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Rheological evaluation of thermosensitive and mucoadhesive vaginal gels in physiological conditions

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Abstract

The timely gelation and retention of in situ-gelling vaginal formulations would be fundamental to improve the efficacy of drugs. In this study, various rheological properties of clotrimazole gels were evaluated for predicting their performance in vagina. Two kinds of thermosensitive and mucoadhesive formulations were composed of poloxamer 407 (P407, 15%), polycarbophil (0.2%), and different amounts of P188 (15 vs. 20%). Both formulations were Newtonian at 20 °C but non-Newtonian at 37 °C. Although both liquid formulations gelled below the vaginal temperature, they differed in gelation time and viscoelastic properties in the presence of vaginal fluid simulant. At body temperature, the formulation with 20% of P188 gelled within 35 s but it took two times longer for the other one gelled. Upon dilution with simulated vaginal fluid, the formulation with 20% of P188 retained the rheology of a gel, but the other one lost the viscoelastic properties typical for a gel. Moreover, after dilution with simulated vaginal fluid, the elastic modulus was orders of magnitude higher in the formulations with 20% of P188 relative to the other one. These results indicate that the rheological evaluation at the physiologic conditions needs to be preceded to develop more effective in situ-gelling vaginal formulations.

Keywords: Rheology; Poloxamer; Thermosensitive gel; Clotrimazole; Vaginal formulation

1. Introduction

In vaginal drug delivery, the physiological conditions imposed by the protective mechanisms of the vagina often lead to the limited contact time of administered drugs with vaginal mucosa and a short duration of therapeutic efficacy, making a frequent dosing regimen necessary. Moreover, conventional vaginal dosage forms such as inserts and ointments give discomfort to the patients. Although the patients are known to tolerate gels better than inserts or ointments, the direct appli-

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cation of gels into the disease sites of vagina might be difficult as well as inconvenient. Vaginal therapy would be thus significantly improved if an intravaginally administered drug can retain at the site of administration for prolonged period after more convenient dosing (Robinson and Bologna, 1994; Mandal, 2000; Garg et al., 2001; Valenta et al., 2001; D'Cruz and Uckun, 2001).

Recently, in situ-gelling liquids have been investigated as a more convenient dosage form of topical applications. The liquids applied to the topical areas such as eyes can make transition to gels as a result of a chemical/physical change induced by the physiological environments. The transition could be induced by the concentration of calcium ions as for Gelrite® (Rozier et al., 1989), and temperature as for poloxamers (Miller and Donovan, 1982; Gilbert et al., 1987). Poloxamer, a block copolymer made of polyoxyethylene and polyoxypropylene, is known for its excellent compatibility with other chemicals, high solubility capacity for different drugs, and good drug release characteristics (Morishita et al., 2001). Bioadhesion can be used as a means to improve intimacy of contact, as well as a way to increase dosage form residence time to various administration routes (Park and Robinson, 1985; Robinson et al., 1987; Lee et al., 2000).

Furthermore, in order to fortify the adhesion of administered drugs onto the mucosal surfaces, mucoadhesive polymers such as polycarbophil, carbopol, hydroxypropyl cellulose, polyvinylpyrolidone have been added to the in situ-gelling liquids (Park and Robinson, 1985; Chu et al., 1991; Jones et al., 1996). There are several ways to sustain the release of a drug from gels in order to take full advantage of the contact time. The drug can be dispersed in the gel, giving a concentration that is higher than that corresponding to the solubility of the drug (Veyries et al., 1999), formulated as particles (Albertsson et al., 1996; Desai and Blanchard, 2000), distributed in liposomes (Bochot et al., 1998; Paavola et al., 2000), interacting with an oil phase that has been included in the gel (Gao et al., 1995).

The evaluation of rheological properties for the gel type dosage forms would be important for predicting their behavior in vivo. The rheological properties of eye gels were reported to affect the ocular residence time of the gels (Carlfors et al., 1998; Edsman et al., 1998; Desai and Blanchard, 2000). The flow properties of semi-solid vaginal dosage forms might be of use to predict the spreading and coating of the formulations over the vaginal epithelia. Especially in the thermosensitive gels containing a mucoadhesive polymer, the rheologcal characteristics need to be controlled and understood since the multi-component gels might exhibit complex flow behaviors due to the feasible interaction among the components.

Despite the importance of rheologic information, there has been little attempt to evaluate the rheological properties of thermosensitive and mucoadhesive vaginal gels in physiological conditions. In most studies, the rheologic evaluation has been limited to test the sol/gel transition temperatures of thermosensitive gels. In this study, using the antimicrobial agent clotrimazole as a model drug, we formulated two kinds of thermosensitive and mucoadhesive gels for vaginal delivery, and evaluated their rheological properties in the conditions that mimic the vaginal environments. Here, we report that two thermosensitive and mucoadhesive formulations, although both look suitable in terms of gelation temperatures, could reveal dramatic differences in the viscoelastic properties upon dilution with simulated vaginal fluid.

2. Materials and methods

2.1. Materials

Poloxamers (P407, P188) were supplied from BASF (Ludwigshafen, Germany). Polycarbophil (PC) was from BF Goodrich (Brecksville, OH, USA). Clotrimazole was kindly provided by Bayer Pharmaceuticals. All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of formulations

Thermosensitive and mucoadhesive gels were prepared by using the cold method (Choi et al.,

1998). In brief, PC (0.2 w/v%) was slowly added to citrate phosphate buffer (0.1 M, pH 4.0) at 4 °C with gentle mixing. P407 (15 w/v%) and P188 (15 or 20 w/v%) were then added to PC solution and allowed to dissolve overnight at 4 °C. Clotrimazole (1%) was initially dissolved in the mixture of ethanol and polyethylene glycol 400 (3:5), and added to the cold P 407/P188 solution containing various content of PC (0.2–1.0%) with gentle mixing.

2.3. Preparation of vaginal fluid simulant

Simulated vaginal fluid was prepared as described (Owen and Katz, 1999). To 1 l of distilled water, 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 dissolved. The pH of the mixture was then adjusted to 4.2 using HCl.

2.4. Steady shear viscosity

The flow curves of thermosensitive and mucoadhesive gels were determined using a Carri-Med CSL2 rheometer (TA instrument, USA) at the controlled-rate mode. The measurements were performed in a cone-and-plate geometry with a diameter of 40 mm (cone angle 2°). The shear rates ranged from 0.1 up to 100 s^{-1}. Samples were applied to the lower plate using a spatula to ensure that formulation shearing did not occur (Jones et al., 1997). To test the effect of temperatures, the measurements were made at 20.0 and 37.2 °C. Each datum point is the mean of at least triplicate analysis. Error bars have been omitted to retain clarity, however, in all cases the coefficient of variation of replicate analyses was less than 5%.

2.5. Temperature and rate of gelation

The sol/gel transition temperatures of poloxamers were determined using a Carri-Med CSL2 at 1 Hz of the oscillation mode. The gelation temperature was defined as the point where the elasticity modulus was halfway between the values for the solution and for the gel (Edsman et al., 1998). Oscillation measurement was conducted in the linear viscoelastic range using a 40-mm cone with 2° angle. The rheometer was also used to characterize the time-dependent changes in the elasticity modulus at 37.2 °C, as such process is associated with both the rate and extent of thermosensitive gel curing (Jones, 1999).

2.6. Dynamic mechanical analysis

The frequency dependence of dynamic viscoelastic parameters was determined using the rheometer at the oscillatory mode. The rheometer was installed with a data-processing oscillation software (TA instruments, USA). Samples were applied to the lower plate using a spatula to ensure that formulation shearing did not occur (Jones et al., 1997). The instrument measured the response (strain) induced at the time of sinusoidal stress application to the sample. Oscillation measurement was conducted in the linear viscoelastic range using a 40-mm cone with 2° cone angle. The elasticity modulus G' (storage modulus) and the viscosity modulus G'' (loss modulus) were obtained under dynamic conditions of non-destructive oscillatory tests in the frequency range of 0.1–10 Hz at a stress of 15 Pa at 37.2 °C. In some cases, to mimic the situation in the vagina, 3 ml of the liquid formulation was mixed with 0.9 ml of simulated vaginal fluid, and tested for the viscoelastic properties. Each datum point is the mean of at least triplicate analysis. Error bars have been omitted to retain clarity, however, in all cases the coefficient of variation of replicate analyses was less than 5%.

3. Results and discussion

3.1. Steady shear behavior

The steady shear behavior of the poloxamer-based formulations was influenced by the temperature and the content of P188 (Fig. 1). At 20.0 °C, two formulations composed of 15/15/0.2 or 15/20/0.2 of P407/P188/PC did not show notable changes in the viscosity over a broad range of

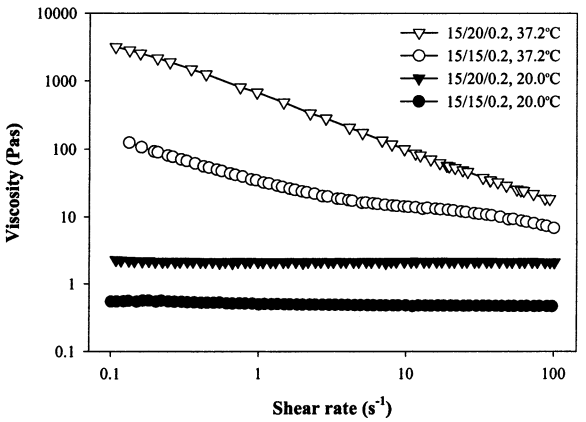

Fig. 1. Shear viscosity of the thermosensitive and mucoadhesive formulations as a function of shear rate. Shear viscosity (Pa) was measured using a Carri-Med CSL2 rheometer at the controlled-rate mode. Two formulations were composed of P407, P188, and PC (15/15/0.2 or 15/20/0.2). Both formulations contained 1% of clotrimazole. The open symbols are for 37.2 °C and the closed ones for 20.0 °C.

shear rates. Both formulations, existing as a liquid form at 20.0 °C, similarly broad range of shear rates. Both formulations, existing as a liquid form at 20.0 °C, similarly exhibited a Newtonian behavior. But, the formulation with 20% P188 showed at least 3.5-fold higher levels of viscosity relative to the other formulation. It has been reported that the thickening power of poloxamers in water increased with hydrophobic molecular weights and the ethylene oxide/propylene oxide ratios (Schmolka, 1977). The data in Fig. 1 suggest that an increase in the hydrophilicity of poloxamer could enhance the apparent viscosity at a given poloxamer concentration. In contrast to the shear viscosity pattern at 20.0 °C, a dramatic shear-thinning behavior was observed in the corresponding profiles of the formulations at 37.2 °C. Moreover, the content of P188 affected the extent of shear thinning. As the shear rates increased from 0.1 to 100 s^{-1}, the viscosity dropped 18- and 173-fold for the formulation with 15 and 20% of P188, respectively. Unlike the shear viscosity pattern at 20.0 °C, the remarkable shear-thinning behavior at 37.2 °C indicates the temperature-induced gel structure formation of the poloxamer and PC-based formulations. The sample had Newtonian behavior below the gelation temperature and non-Newtonian behavior above this temperature (Miller and Drabik, 1984; Lenaerts et al., 1987) (Fig. 1).

Regardless of the measurement conditions, all the shear viscosity curves showed relatively constant slop. Such constant slopes indicate that, under these flow conditions, all products can be modeled using a power law constitutive equation for the viscosity (η) as a function of shear rate ($\bar{y}$), $\eta = m\bar{y}^{n-1}$ where m is consistency index and n is so-called flow index (copetti et al., 1997; Owen et al., 2000). As n tends to 1, the shear-thinning properties are less and less pronounced, so that Newtonian behavior is achieved when $n = 1$.

At 20.0 °C, the n values of both formulations were close to 1, indicating a Newtonian behavior (Table 1). However, when the temperature was increased to 37.2 °C, two formulations clearly differed in the n values. For the non-Newtonian fluids, the lower the value of n, the more shear thinning the formulation. The higher value of m in the gel containing 20% of P188 implies that P188 may exert substantial contribution to the shear viscosity of the thermosensitive gel.

3.2. Gelation temperature

The themosensitive property of gels was evaluated by sol/gel transition temperature. The sol/gel transition temperatures correspond to the temper-

Table 1
Power law parameters of the thermosensitive and mucoadhesive gels containing clotrimazole

Composition (P407/P188/PC)	Temperature	m	n	Rheologic behavior
15/15/0.2	20.0	0.511	0.975	Newtonian
15/15/0.2	37.2	44.585	0.527	Non-Newtonian
15/20/0.2	20.0	2.170	0.993	Newtonian
15/20/0.2	37.2	479.000	0.308	Non-Newtonian

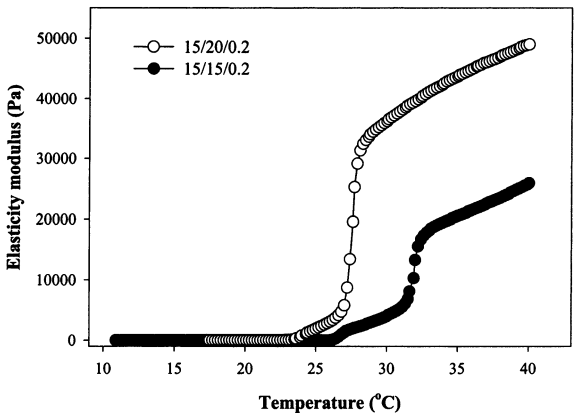

Fig. 2. Temperature-dependent changes of the elasticity modulus.

ature characterized by a drastic change of the Theological behavior and the elasticity modulus. The elasticity modulus is a measure of the energy stored and recovered per cycle of deformation and reflects the solid-like component of elastic behavior (Almdal et al., 1993). The elasticity modulus is thus low at solution stage but increases drastically at the gelation temperature. The gelation temperatures were 27.6 and 32.0 °C for the formulations with 20 and 15% of P188, respectively (Fig. 2). However, we further studied to test whether the gelation temperatures of the thermosensitive formulations, although looked suitable, could guarantee the gel-like behavior in the vagina.

Both formulations existed as a liquid form at the room temperature and might gel at the human vaginal temperature, known to be 37.2 °C (Rashad et al., 1992). Usually, the gelation temperatures have been considered to be suitable if they were in the range of 25–37 °C. If the gelation temperature of a thermosensitive formulation is lower than 25 °C, a gel might be formed at room temperature leading to difficult manufacturing, handling, and administering. If the gelation temperature is higher than 37 °C, a liquid dosage form still exists at the body temperature, resulting in the leakage of the administered antimicrobial agents from the vagina.

3.3. Gelation time

Two formulations, both showed gel-like rheologic properties at 37.2 °C, showed different rate of gelation depending on the content of P188 (Fig. 3). Gelation time was defined as the time when the elasticity modulus became higher than the viscosity modulus. The gelation of P407/P188/PC (15/20/0.2) was observed at 35 s, but it took longer time for P407/P188/PC (15/15/0.2) which began to show the viscoelastic property of a gel at 72 s.

At 37.2 °C, the formulation with 20% of P188 gelled within 34 s. The higher gelation rate of the formulation with 20% of P188 might have resulted from the stronger association of P188 with other components via hydrogen-bonding and ionic interaction. After intravaginal application, the shorter gelation time observed in the formulation (15/20/0.2) would be advantageous in that the rapidly gelled formulation might face the less change of drainage from the site of application, leading to a prolonged retention of clotrimazole in the vaginal cavity.

3.4. Viscoelastic properties of thermosensitive and mucoadhesive formulations

Dynamic mechanical analysis revealed that the viscoelastic properties of two formulations dif-

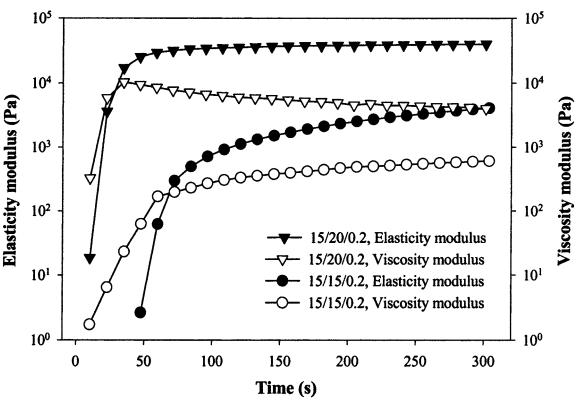

Fig. 3. Time-dependent changes of viscoelastic properties of the formulations. Each modulus was measured at 37.2 °C using the oscillatory mode of a Carri-Med CSL2 rheometer. The frequency was fixed at 1 Hz during the measurement.

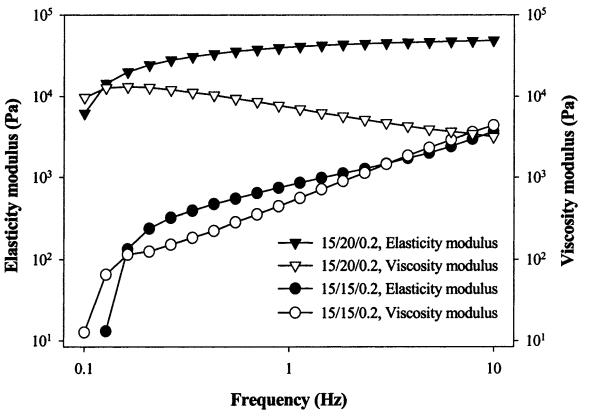

Fig. 4. Frequency-dependence of the viscoelastic moduli of the formulations. Each modulus was measured between 0.1 and 10 Hz at a stress of 15 Pa at 37.2 °C.

fered in the frequency dependence. For each formulation, the elasticity modulus and the viscosity modulus were measured as a function of the oscillatory frequency (Hz). In this study, the gel structure was examined over the frequency range, 0.1–10 Hz, which was frequently tested region in other rheological studies (Hägerström et al., 2000; Eouani et al., 2001; Riley et al., 2001).

Oscillatory measurements by varying the frequency (Fig. 4) showed that the elasticity modulus of the formulation with 20% of P188 was higher than the viscosity modulus over almost all the frequency range except the lowest frequency point (0.1 Hz). Moreover, only slight frequency dependence was observed in the elasticity modulus of the formulation with 20% of P188. Such little frequency dependence is typical for gels, indicating that the formulation with 20% of P188 was in the gel form at 37.2 °C. As compared with the formulation (15/20/0.2), the formulation with 15% of P188 displayed stronger frequency dependence.

Our observation that the elasticity modulus was more than one order of magnitude higher in the formulation with 20% of P188 than the other one (Fig. 4) implies that the formulation with 20% of P188 could be more elastic at 37.2 °C. The different viscoelastic properties of two formulations at 37.2 °C might be attributed to the different gelation temperatures (Fig. 2). Notably, the formulation with 20% of P188 showed the decrease of the viscosity modulus with increasing frequency. This phenomenon could be due to the higher crosslink between the poloxamer molecules and PC at the increased P188 concentration.

We measured all the viscoelastic parameters within the linear response region, i.e. below the maximum strain. The destruction of the three-dimensional network of the gel was observed above the maximum strain. Analysis of viscoelastic materials is designed not to destroy the structure, so that measurements can provide information on the intermolecular and interparticle forces in the material (Lippacher et al., 2000; Korhonen et al., 2000).

It has been reported that the rheological behavior could be affected by various factors such as copolymer compositions and solutes (Gilbert et al., 1987). Although we did not directly measure the effect of clotrimazole on the rheological properties of the formulations, we can not exclude the possibility that clotrimazole might affect the rheological behavior.

In vivo, vaginal formulations will experience the dilution with vaginal fluids. The volume of vaginal fluids is known to be about 0.75 ml (Owen and Katz, 1999), and the liquid dosage form would be applied in volumes of 1–3 ml, resulting in dilution when thoroughly mixed. To mimic the situation in the vagina, we mixed the formulations (3 ml) with simulated vaginal fluid (0.9 ml), and tested the influence of the dilution on the elastic modulus.

The dilution with simulated vaginal fluid reduced the level of elasticity modulus in both formulations but to the different extent (Fig. 5). The formulation with 20% of P188 showed about 10-fold decrease in the elasticity modulus over a wide range of frequency except the lowest range, whereas the other one with 15% of P188 revealed at least two orders of magnitude decrease in the elasticity modulus. Upon dilution with simulated vaginal fluid, the elasticity modulus of the formulation with 15% P188 dropped down to lower than the detectable limit at the frequency range of 0.1–2.3 Hz.

Elasticity could be affected by various factors such as dilution, and the concentrations of salts and proteins. In this study, the formulations were

diluted with simulant vaginal fluids to resemble the vaginal circumstance where various salts and proteins exist. Similar to our results, Edsman et al. (1998) reported that the rheological property of ocular gel formulations changed upon dilution in simulated ocular fluids.

The possibility exists that the mucoadhesiveness of the formulations might be affected by dilution with environmental fluids. Previously, Hassan and Gallo (1990) evaluated the mucoadhesiveness of bioadhesive polymers using the elasticity modulus as an indicator. In this study, the decreased elasticity modulus of the formulations upon dilution with simulated vaginal fluids (Fig. 5) indicates that the mucoadhesivenss of the formulation may also be reduced by dilution.

It is possible that the dilution of the formulation with simulated vaginal fluid may affect the gelation temperatures. Edsman et al. (1998) reported that gelation temperatures of poloxamer 407-based formulation (20% of P407) increased up to 5 °C by dilution with simulated tear fluids. In this study, the gelation temperature of 15/15/0.2 (P407/P188/PC) was about 32 °C, it may increase inside the vaginal by dilution with physiological fluids. The feasible increase of gelation temperature might partly explain the lost gelation of 15/15/0.2 (P407/P188/PC) formulations upon dilution with simulated vaginal fluid. However, the decrease of elasticity modulus observed in this study may also attribute to the lost gelation of the formulation.

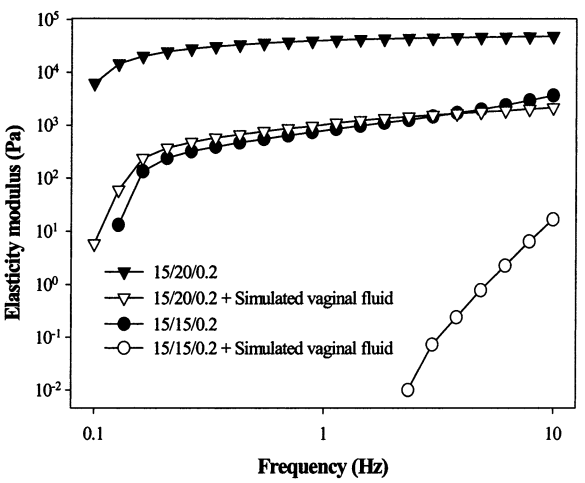

Fig. 5. Effect of simulated vaginal fluids on the frequency dependence of the elasticity modulus. The elasticity modulus was measured between 0.1 and 10 Hz at a stress of 15 Pa at 37.2 °C. Each formulation (3 ml) was diluted with simulated vaginal fluid (0.9 ml) before the measurements.

After dilution with simulated vaginal fluid, the frequency dependence of elasticity modulus changed depending on the contents of P188. Upon dilution, the dependence of elasticity modulus on the frequency was highly increased in the formulation with 15% of P188, but not in the one with 20% of P188. Regardless of the presence of simulated vaginal fluid, the formulation with 20% of P188 showed little dependence of elasticity modulus on the frequency, indicating that the formulation retained the gel shape upon dilution.

We observed that the formulation with 20% of P188 displayed more than five orders of magnitude higher elasticity modulus as compared with the other formulation at the frequency of 2.34 Hz after dilution with simulated vaginal fluid (Fig. 5). The formulation with a lower elasticity will more easily drain away from the vagina and reduce the availability of the entrapped drug, clotrimazole, to the site of application. The remarkably differential impact of vaginal fluid simulant dilution on the mechanical spectra of two formulations clearly suggests that the elucidation of viscoelastic properties on the biopolymer-based vaginal formulations would be of great importance for predicting the in vivo behavior and retention of vaginal dosage forms.

4. Conclusions

We demonstrated that the subtle difference in the composition of the thermosensitive and mucoadhesive gel formulations might lead to a dramatic change in the rheological behaviors. Although the gelation temperatures of both formulations with 15 or 20% of P188 were below the human vaginal temperature, they differed in the rate of gelation and viscoelastic properties. Upon dilution with simulated vaginal fluid, the formulation with 15% of P188 lost the gelation property whereas the other formulation retained the rheology of a gel. Taken together, the thermosenstive

and mucoadhesive formulation, P407/P188/PC (15/20/0.2), could be more suitable as an intravaginal delivery system of clotrimazole. Furthermore, our results suggest that the rheological analysis in the physiological conditions would be a powerful tool for the prediction of gel behavior and drug efficacy after intravaginal application.

Acknowledgements

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