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A New *In-Situ* Gel Formulation of Itraconazole for Vaginal Administration

Sinem Yaprak Karavana*, Seda Rençber, Zeynep Ay Şenyiğit, Esra Baloğlu

Department of Pharmaceutical Technology, Faculty of Pharmacy, Ege University, Bornova, Turkey.
Email: *sinem.yaprak.karavana@ege.edu.tr

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ABSTRACT

In this paper, mucoadhesive *in-situ* gel with poloxamer and hydroxypropylmethylcellulose formulations of itraconazole were prepared for vaginal application. In addition, rheological, mechanical and mucoadhesive properties and syringeability of the formulations were characterized. The mixtures of Poloxamer 407 and 188 with two different types of hydroxypropylmethylcellulose were used as polymers for gel formulations. Flow rheometry studies and oscillatory analysis of each formulation were performed at 20˚C ± 0.1˚C and 37˚C ± 0.1˚C. All formulations exhibited pseudoplastic flow and typical gel-type mechanical spectra (G′ > G″) after the determined frequency value at 37˚C. Texture profile analysis presented that F3 formulation containing 20% poloxamer 407, 10% poloxamer 188 and 0.5% hydroxypropylmethylcellulose appeared to offer more suitable mechanical and mucoadhesive performance. Using different hydroxypropylmethylcellulose type in formulations didn't significantly change syringeability values. The evaluation of the entire candidate formulations indicated that vaginal formulation of itraconazole will be an alternative for the treatment of vaginal candidiasis with suitable textural and rheological properties. Our results showed that the developed formulations were found worthy of further studies.

Keywords: Itraconazole; Poloxamer; Hydroxypropylmethylcellulose; Gel; Vaginal Candidiasis

1. Introduction

The azole antifungal agents represent a major advance in the treatment of both superficial and systemic fungal infections. These drugs can be divided in two main groups: the imidazoles and the triazoles [1]. Itraconazole is a broad-spectrum antifungal agent, which can be used either orally or intravenously. However, a vaginal formulation of itraconazole has not been developed yet [2-4]. It is a safe and effective active substance in the treatment of vulvovaginal candidiasis. It was shown that, active amount of the itraconazole may persist in vaginal epithelium for four days after a one-day treatment. It has been suggested that a cause of relapse in women with vaginal candidiasis is the re-emergence of *Candida* organisms from deeper layers of vaginal tissue [5,6].

Local drug delivery is frequently utilized for the treatment of localized disorders. The main advantages of this of administration are the ability to deliver the active agent directly to the site and the maintenance of the required concentration of active substance at the site for a prolonged period [7]. For the treatment of vaginitis, local antimicrobial administration of imidazole derivatives has been favored due to the numerous side effects of systemically applied drugs. To achieve desirable therapeutic effect, vaginal delivery systems need to reside at the sites of infection for a prolonged period [1]. For a long time, a great deal of attention has been devoted to the development of mucoadhesive drug delivery systems. Mucoadhesives may localize in a particular region and prolong the residence time, thereby improve the bioavailability of drugs [8]. Nowadays, *in situ*-gelling liquids have also proved as more convenient dosage forms for local applications because they are easy to administer into desired body cavities [9]. Poloxamers (Plx) which is chosen to prepare *in-situ* gel formulation, are synthetic triblock copolymers of poly(ethyleneoxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO) that exhibit thermoreversible behaviour in aqueous solutions [10-12]. A change in micellar properties occurs as a function of both environmental temperature and the concentration of Plx and a reversible gelation can occur at physiological temperature [12,13]. The use of such systems for local administration of therapeutic agents to the vagina offers several advantages, including ease of application and high spreadability at temperatures below the sol-gel temperature, rheological structuring and hence enhanced retention at body temperature. They have excellent compatibility and good characteristics of prolonged release of

*Corresponding author.

the active ingredient. On the other hand, they have low mucoadhesive properties.

Hydroxypropylmethylcellulose (HPMC), a well-known cellulose derivative, is generally used to provide sustained release. HPMC is frequently used for mucoadhesive formulations due to its nontoxic, nonirritant, high mucoadhesive characteristics, easy incorporation with the drugs and stability at vaginal pH [14,15].

It is available in a wide range of molecular weights and is classified by the viscosities of their 2% (w/w) aqueous solution [16]. In this study, two types of HPMC were added to improve the mucoadhesive and mechanical properties of *in-situ* gel formulations.

The objective of this study was to prepare a suitable mucoadhesive *in-situ* gel formulation of itraconazole with Plx and HPMC that possess appropriate mechanical and rheological properties, retain on the vaginal mucosa for a long period of a time.

2. Experimental

2.1. Materials

Plx 188 and 407 were kindly gifted by BASF Chemical Company (GERMANY). Itraconazole was selected as a model drug and was obtained from Nobel Pharmaceutical Company (TURKEY). HPMC E50 (40 - 60 cps) and K100M (80 - 120 cps) were donated by Colorcon (ENGLAND). All other materials were of analytical grade.

2.2. Preparation of Formulations

Vaginal mucoadhesive gel formulations of itraconazole was prepared with 20% Plx 407:10% Plx 188 mixture; and adding either 0.5% HPMC E50 or 0.5% HPMC K100M as mucoadhesive agent. Plx mixture ratio was decided according to our previous study [6]. Gels were prepared by a modification of the cold method [17]. Distilled water was cooled to 4˚C. Plx 188 and 407 were then slowly added to the distilled water with continuous agitation. The gels were left at 4˚C until a clear solution was obtained. Then, 0.5% HPMC K100M or HPMC E50 were gradually added and these gels were left at room temperature for 24 hours. Finally, 2% itraconazole was added with vigorous stirring. The compositions of gels are given in **Table 1**.

2.3. Determination of pH

To investigate the compatibility of the gel bases for vaginal application, their pH values were measured by a pH meter (NEL Mod.821) at room temperature (n = 5).

2.4. Measurement of Gelation Temperature and Gelation Time

Determination of gelation temperature and gelation time were carried out on Haake Mars rheometer and were determined graphically. The geometry was a stainless steal plate/plate (diameter 40 mm), which provided a homogeneous shear of the sample. The sol-gel transition temperatures and gelation times of the gels were determined from oscillation measurements with a fixed frequency of 0.01 Hz. The samples were heated at a rate of 2˚C every 60 s, the temperature changed between 7˚C - 70˚C during the procedure (n = 5). The sol-gel transition temperature graph was determined by plotting temperature as a function of the viscosity (η') and the transition point was defined as the point where the viscosity was halfway between the values for the solution and the gel [6].

2.5. Mechanical Properties of Polymer Solutions

Textural analysis was performed using Software-controlled penetrometer [TA-TX Plus, Stable Micro System, UK] equipped with 5 kg load cell in Texture Profile Analysis (TPA) mode. Formulations were transferred into jacketed glass vial (20 mL) at 20˚C and 37˚C. In this, an analytical probe was twice compressed into each formulation to a defined depth (15 mm) and at a defined rate (2 mm/s), allowing a delay period (15 s) between the end of the first and beginning of the second compression. Mechanical parameters (hardness, compressibility, adhesiveness, cohesiveness and elasticity) were derived and calculated from the resultant force-time curve [18]. Experiments were carried out at least three times. From the resultant force-time plots, several mechanical parameters may be derived [19]. These include:

- hardness (the force required to attain a given deformation)

Table 1. The composition of formulations.

Codes of formulation	Plx 407 (%)	Plx 188 (%)	HPMC K100M (%)	HPMC E50 (%)	Itraconazole (%)	Distilled water (%)
F1	20	10	0.5	-	-	69.5
F2	20	10	-	0.5	-	69.5
F3	20	10	0.5	-	2	67.5
F4	20	10	-	0.5	2	67.5

- compressibility (the work required to deform the sample during the first compression of the probe)
- adhesiveness (the work required to overcome the attractive forces between the surface of the sample and the surface of the probe)
- cohesiveness (the ratio of the area under the force-time curve produced on the second compression cycle to that on the first compression cycle, where successive compressions are separated by a defined recovery period)
- elasticity (the rate at which the deformed sample returns to its undeformed condition after the removal of the deforming force)

2.6. Evaluation of the Mucoadhesive Properties

The mucoadhesive strength of the formulations was evaluated by measuring the force required to detach the formulation from a mucin disc using a 5 kg load cell TPA in tension mode [18,20]. Mucin discs (250 mg) were hydrated with 50 µL mucin solution before the experiment and they were attached to the lower end of the probe (P 10 Perspex, θ: 10 mm). The gels were packed into the beaker. The probe holding the mucin disc was lowered on to the surface of the gel with a constant speed of 0.1 $mm \cdot s^{-1}$ and a contact force of 0.05 N were applied. After keeping in contact surfaces for 120 s, the probe was then moved vertically upward at a constant speed of 0.1 $mm \cdot s^{-1}$. Maximum detachment force (F) was obtained from the force-distance graph. The area under the curve (AUC) was calculated from force-distance plot as the mucoadhesion (M). The tests were conducted at 37˚C and each experiment was carried out five times.

2.7. Syringeability of the Formulations

The syringeability of the formulations was examined using a software controlled penetrometer in compression mode. A filled 2 mL syringe was held in place with a clamp and the upper probe of the texture analyzer moved downwards until it came in contact with the syringe barrel base. A constant force of 0.5 N was applied to the base and the work required to expel the contents for a barrel length of 30 mm was measured. The area under the resulting curve was used to determine the work of expulsion ([22]). The tests were conducted at room temperature and each experiment was carried out five times. The syringeability device is described in **Figure 1**.

2.8. Rhelogical Studies

All the formulations were characterized rheologically using Haake Mars rheometer. Continuous shear analysis of each formulation was performed at 20˚C ± 0.1˚C and 37˚C ± 0.1˚C, in flow mode, and in conjunction with parallel steel plate geometry (diameter 40 mm) and gap

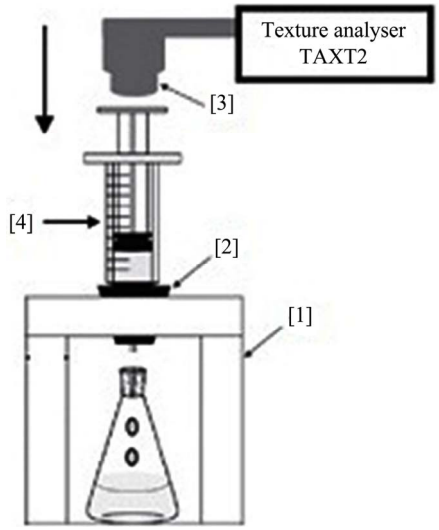

Figure 1. Experimental set-up for the measurement of the syringeability force developed during injection: 1) metallic support; 2) plastic clamping ring; 3) force transducer and 4) syringe (adapted from reference [22]).

of 0.3 mm. Samples were carefully applied to the lower plate of enstrument, ensuring that formulation shearing was minimized and allowed to equilibrate for at least 1 min prior to analysis. Upward and downward flow curves were measured over a range of shear rates (10 - 1000 s^{-1}). The flow properties of at least five replicate samples were determined [23,24].

Oscillatory analysis of each formulation under examination was performed after determination of its linear viscoelastic region at 20˚C ± 0.1˚C and 37˚C ± 0.1˚C, where stress was directly proportional to strain and the storage modulus remained constant. Frequency sweep analysis was performed over the frequency range of 0.1 - 10 Hz following application of a constant stress and standard gap size was 0.3 mm for each sample. Storage modulus (G′) and loss modulus (G″), the dynamic viscosity (η'), and the loss tangent (tanδ) were determined. In each case, the dynamic rheological properties of at least five replicates were examined [25,26].

2.9. Statistical Data Analysis

Statistical data analysis was performed using the Student *t*-test with $P < 0.05$ as the minimal level of significance.

3. Results

The gelation temperatures of the F1, F2, F3 and F4 formulations were found to be 34.47˚C ± 0.03˚C, 34.47˚C ± 0.02˚C, 34.47˚C ± 0.02˚C and 34.46˚C ± 0.03˚C, respectively. Gelation time is also an important parameter for determining vaginal retention of formulation [21]. For our formulations these value were between 326.63 ± 0.35 sec and 326.98 ± 0.17 sec. The pH values of the F1, F2,

F3 and F4 formulations were found to be 6.52 ± 0.06, 7.25 ± 0.07, 6.95 ± 0.04 and 7.01 ± 0.04, respectively. The TPA graphs of formulations at 37˚C are presented in **Figure 2** and the mechanical properties of formulations are presented in **Table 2**.

Mucoadhesive formulations have been reported to prolong the residence time of the formulation at the site of application [6]. Mucoadhesive studies were carried out only at body temperature because the formulations were in liquid form at room temperature. In this study the work of adhesion was used to quantify adhesion. This measure provides a more comprehensive evaluation of the detachment phenomenon [27]. The related data of the detachment force, mucoadhesion and work of adhesion of the formulations are listed in **Table 3**. The syringeability of each formulation is also presented in **Table 3**.

Representative flow curves of *in-situ* gel formulations were graphically presented in **Figure 3**.

Oscillatory analysis was also carried out at both room and body temperature. Thereby, the changes of the struc-

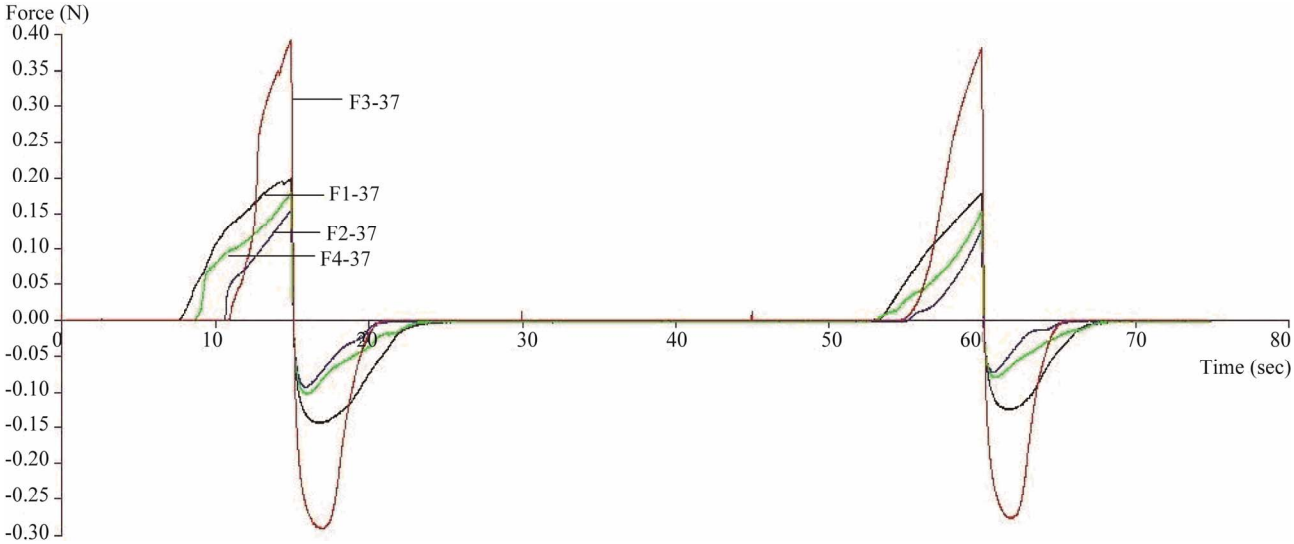

Figure 2. TPA analysis graphs of F1, F2, F3 and F4 at 37˚C.

Table 2. Mechanical properties of formulations.

Codes	H (N) ± SD	C (N.mm) ± SD	A (N.mm) ± SD	E ± SD	Ch ± SD
F1-20˚C	0.016 ± 0.003	0.103 ± 0.037	0.036 ± 0.006	0.931 ± 0.047	0.915 ± 0.009
F1-37˚C	0.196 ± 0.011	0.779 ± 0.030	0.662 ± 0.026	1.102 ± 0.137	0.725 ± 0.041
F2-20˚C	0.017 ± 0.003	0.125 ± 0.058	0.039 ± 0.006	0.954 ± 0.066	0.889 ± 0.012
F2-37˚C	0.156 ± 0.009	0.426 ± 0.048	0.38 ± 0.029	1.097 ± 0.025	0.572 ± 0.007
F3-20˚C	0.009 ± 0.002	0.021 ± 0.001	0.035 ± 0.000	0.944 ± 0.036	0.864 ± 0.069
F3-37˚C	0.394 ± 0.053	0.900 ± 0.026	0.961 ± 0.018	1.713 ± 0.447	1.050 ± 0.095
F4-20˚C	0.007 ± 0.000	0.011 ± 0.0001	0.028 ± 0.004	0.823 ± 0.050	0.751 ± 0.057
F4-37˚C	0.131 ± 0.003	0.762 ± 0.013	0.579 ± 0.021	0.993 ± 0.107	0.714 ± 0.152

*H: Hardness, C: Compressibility, A: Adhesiveness, E: Elasticity, Ch: Cohesiveness.

Table 3. Results of mucoadhesion studies of the formulations with mucin disc and syringeability studies.

Codes	F (N) ± SD	M (mJ) ± SD	W (mJ/cm^2) ± SD	Syringeability (N.sn) ± SD
F1	0.151 ± 0.035	0.077 ± 0.056	0.094 ± 0.068	11.130 ± 1.986
F2	0.215 ± 0.052	0.082 ± 0.040	0.100 ± 0.048	13.663 ± 2.893
F3	0.230 ± 0.067	0.064 ± 0.037	0.078 ± 0.045	16.425 ± 2.065
F4	0.163 ± 0.046	0.042 ± 0.011	0.051 ± 0.013	16.900 ± 2.788

*F: Detachment force, M: Mucoadhesion, W: Work of adhesion.

cture of the formulation were investigated in both temperatures. **Figure 4** shows the rheological properties of the formulations.

In **Table 4** and **Figure 5**, effect of temperature on the loss tangent and dynamic viscosity of formulation at certain frequencies were presented.

4. Discussion

Poloxamer molecules in solution exhibit a zigzag configuration, initially transforming into a close-packed configuration and then to a viscous gel due to the increasing temperature [28]. Sol-gel transition temperature is the temperature at which the liquid phase makes transition into a gel. The transformation from the solution to the form after the application is important for efficient therapy due to the covering mucosal tissue and the decreasing the vaginal leakage. Ideally the gelation temperature of mucosal formulations should be 30˚C - 36˚C [11,17,29]. If the gelation temperature is high, the formulation exhibits liquid properties at physiological temperatures and leakage results. Conversely, lower gelation temperatures may result in problems concerning application due to the viscous nature of the formulation. It is known that the sol-gel transition temperature can be changed by addition of the active substance or additivies [30]. But for our formulations, addition of active substance didn't affect gelation temperatures. Gelation temperatures of our formulations were found suitable for the vaginal

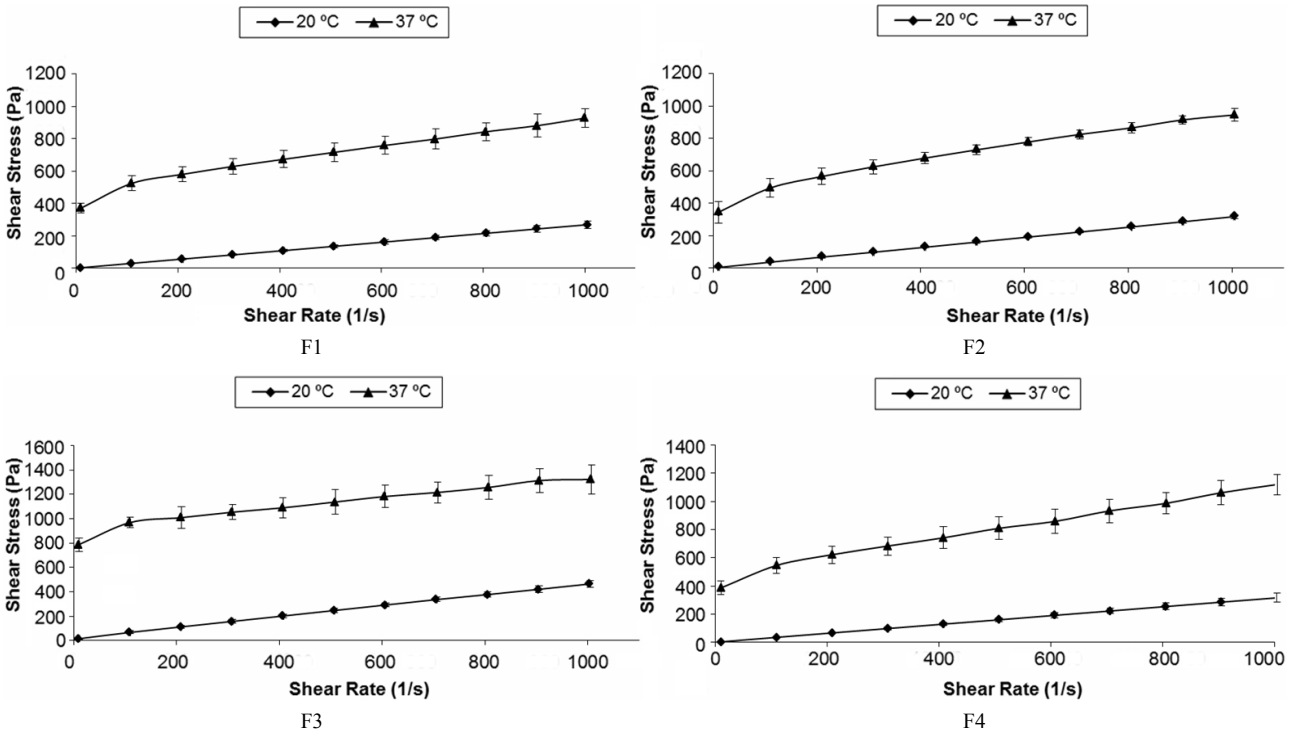

Figure 3. Flow curves of itraconazole formulations at 20˚C and 37˚C.

Table 4. Effect of temperature on the dynamic viscosity (η') of formulations at five representative frequencies.

Codes of Formulations	Temperature (˚C)	η^* (Pa.s) values at different ossilasion frequency				
		0.60 Hz	2 Hz	5 Hz	7 Hz	10 Hz
F1	20	14.481 ± 0.880	3.465 ± 0.764	1.482 ± 0.659	1.338 ± 0.538	1.152 ± 0.494
	37	679.625 ± 0.500	213.800 ± 0.431	92.988 ± 0.324	67.330 ± 0.510	50.143 ± 0.402
F2	20	10.433 ± 0.235	2.886 ± 0.12	1.288 ± 0.096	0.925 ± 0.023	0.601 ± 0.052
	37	471.300 ± 0.654	203.267 ± 0.235	101.093 ± 0.365	77.237 ± 0.652	58.403 ± 0.265
F3	20	30.347 ± 0.485	5.158 ± 0.251	4.335 ± 0.159	1.674 ± 0.571	2.049 ± 0.485
	37	804.850 ± 0.396	126.743 ± 0.254	44.638 ± 0.158	17.790 ± 0.654	14.264 ± 0.478
F4	20	10.415 ± 0.096	3.907 ± 0.098	2.010 ± 0.065	1.009 ± 0.030	0.917 ± 0.002
	37	1005.600 ± 0.216	299.300 ± 0.365	117.650 ± 0.652	79.205 ± 0.521	47.640 ± 0.321

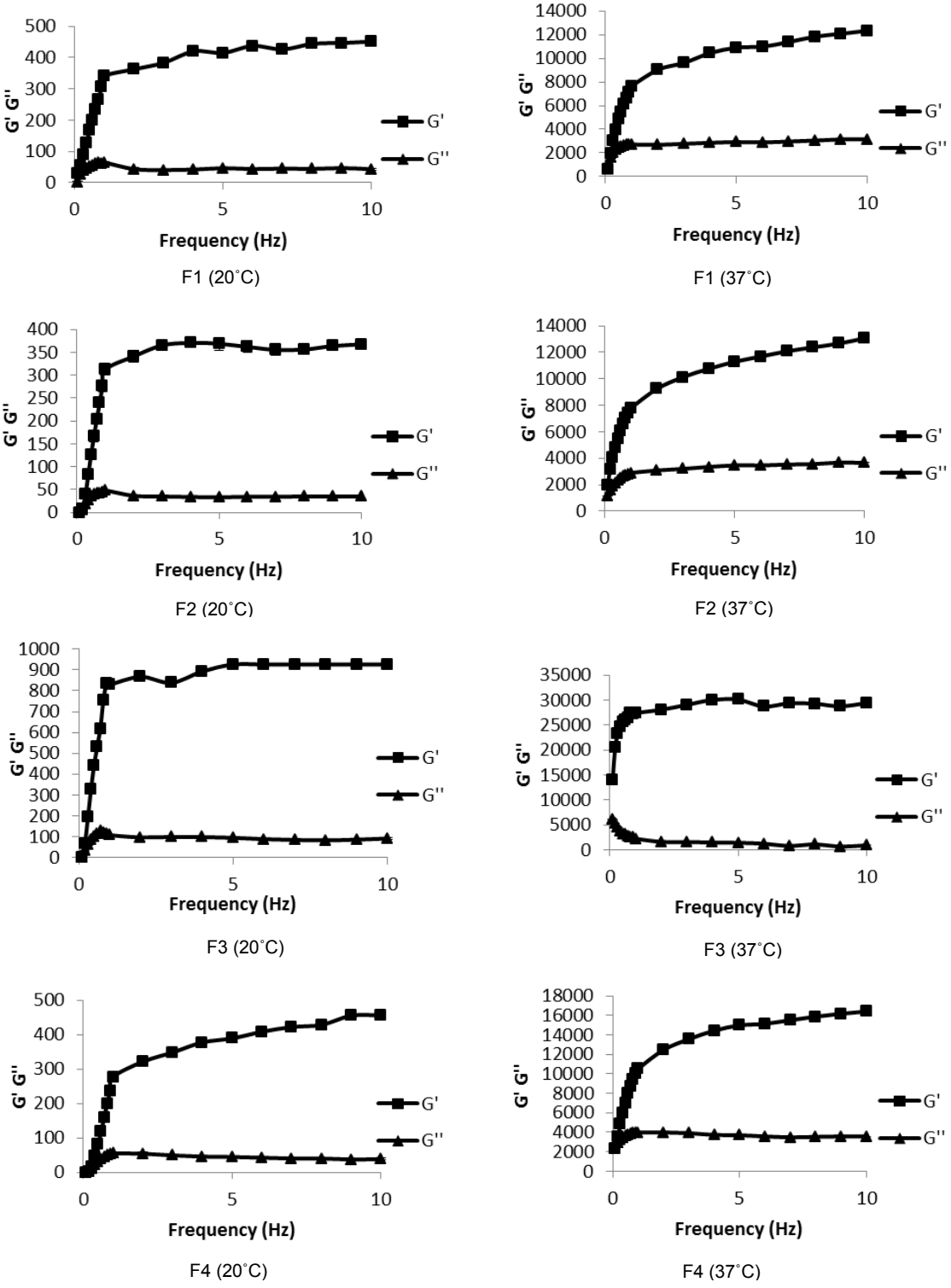

Figure 4. Frequency-dependent changes of viscoelastic properties of the formulations.

application. Our formulations behaved as viscous liquid at room temperature and transformed to gel at body temperature. So, their application will be easy with a catheter and increased viscosity could be a solution of leakage.

Most of the studies which used Plx as a polymer have focused only on rheological properties, and sustained release action of thermosensitive hydrogels. There is a lack of knowledge on practical administration of formulation to the body such as syringeability and gelation time. But, these two factors are crucial in the develop-

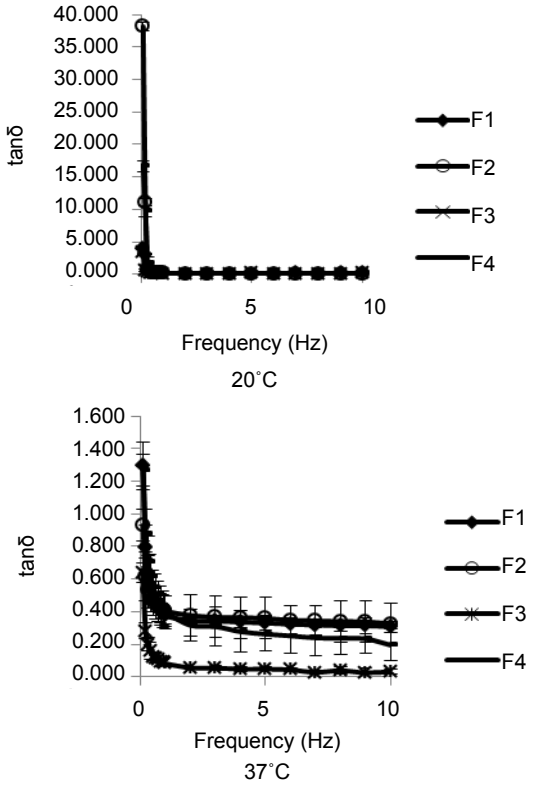

Figure 5. Frequency-dependent changes of loss tangent (tanδ) of the formulations.

ment of a desirable thermosensitive hydrogel that is easy to administer to the body and gels rapidly, enabling practical use in pharmaceutical preparations [31]. Because of this reason, we examined these two parameters of formulation with other properties. The determination of the gelation time of a gel formulation requires a knowledge of the viscosity of the solution as a function of time. The short gelation time was advantageous to prevent the drainage from the site of application leading to a prolonged retention of the active substance on the mucosal tissue [32]. In our previous studies, we know that the formulations include Plx alone has short gelation times [7]. Adding HPMC to the formulations caused increased gelation time of the formulations. However, gelation time values were still suitable for vaginal applications.

The pH values of the prepared formulations were found in physiological limitations and they were deemed to be suitable for vaginal administration.

TPA is a mechanical test that describes the resistance of pharmaceutical formulations to compressive stresses and subsequent relaxation. The parameters derived from this technique (hardness, compressibility, adhesiveness, elasticty and cohesiveness) have been proven to be relevant to the performance of local formulations, e.g. ease of removal from the container, ease of application to the surface and retention of the product at the site of application. For this reason, TPA is frequently used to identify formulations that may be suitable for clinical application [22]. Hardness and compressibility describe the stress/work required to remove the sample from the container and to subsequently apply this to the site of application. These characteristics quantify sample deformation under compression and should be low to allow the gel to be easily removed from the container and spread onto the mucosal epithelia. The hardness and compressibility values of the gels increased significantly due to the increases in polymer concentration. Adhesiveness, a property related to mucoadhesion, is defined as the work required to detach probe from the sample in which its cohesive bonds were broken and describes the relative properties of each candidate formulation. Product elasticity represents the rate at which the deformed sample returns to its undeformed condition. Lower numerical values as determined by TPA in the elasticity mode indicate greater product elasticity [33]. TPA also provides information on the effects of repeated shearing stresses on the structural properties of formulations, a property termed its "cohesiveness" [34-36]. As it can be seen from the **Table 2**, compressibility, adhesiveness and cohesiveness values of the gels not significantly increased with addition of active substance. The gel structure of F3, containing 0.5% HPMC K100M and itraconazole 2%, exhibited the greatest compressibility, adhesiveness and cohesiveness. Based on these properties, F3 appeared to offer more suitable performance than other formulations.

The contact time of a formulation on the mucosa is of high importance for vaginal drug delivery. Mucoadhesive formulations have been reported to prolong the residence time of the formulation at the site of application. Quantification of mucoadhesion is important to ensure that the adhesion offered by formulations is sufficient to ensure prolonged retention at the site of application [37]. Importantly, the formulations under examination displayed significant mucoadhesion, similar to other systems that have been used for implantation into body cavities. It is known that HPMC exhibits a mucoadhesive property. Although Plx is not as mucoadhesive as HPMC, its sol-gel transition ability increases the viscosity of the solution at physiological temperature. Hence, combinations of HPMC and Plx showed higher mucoadhesiveness at 37˚C. Using two different viscosity type of HPMC did not significantly effect the mucoadhesive properties of the formulations. Also, our experimental datas indicated that F3 formulation has higher mucoadhesive properties than other formulations. Result of mucoadhesive studies showed similarity with TPA analysis.

Syringeability of the *in-situ* gel formulations presented the effect that content of the formulation have on the force required to expel the product. Although, our formulations viscous liquid at 20˚C, syringeability is still

important parameter to show easy application of our formulations. According to the result our study, addition of active substance did not significantly affect syringeability of formulations. On the other hand, using different HPMC polymer types in formulation did not significantly change syringeability values of formulations.

The evaluation of the rheological properties for the gel type dosage forms would be important for predicting their behavior *in vivo*. The shear stress changes upon shear rates have been used to determine whether the rheological behavior of the formulation is Newtonian or non-Newtonian. Non-newtonian flow is typical for poloxamer formulations at higher temperatures than sol-gel transition temperature [18]. In continuous shear rheometry, all formulations exhibited pseudo-plastic flow at 37˚C as it was expected due to its thermoresponsive property. Our results showed similarity with the literature [6,21]. Among the all formulations, high shear stress values were obtained with F3 formulation.

The rheological properties of *in-situ* gel formulations affect both the ease of application and retention within the vagina. Following local application to the vagina, it is accepted that the equilibrium rheological properties of the formulations will dominate the subsequent physicochemical properties. In polymer solutions, at a sufficiently high concentration, there are entanglements among the polymer chains but there is sufficient time for polymer chains to distangle and flow during a single oscillation at low frequencies ($G'' > G'$). Conversely, as the elastic properties of the sample increase, interchain entanglements do not have sufficient time to come apart within the period of single oscillation and G' becomes higher than G'' [34, 38]. A gel should exhibit a solid-like mechanical spectrum, that is, $G' > G''$ throughout the experimentally accessible frequency range, and there should be little frequency dependence of the moduli [39].

In oscillatory rheometry the effects of oscillatory stresses on the viscoelastic properties are measured, from which two dynamic moduli, namely, the storage modulus, G', a measure of the elasticity, and the loss modulus, G'', representing viscous components at a given frequency of oscillation, are obtained [20,21]. Frequency-independent behaviour presents a gel like material whereas the frequency dependence shows the viscous fluid. According to the results, F3 formulations were found nearly frequency independent after certain frequency values and this formulation exhibited typical gel-type mechanical spectra ($G' > G''$) at 37˚C. It was also investigated that presence of itraconazole and HPMC K100M provided higher elasticity value for F3 formulation comparing to other formulations. Greater elasticity of this formulation would be expected to enhance retention at the site of application.

The value of phase angle ($\tan\delta = G''/G'$), which is a measure of the relative contribution of viscous components to the mechanical properties of the materials, was <1 for all of the formulations at 37˚C (solid gel response) but was >1 for all of the formulations at 20˚C (liquid-like response). Thus, as $\tan\delta$ becomes smaller, the elasticity of the formulation increases, while the viscous behavior is reduced. As it was expected, $\tan\delta$ values were found higher for all the formulations at 20˚C than 37˚C [20]. F4 formulation showed more elastic property than other formulations and this result is accordance with TPA analysis.

Dynamic viscosity (η') is described as the flow resistance of the sample in the structure state, originating as viscous or elastic flow resistance to oscillating movement. The higher value of dynamic viscosity means the greater the resistance to flow in the structured state [20]. In our study, the highest η' was obtained with F4 formulation due to its more consistent gel structure. The observed large dynamic viscosities of gels at low oscillatory frequencies are characteristic of viscoelastic systems.

5. Conclusion

This study has described the *in-situ* gel formulations of itraconazole and evaluated their textural and rheological properties. Plx has low mucoadhesive properties but its termal sensitivity lead to easy application and covering over the mucosa. Adding HPMC to the formulation decreased the sol-gel transition temperature, and affected the mucoadhesive, mechanical and rheological properties of the formulation. The results showed that the texture characterization was in agreement with rheological results confirming the improved mechanical properties of Plx-HPMC formulations. As a result, the evaluation of the entire candidate formulations indicated that vaginal formulation of itraconazole will be a new alternative for the treatment of vaginal candidiasis with suitable textural and rheological properties. Our results showed that the developed formulations were found worthy of further studies.

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