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Research Paper

In-situ gel formulations of econazole nitrate: preparation and in-vitro and in-vivo evaluation

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Abstract

Objectives This study describes the in-situ gelling of econazole nitrate containing thermosensitive polymers composed of poloxamer 407 and 188 as a novel treatment platform for vaginal candidiasis.

Methods Aqueous thermosensitive formulations containing 1% of econazole nitrate and poloxamer 407 and/or 188 were prepared and their rheological, mechanical and drug-release properties determined at 20 ± 0.1°C and/or 37 ± 0.1°C. Based on their biologically suitable thermorheological properties, formulations containing the mixtures of poloxamer 407 and 188 in ratios of 15:15 (F1), 15:20 (F2) and 20:10 (F3) were chosen for comprehensive analysis.

Key findings Formulations based on F3 exhibited typical gel-type mechanical spectra ($G' > G''$) at 37°C whereas formulations based on F1 and F2 exhibited properties akin to weakly cross-linked gels. Texture profile analysis demonstrated that F3 showed the highest cohesiveness, adhesiveness, hardness and compressibility. No statistically significant differences ($P > 0.5$) were observed in the release of econazole nitrate from the formulations at pH 4.5, which in all cases followed anomalous diffusion kinetics. Formulations based on 20% poloxamer 407:10% poloxamer 188 were chosen for in-vivo studies and were shown to be effective for the treatment of the vaginal candidiasis. Histopathologic evaluation also supported the effectiveness of the thermosensitive formulation administered intravaginally.

Conclusion By careful engineering of the rheological properties, in-situ thermosensitive gel formulations of econazole nitrate were prepared and were shown to be efficacious in the treatment of vaginal candidiasis.

Keywords Econazole nitrate; in-situ gel; poloxamer; rheology; texture profile analysis

Introduction

Vulvovaginal candidiasis is the most frequent gynaecologic condition encountered by physicians and approximately 75% of women experience at least one episode during their lifetime. *Candida albicans* is recognised as the most frequent etiologic agent of vulvovaginal candidiasis.[1–3] For many years, imidazole derivatives have been used for the treatment of fungal infections caused by *Candida* species.[4,5] Treatment of acute vulvovaginal candidiasis requires at least 5–7 days but a shorter regimen is more preferable and achieves better compliance. The longer regimen is required for and is effective for the treatment of recurrent vaginal infections. Econazole nitrate (EN) is used mainly for vaginal infections and other diseases caused by fungi. It is usually administered topically as a pessary of 150 mg for three consecutive days or as a 1% EN cream in a regimen of at least 15 days.[6,7] Traditionally available vaginal dosage forms include solutions, emulsions, suspensions, vaginal tablets, suppositories, creams, ointments and gels. Among these formulations, gels have significant advantages, including safety, versatility, higher bioavailability and cheapness. Although there are some aesthetic disadvantages of gels, they are better tolerated by vaginal candidiasis patients than inserts or ointments. Furthermore, the ease of application and spreadability of gels are greater than those of other semi-solid systems, e.g. ointments.[8]

In-situ gelling drug delivery systems release drugs in response to various environmental conditions as a result of stimulus-dependent changes in the rheological properties of the

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polymer platform.[9–12] Such systems may therefore be formulated to provide sufficient coverage of the vagina and longer retention of the formulation on the mucosal tissue. Poloxamers (Plx) are triblock, synthetic copolymers of poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (PEO–PPO–PEO) that exhibit thermoreversible behaviour in aqueous solutions.[9,13] A change in micellar properties occurs as a function of both environmental temperature and the concentration of poloxamer and a reversible gelation can occur at physiological temperature.[13,14] The use of such systems for topical administration of therapeutic agents to the vagina offers several advantages, including ease of application and high spreadability at temperatures below the sol–gel temperature, and rheological structuring and hence enhanced retention at body temperature.

In this context, the aims of this study were to develop an in-situ vaginal gel platform containing EN for the effective treatment of vulvovaginal candidiasis with a single-dose application. To achieve this goal, the mechanical and rheological properties of the prepared formulations were examined and the optimised formulation was examined within a clinical setting.

Materials and Methods

Materials

Econazole nitrate was purchased from Sigma-Aldrich Chemie (Germany). Poloxamer 407 (Plx 407) and Poloxamer 188 (Plx 188) were donated by BASF Chemical Company (Germany). All the other chemicals were of analytical grade.

Preparation of the thermosensitive gel formulations

The vaginal thermosensitive gel formulations of EN with poloxamers were prepared using the cold method. Distilled water was cooled to 4°C. Poloxamer was then slowly added to the distilled water with continuous agitation. The gels were left at 4°C until a clear solution was obtained.[9,15] Each formulation contained 1% w/w EN.

Measurement of the sol–gel transition temperatures

The sol–gel transition temperatures of test formulations were determined rheologically ($n = 5$) using an AR 2000 controlled stress/controlled rate rheometer (T.A. Instruments, Surrey, England). The geometry was a stainless steel plate/plate (diameter 40 mm), which provided a homogeneous shear of the sample. The sol–gel transition temperatures of the formulations 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 min, the temperature changing between 7 and 80°C during the procedure. 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.[9,16]

Rheological studies

The rheological analysis of the formulations was performed both at 20 ± 0.1°C and 37 ± 0.1°C using an AR 2000 controlled stress/controlled rate rheometer in flow mode and in conjunction with parallel steel plate geometry (40 mm diameter). In continuous shear analysis, the upward and downward flow curves for each formulation were measured over shear rates ranging from 10 to 900 s^{-1}.[17,18]

Oscillatory analysis of each formulation was performed after determination of its linear viscoelastic region at 20 ± 0.1°C and 37 ± 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 0.1–10.0 Hz following application of a constant stress and the standard gap size was 0.1 mm for each sample. Storage modulus (G') and loss modulus (G''), the dynamic viscosity (η'), and the loss tangent ($\tan \delta$) were determined as previously described by the authors.[19,20] In each case, the dynamic rheological properties of at least five replicates were examined.

Mechanical (texture profile) analysis of poloxamer formulations

Textural analysis was performed using a TA-XT Plus Texture Analyser (Stable Micro Systems UK) equipped with 5 kg load cell in texture profile analysis (TPA) mode.[17,18] Formulations were initially transferred into a jacketed glass vial (20 ml) at 37°C. An analytical probe was twice inserted 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 the beginning of the second compression. From the resultant force–time curve the mechanical parameters (hardness, compressibility, adhesiveness and cohesiveness) were derived.[21] TPA studies were only performed on formulations having suitable sol–gel temperatures. Experiments were carried out at least five times.

Mucoadhesion studies

The mucoadhesive properties of the various formulations were evaluated by means of a tensile test, where the measurement of maximum force, mucoadhesion and work of adhesion required to detach the formulations from a section of mucosal tissue was assessed. Mucosal tissue was obtained from newly sacrificed abattoir animals and was separated from underlying tissues, washed, cut into smaller pieces and rinsed carefully. The samples were frozen at 20°C until required. The mucoadhesive properties of formulations were evaluated using the TA-XT *Plus* texture analyser equipped with a 5 kg load cell. Sections (>2 mm in thickness) that had been taken from the inner part of the surface of the mucosal membrane were attached to the lower end of the texture analyser probe (10 mm diameter) using cyanoacrylate glue. The gels were packed into a holder and maintained at 37°C. The probe holding the mucosa was lowered onto surface of the gel with a constant speed of 0.1 mm/s until in contact with the gel surface and a contact force of 0.05 N was then applied for 2 min. The probe was then moved vertically upwards at a constant speed of 0.1 mm/s and the maximum detachment force (F) and the area under the curve (AUC, termed the mucoadhesion) were determined from the resultant force–distance graph. The work of mucoadhesion (Work, mJ cm^{-2}) was calculated from the AUC using the following equation:

$$work = \frac{AUC}{\pi r^2}$$

where, πr^2 = the mucosal surface in contact with gel. All analyses were repeated at least five times.

Release studies

In-vitro release studies were carried out using Spectra/Por regenerated cellulose dialysis membrane tubes. The membranes onto which the gel samples were placed were soaked in distilled water for 24 h before use. The chosen receptor medium was composed of phosphate buffer (pH 4.5, 65% v/v) and dioxane (35% v/v), and was continuously stirred during the release experiments.[22] At defined time intervals, samples were withdrawn and the EN content of each sample was analysed using UV spectrophotometry (λ_{max} = 278 nm). All the experiments were repeated five times and the data were expressed as the mean ± SD. The mass of EN released from the formulations was calculated using a calibration curve. There was no analytical interference from the polymers.

To investigate the mechanism of drug release, the data generated from the study were fitted to the power law equation (below) using logarithmic transformations and least squares regression analysis:[23]

$$\frac{M_t}{M_\infty} = kt^n$$

where M_t/M_∞ is the fraction of drug released at time t, k is a constant, incorporating structural and geometric characteristics of the delivery system, and n is the release exponent.

In-vivo studies

Experimental rat vaginitis

Animal models require the animal to be in a state of pseudoestrus at the time of intravaginal inoculation to obtain a persistent infection that can be studied for therapeutic purposes.[24] In-vivo experiments were performed using female Wistar albino rats, body weight 150–200 g, and the study was performed following institutional approval from the Ethics Committee of Ege University, Faculty of Pharmacy for Animal Research.

All rats were maintained in pseudoestrus with a subcutaneous injection of estradiol valerate (25 mg/kg) 48 h prior to vaginal inoculation and weekly thereafter. Prior to the initiation of the experiments, vaginal cultures of each rat were performed and no *Candida* sp. were found. The animals were inoculated intravaginally with 10^7 blastoconidia/ml in 20 µl of sterile saline solution. Administration was via a tuberculin syringe without a needle. Vaginal fluid was taken from each animal every 2 days with a sterile swab. Each swab was streaked over a Sabouraud dextrose agar (SDA) plate and the plates were incubated at 35°C for 72 h. One vaginal sample per rat was evaluated and the viability of the inoculum was confirmed by counting the number of colony units using a serial dilution method. The infected animals were separated randomly into three groups as follows (12 animals per group): group 1 (G1: control group: no treatment), group 2 (G2: administered only formulation base without EN to observe the effect of the gel base of formulation F3) and group 3 (G3: administered formulation F3 designed to treat vaginal candidiasis). A single thermosensitive formulation F3, containing or devoid of EN, was applied vaginally using an injector without a needle.

The evaluation of vaginal burden was performed on samples collected by rolling a sterile cotton swab over the vaginal cavity. The swab was then streaked over Sabouraud dextrose agar (SDA) plates and incubated at 35°C for 3 days prior to analysis. Vaginal swabs were taken on days 2, 5, 7 and 21 after intravaginal administration of the formulations.

Twenty-one days after the administration of the F3 formulation, the animals were euthanised with an excess dose of pentobarbital and the vaginal tissues were removed. The samples were fixed in 10% neutral buffered formalin and standard procedures were applied to prepare paraffin blocks and 4 µm-thick tissue sections. The specimens were stained with hematoxylin-eosin. Histopathological evaluation of the sections from the vagina was carried out to investigate for inflammation (judged by presence of neutrophil accumulation). Inflammation was also graded subjectively as low, moderate or extensive by assessing the inflammatory infiltrate, fibrosis and lamina propria infiltration by inflammatory cells.

Statistical analysis

The effects of formulation variables on the various rheological properties were statistically assessed using one-way ANOVA. Individual differences between the various treatments were assessed using Tukey's post-hoc test. In all cases $P < 0.05$ denoted significance.

Results and Discussion

The composition, gelation temperatures and pH values of the formulations are shown in Table 1. The pH values of the formulations ranged from 4.08 to 4.89 and were deemed to be suitable for vaginal administration. Individual differences in the gelation temperatures of the formulations were observed.

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.[25] The sol–gel transition temperature is the temperature at which the rheological properties of the system change from Newtonian/elastoviscous to viscoelastic. Ideally the gelation temperature for mucosal formulations should be 30–36°C.[9,15,26] 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. In this study effective control of the sol/gel transition temperature was achieved by combining Plx 407/ and Plx 188 as shown in Table 1. Formulations F1, F2 and F3 behaved as a mobile viscous liquid at room temperature and transformed into a semi-solid transparent gel at body temperature. The sol–gel transition temperature of the F3 formulation was the most suitable for the given application.

Table 1 The composition, gelation temperatures and pH values of formulations containing 1% EN

Formulation	PLX 407 (%)	PLX 188 (%)	Mean (±SD) gelation temperature (°C)	pH
F1	15	15	43.5°C ± 0.3	4.45 ± 0.04
F2	15	20	39.5°C ± 0.2	4.29 ± 0.04
F3	20	10	38.0°C ± 0.5	4.37 ± 0.02
F4	10	25	≥40	4.41 ± 0.03
F5	5	25	≥40	4.42 ± 0.03
F6	12	20	≥40	4.34 ± 0.02
F7	10	20	≥40	4.36 ± 0.01
F8	25	5	24 ± 0.4	4.23 ± 0.03
F9	20	–	25 ± 0.3	4.08 ± 0.05
F10	–	20	23 ± 0.2	4.89 ± 0.04

Rheological studies

The rheological properties of topical formulations affect both the ease of application/spreadability and retention within the vagina. In continuous shear rheometry, the various poloxamer formulations were Newtonian below the sol–gel temperature but exhibited pseudo-plastic flow at 37°C, as was expected due to their thermoresponsive properties. Of these formulations, F2 exhibited the greatest degree of pseudoplasticity. Representative rheograms are shown in Figure 1.

Following topical application to the vagina, it is accepted that the equilibrium rheological properties of the formulations will dominate the subsequent physicochemical properties. 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.[27] 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″.[27,28] 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.[29] Tables 2 and 3 represent the frequency dependence of G′ and G″ of EN containing formulations at 20 and 37°C. At 20°C and all frequencies, G″ exceeded G′ in all the formulations and accordingly these formulations may be described as elastoviscous systems. Raising the temperature caused an increase in G′ and G″. The magnitudes of both G′ and G″ are directly related to the gel strength.[29,30] F3 formulations exhibited typically strong gel structures with the highest magnitude of G′ at 37°C and a significant disparity in the magnitudes of G′ and G″. Above 2.58 Hz, G′ values of F3 were relatively stable. The frequency dependencies of F1 and F2 were more pronounced than F3 and were more typical of a weakly cross-linked gel.[30]

The value of the loss tangent, 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 but was >1 for all of the formulations at 20°C, as expected. The situation at 37°C was indicative of a solid gel response and the situation at 20°C reflected a liquid-like response.[16,29,30] According to the data in Table 4, formulation F3 displayed the lowest value of the loss tangent, providing further evidence of the greater elasticity of this system. This behaviour may be directly accredited to the increased concentration of Plx 407 due to the multimolecular aggregates formed, which results in an increased resistance to polymer deformation.[31] The dynamic viscosity η' describes the flow resistance of the sample, originating as viscous or elastic flow that resists oscillating movement. The observed dynamic viscosities of gels at low oscillatory frequencies are characteristic of viscoelastic systems (Table 5).[32]

Texture profile analysis of poloxamer formulations

Texture profile analysis 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, cohesiveness) have been shown to be relevant to the performance of topical 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.[18,21,33] For this reason texture profile analysis is frequently used to identify formulations that may be suitable for clinical application. Table 6 shows the textural properties of candidate gel formulations. As may be observed, the textural properties of the three formulations differed.

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. Gel hardness and compressibility 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. TPA also provides information on the effects of repeated shearing stresses on the structural properties of formulations, a property termed its ‘cohesiveness’. Each gel formulation

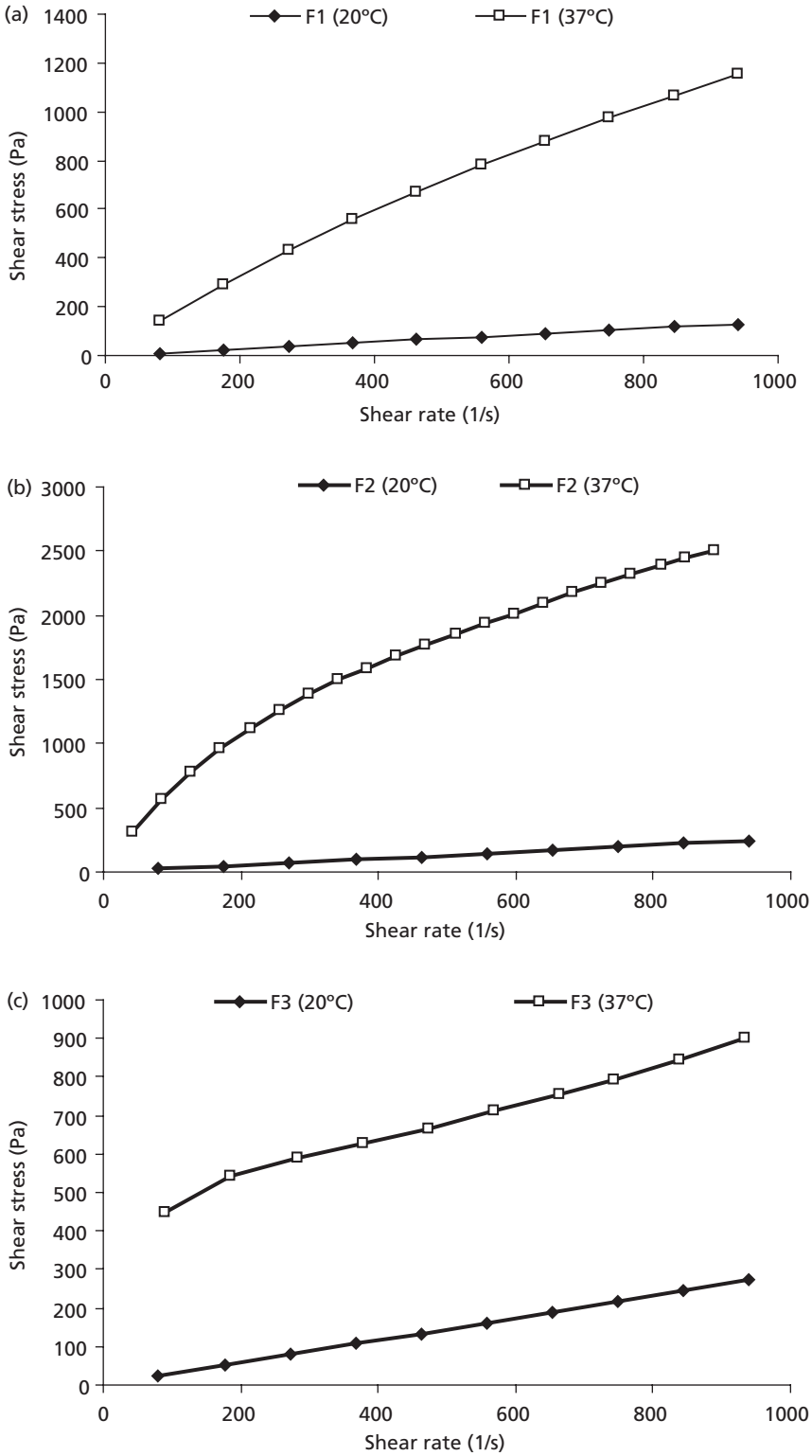

Figure 1 Flow curves of econazole nitrate formulations at 20 and 37°C.

Table 2 Effect of temperature on the storage modulus (G′) of formulations at five representative frequencies

Formulation	Temperature (°C)	G′ (Pa) at representative oscillatory frequencies*				
		0.60 Hz	2.58 Hz	5.05 Hz	7.53 Hz	10.00 Hz
F1	**20**	0.04 ± 0.00	0.26 ± 0.05	0.38 ± 0.04	0.488 ± 0.02	0.57 ± 0.02
	37	201.73 ± 0.93	1296 ± 10.3	2835 ± 12.50	4294 ± 13.31	5153 ± 0.35
F2	**20**	0.14 ± 0.07	0.68 ± 0.01	1.28 ± 0.03	1.64 ± 0.09	2.034 ± 0.07
	37	362.70 ± 0.03	972.8 ± 0.08	2727 ± 0.10	4804 ± 0.57	6816 ± 0.94
F3	**20**	0.20 ± 0.03	0.82 ± 0.05	1.59 ± 0.08	2.44 ± 0.10	3.343 ± 0.04
	37	17470 ± 1.99	23630 ± 2.03	25080 ± 3.44	25590 ± 1.38	25950 ± 2.08

*Each value represents the mean (±SD) of five replicates.

Table 3 Effect of temperature on the loss modulus (G″) of formulations at five representative frequencies

Formulation	Temperature (°C)	G″ (Pa) at representative oscillatory frequencies*				
		0.60 Hz	2.58 Hz	5.05 Hz	7.53 Hz	10.00 Hz
F1	**20**	0.55 ± 0.03	2.250 ± 0.05	4.47 ± 0.06	6.68 ± 0.07	9.160 ± 0.07
	37	32.64 ± 0.02	74.09 ± 0.03	120.5 ± 0.11	170.3 ± 0.21	206.9 ± 0.10
F2	**20**	1.25 ± 0.03	5.40 ± 0.04	10.37 ± 0.09	14.93 ± 0.07	19.7 ± 0.05
	37	132.4 ± 0.07	255 ± 0.062	488.6 ± 0.03	682.8 ± 0.04	728.7 ± 0.09
F3	**20**	1.58 ± 0.05	6.18 ± 0.05	12.01 ± 0.07	17.98 ± 0.04	24.05 ± 0.07
	37	6362 ± 3.04	3225 ± 2.06	1834 ± 3.07	1389 ± 2.93	1161 ± 3.07

*Each value represents the mean (±SD) of five replicates.

Table 4 Effect of temperature on the loss tangent (tan δ) of formulations at five representative frequencies

Formulation	Temperature (°C)	Tan δ (Pa) at representative oscillatory frequencies*				
		0.60 Hz	2.58 Hz	5.05 Hz	7.53 Hz	10.00 Hz
F1	**20**	15.66 ± 0.08	8.66 ± 0.15	11.88 ± 0.08	13.68 ± 0.09	16.10 ± 0.05
	37	1.57 ± 0.00	0.06 ± 0.02	0.04 ± 0.03	0.04 ± 0.07	0.04 ± 0.02
F2	**20**	9.14 ± 0.01	7.94 ± 0.04	8.07 ± 0.05	9.09 ± 0.08	9.68 ± 0.08
	37	0.37 ± 0.02	0.26 ± 0.05	0.18 ± 0.04	0.14 ± 0.06	0.11 ± 0.04
F3	**20**	8.10 ± 0.08	7.53 ± 0.08	7.56 ± 0.06	7.38 ± 0.04	7.19 ± 0.06
	37	0.36 ± 0.02	0.14 ± 0.07	0.07 ± 0.05	0.05 ± 0.00	0.04 ± 0.01

*Each value represents the mean (±SD) of five replicates.

Table 5 Effect of temperature on the dynamic viscosity (η') of formulations at five representative frequencies

Formulation	Temperature (°C)	η' (Pa) of formulations at representative oscillatory frequencies*				
		0.60 Hz	2.58 Hz	5.05 Hz	7.53 Hz	10.00 Hz
F1	**20**	0.15 ± 0.05	0.14 ± 0.02	0.14 ± 0.03	0.14 ± 0.02	0.15 ± 0.04
	37	8.73 ± 0.01	4.58 ± 0.01	3.80 ± 0.00	3.60 ± 0.00	3.30 ± 0.01
F2	**20**	0.33 ± 0.01	0.33 ± 0.06	0.33 ± 0.02	0.32 ± 0.09	0.31 ± 0.03
	37	35.42 ± 0.03	15.8 ± 0.02	15.4 ± 0.02	14.44 ± 0.03	11.60 ± 0.09
F3	**20**	0.42 ± 0.02	0.38 ± 0.06	0.38 ± 0.07	0.38 ± 0.09	0.38 ± 0.04
	37	1702.00 ± 1.99	199.30 ± 0.46	57.79 ± 0.14	29.38 ± 0.04	18.49 ± 0.02

*Each value represents the mean (±SD) of five replicates.

was evaluated and the values were compared according to their mechanical properties. The gel structure of F3, containing P407/P188 (2 : 1), exhibited the greatest hardness, compressibility, adhesiveness and cohesiveness. Based on these properties F3 appeared to offer optimal performance.

Mucoadhesion studies

Mucoadhesive formulations have been reported to prolong the residence time of the formulation at the site of application.[34] Quantification of mucoadhesion is important to ensure that

Table 6 Mean (±SD) mechanical and drug-release properties of the formulations

Formulation	Hardness (N) ± SD	Adhesiveness (N.mm) ± SD	Cohesiveness ± SD	Compressibility (N.mm) ± SD	Release component (n)	Kinetic constant (k)
F1	0.23 ± 0.04	0.60 ± 0.16	0.56 ± 0.05	0.79 ± 0.18	0.82 ± 0.04	1.02 ± 0.06
F2	0.56 ± 0.08	1.88 ± 0.57	0.81 ± 0.01	2.60 ± 0.71	0.94 ± 0.04	0.93 ± 0.04
F3	0.92 ± 0.29	3.90 ± 0.45	0.84 ± 0.11	4.28 ± 0.1.0	0.70 ± 0.03	1.10 ± 0.07

Table 7 Mucoadhesion studies of EN-containing formulations with bovine vaginal mucosa

Formulation	Mean (±SD) mucoadhesion to vaginal mucosa		
	Maximum detachment force (N)	Mucoadhesion (mJ)	Work of adhesion (mJ/cm^2)
F1	0.39 ± 0.03	0.05 ± 0.01	0.06 ± 0.01
F2	0.41 ± 0.01	0.08 ± 0.01	0.11 ± 0.01
F3	0.44 ± 0.02	0.11 ± 0.01	0.14 ± 0.00

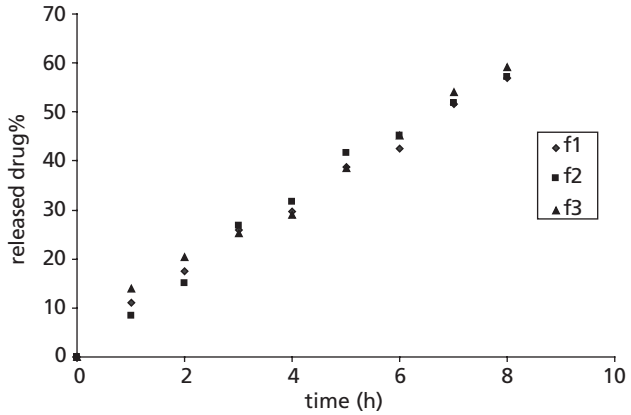

Figure 2 In-vitro release profiles of econazole nitrate from selected formulations: ◆, F1; ■, F2; ▲, F3.

the adhesion offered by formulations is sufficient to ensure prolonged retention at the site of application, but not excessively so, as this may be associated with damage to the mucous membrane.[8] In this study the work of adhesion was used to quantify adhesion. This measure provides a more comprehensive evaluation of the detachment phenomenon.[35] Importantly, the formulations under examination displayed significant mucoadhesion, similar to other systems that have been used for implantation into body cavities.[36] Of the various formulations under examination, formulation F3 (containing 20% poloxamer 407 and 10% poloxamer 188) showed the highest mucoadhesion amongst the various thermosensitive gel formulations (Table 7).

Release studies

Whilst small differences in the mass of drug released from the various formulations were observed at the early stages of drug release, after 3 h the mass of drug released from the various formulations was similar (Figure 2).

Application of the general release equation enabled calculation of n and hence the identification of mechanism of release from the formulations. In this context, $n = 0.5$ indicates release controlled only by Fickian diffusion and $n = 1$ indicates release controlled only by relaxation of polymer chains.[23] The release exponent (n) ranged from 0.5 to 1.0, indicating non-Fickian drug transport (Table 7). Similar kinetic results have been investigated with poloxamer formulations in the literature.[37,38]

In-vivo studies

In the in-vivo studies vaginal swabs were taken after the second, fifth, seventh, and twenty-first days of treatment. For the untreated group of infected animals the vaginal swabs were all positive for *C. albicans* up to the twenty-first day. Among the groups administered thermosensitive gel base without EN, only one rat showed negative culture after the seventh day. Conversely, for all the infected rats treated by the administration of F3 intravaginally, with one exception, the single dose of F3 resulted in a cure rate of over 90% (91.7%).

Histopathological studies were performed to assess the biocompatibility of the EN-containing thermosensitive formulations. The microscopic features of G1 and G2 were consistent with a fungal infection and they were consistent with the microbiological culture findings. Histopathologic evaluation of the formulations showed that there was no evidence of inflammation with the exception of one animal under treatment which presented with low inflammation (G3). Conversely the non-treated (G1) and thermosensitive base administered group (G2) showed visible signs of inflammation (12/12 extensive inflammation for G1 and 11/12 extensive and 1/12 low inflammation for formulation G2). Figures 3 and 4 are micrographs of the vaginal mucosa showing inflammation and no inflammation, respectively. Rats treated with F3 showed healthy tissue and no positive microbiological culture except one.

Conclusion

This study focused on the formulation of in-situ thermoresponsive, mucoadhesive gels containing EN and designed for vaginal administration. Among the ten different in-situ gel formulation of EN prepared with Plx 407/Plx 188 mixtures, F1: 15/15, F2: 15/20 and F3: 20/10 were found to exhibit an acceptable gelation temperature for vaginal administration. Formulations were characterised in terms of textural, rheological and mucoadhesive properties. The thermosensitive formulations exhibited non-Newtonian flow at room temperature and an abrupt rise in viscosity at body temperature. Comparison of the results of oscillatory analysis showed that F3 formulations were consistent gels while F1 and F2 exhibited a weak gel property. The mechanical properties of F3 were greater than those of other formulations. In-vivo studies

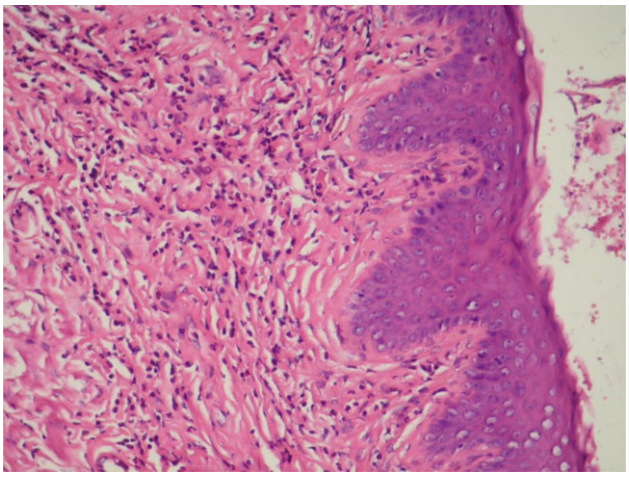

Figure 3 Extensive neutrophil accumulation in lamina propria of the vaginal squamous epithelium (hematoxylin eosin × 200).

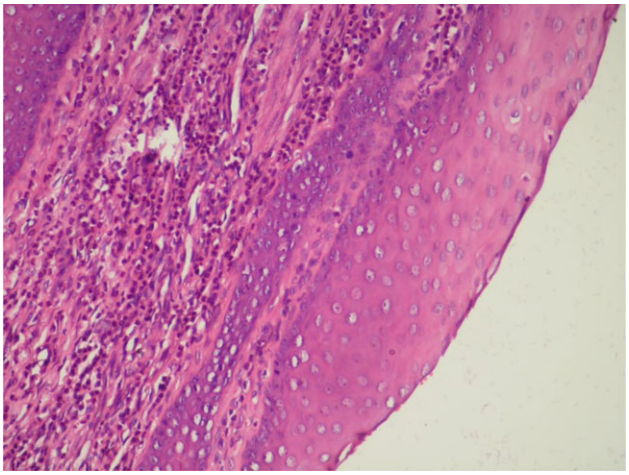

Figure 4 High view appearance of vaginal mucosa without evidence of inflammation (hematoxylin eosin × 200).

demonstrated the efficiency of the formulation for the treatment of vaginal candidiasis with a single-dose intravaginal application. Based on this evidence, it can be inferred that in-situ formulation of EN, with its single-dose regimen, is an effective and promising candidate compound and represents an innovative approach for the treatment of vaginal candidiasis.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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