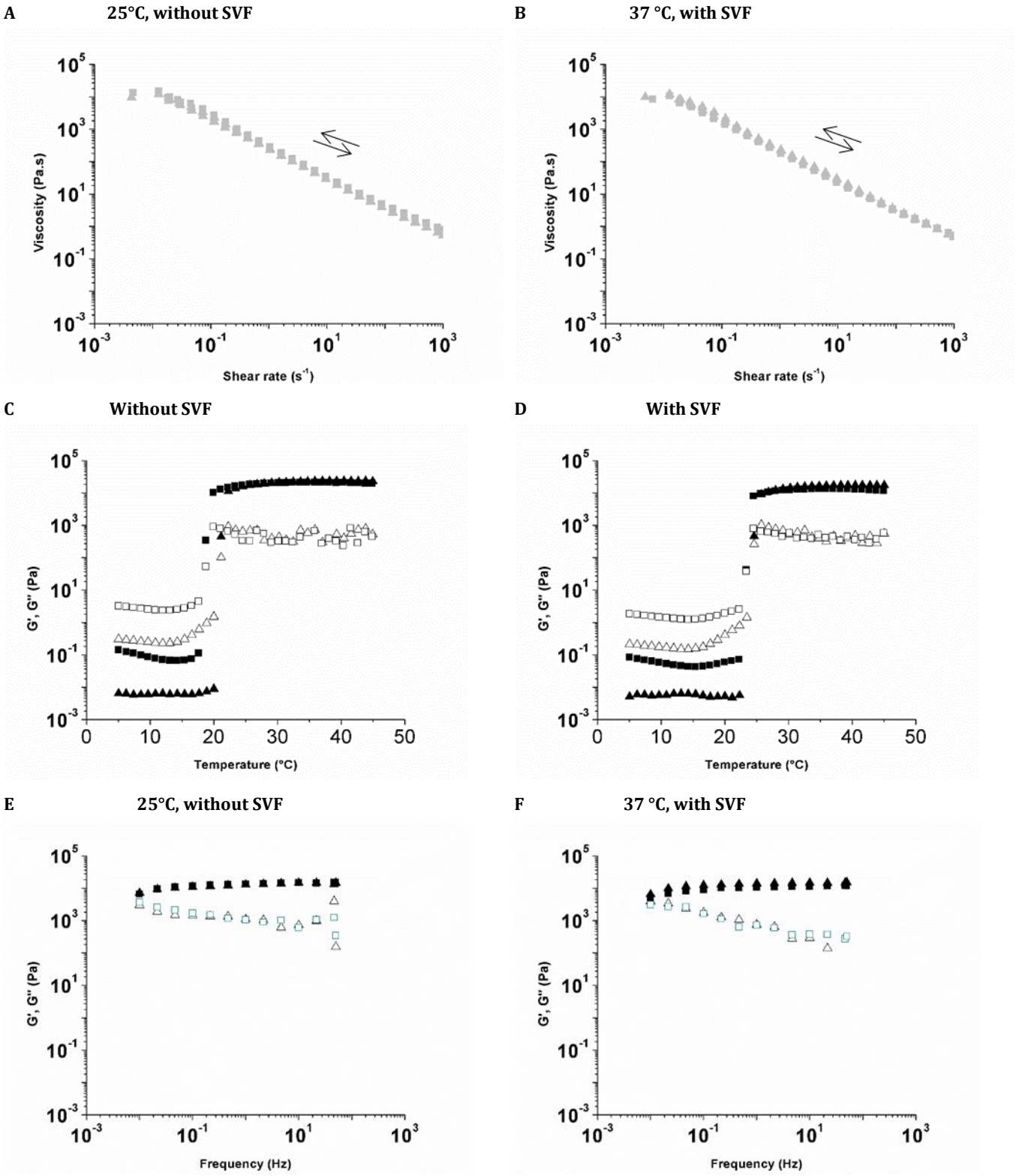

Figure 3. Flow properties (A, B), temperature sweep (C, D) displaying one data point out of ten, frequency sweep (E, F) of P21.5 and P21.5A1 as was (A, C, E) and after dilution with SVF (B, D, F): (▲) η for P21.5, (▲) G' for P21.5, (△) G'' for P21.5, (■) η for P21.5A1, (■) G' for P21.5A1, (□) G'' for P21.5A1.

Table 2. Gelation temperature, viscous and elastic moduli values at a frequency of 1 Hz for the poloxamer formulations and the poloxamer–alginate mixture.

Formulation	T_{gel} (°C)	Conditions	G' (Pa)	G'' (Pa)
P16	32 ± 1	25 °C	(4.0 ± 0.7) 10^{-3}	0.4 ± 0.1
P18.5	25 ± 1		3.0 ± 0.2	40 ± 6
P18.5A1	23 ± 1		3,3 ± 0.2	35,8± 6.2
P21.5	21 ± 1		(12,7 ± 0.2).10^3	(1.1 ± 0.01).10^3
P21.5A1	19 ± 1		(13.5 ± 0.4).10^3	(1.0 ± 0.2).10^3
P18.5 + SVF	29 ± 1	37 °C	(8.4 ± 0.5).10^3	(0.9 ± 0.2).10^3
P21.5 + SVF	25 ± 1		(13.8 ± 0.2).10^3	(0.7 ± 0.1).10^3
P21.5A1 + SVF	24 ± 1		(10.5 ± 0.2).10^3	(0.7 ± 0.2).10^3

The mixtures also remained shear-thinning and non-thixotropic after dilution with SVF (**Fig. 3B**). Flow experiments at 37°C in the presence of SVF were likely to be related to the residence time of the gel in the vagina, important property to ensure the therapeutic efficacy and to avoid leakage. Indeed, both vaginal shearing and dilution with SVF will influence the rheological properties of the administered formulations and, hence, their spreading and retention in the vagina. From these results a good spreadability is expected for the mixtures due to their shear-thinning behavior. Otherwise, after dilution with SVF, the viscosities remained high, which is favorable to a prolonged residence time.

Dilution of P21.5 with SVF also increased the gelation temperature. T_{gel} of P21.5A1 after dilution with SVF was similar to P21.5 sample diluted with SVF (**Fig. 3D**). Therefore, SVF had the same influence on both P21.5 and P21.5A1 gelation properties. Similar observation for P18.5 and P18.5A1.

After dilution with SVF the poloxamer and its mixtures remained viscoelastic with G' that remained higher than G'' (**Table 2**). The elastic modulus value of the diluted mixture P21.5A1 with SVF was lower than the non-diluted one (**Fig 3E** compared to **Fig. 3F**). This could be explained by the decrease in polymer concentration, but also by the more compact conformation of alginate chains due to ionic strength increased, caused by SVF. Moreover, proteins and other electrolytes contained in SVF could also interact with the polymers and decrease the elasticity of the system. The same trend for P18.5A1 mixture was observed.

4. CONCLUSION

In this work, hydrogels containing different concentrations of poloxamer 407 and sodium alginate and intended to be vehicles for vaginal administration were developed. The results confirmed the macroscopic compatibility between both polymers. All the formulations containing P407 were temperature-sensitive. The concentration of poloxamer to preserve the thermogelling properties after dilution with vaginal fluids should be above 18.5 % w/w. Adding 1 % w/w sodium alginate enhanced the formulation elasticity in liquid state. The mixture of P407 at 18.5 or 21.5 % w/w and sodium alginate at 1 % w/w were temperature-sensitive system with increased elasticity and adequate rheological properties, although it was not possible to induce *in-situ* ionic gelation of sodium alginate in these conditions. Their rheological properties at 25 °C were favorable to their administration. Their rheological properties at 37 °C after dilution with SVF were adequate to avoid leakage and ensure their spreading, P215A1 and P18.5A1 are promising candidates for vaginal administration.

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