ORIGINAL ARTICLE, MEDICINE

Temperature-dependent in Situ Gel of Clotrimazole: an Experimental Study

Vipul P. Patel, Harshad M. Damasiya, Pankaj Kapupara, Kalpesh C. Ashara
School of Pharmacy, RK University, Tramba, Rajkot, Gujarat, India

Correspondence: Vipul P. Patel, School of Pharmacy, RK University, Tramba, Rajkot -360020, Gujarat, India
E-mail: vipul.patel@rku.ac.in
Tel: +919712902310
Received: 30 July 2018
Accepted: 27 Nov 2018
Published Online: 27 Dec 2018
Published: 30 June 2019

Key words: candidiasis, clotrimazole, in situ gel, Poloxamer-407, vaginal gel

Citation: Patel VP, Damasiya HM, Kapupara P, Ashara KC. Temperature-dependent in situ gel of clotrimazole: an experimental study. Folia Med (Plovdiv) 2019;61(2):266-76. doi: 10.2478/folmed-2018-0073

Background: The in-situ gel-forming polymeric formulations offer sustained and prolonged action in comparison to conventional drug delivery systems.

Aim: To formulate and evaluate in situ vaginal gel of clotrimazole.

Materials and methods: Poloxamer 407 (20%) was slowly added to freezing water (5°C) with constant stirring. The prepared dispersion was refrigerated for 5 h, the different concentrations of polymers were added for preliminary batches. Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) were performed for clotrimazole-excipients compatibility study. The final batch was prepared and evaluated for physicochemical parameters, in vitro clotrimazole release, in vitro antifungal activity, and in vivo vaginal tissue irritation test.

Results: The compatibility study showed no chemical interaction between clotrimazole and excipients used. The evaluation parameters showed that clotrimazole release was in the range of 8 to 10 h, gelling temperature was in the range of 27–35°C, gelling time was in the range of 28–34 sec, pH was in the range of 4.4–4.8, and viscosities were in the range of 16.4–182.6 cP (solution form) and 10,500–20,756 cP (gel form). The zone of inhibitions for clotrimazole pure drug, the marketed vaginal gel of clotrimazole, and optimized gel formulation was 9.15±0.75 mm, 14.35±1.12 mm, and 18.85±1.56 mm, respectively (p < 0.0001, q = 5.98). An optimized gel formulation was not irritant to vaginal tissue.

Conclusion: It was possible to formulate effective in situ vaginal gel for control release action of clotrimazole.

Level of Evidence: IIc.

BACKGROUND

Vaginal candidiasis is a vaginal mucositis opportunistic infection caused by species of the genus Candida in women, in the fertile period, and also the most frequent fungal disease of vaginal content.1 Women around the world get diagnosed with vaginal candidiasis. It is estimated that 75% of women during the fertile period have at least one episode of vaginal candidiasis. Approximately 40–50% of women have repeated infection. Less than 5% of the adult female population receives repeated, frequent attacks of recurrent vulvovaginal candidiasis. Point-prevalence studies indicate that Candida species may be isolated from the genital tract of approximately 20% (the range 10–50%) of asymptomatic, healthy women in the child-bearing age. Twenty to forty percent of women who are cultured positive for Candida species in the vaginal area are asymptomatic carriers.

The natural history of asymptomatic colonization is unknown, although limited human studies suggest that vaginal carriage may continue for several months and perhaps years. The increased secretion of reproductive hormones during pregnancy favors the formation of infection. The elevated levels of estrogen provide an increased amount of glycogen in the vagina, furthermore providing a reliable source of carbon required for Candida growth and their germination. These hormones accelerate the formation of yeast pseudohyphae. Vaginal candidiasis is rare in postmenopausal women, due to the hormonal dependence of vaginal candidiasis. There is a balance between Candida, normal bacterial flora, and immune defense mechanisms. When this balance is disturbed, colonization is replaced by infection. It is not concretely evident as to what exactly leads to disruption of the balance and origin of infec-
tion. Vaginal candidiasis occurs when there is an increase in the virulence of Candida, and because of the reduction in local defense mechanisms. Vagina has the normal pH range (pH 4.0–4.7), as opposed to mixed infections (bacterial, trichomonas), where pH rises to levels greater than 4.7. The clinical symptoms of vaginal candidiasis are nonspecific, and a broad variety of infectious and noninfectious diseases can cause similar symptoms. Women with vaginal candidiasis do not notice any change in their vaginal secretions. Vulvovaginal itching, irritation, soreness, burning, or dyspareunia are more common symptoms of vaginal candidiasis. Occasionally, vaginal candidiasis causes external dysuria. On vulvar examination, patients may exhibit redness, swelling, fissures, or excoriations, and vaginal signs of erythema or a thick curdy discharge may be seen.2

The in-situ gel-forming polymeric formulations offer several advantages like sustained and prolonged action in comparison to conventional drug delivery systems. It is drug delivery systems that are in sol form before administration in the body, but once administered, undergo gelation in situ, to form a gel. The formation of gels depends on factors like temperature modulation, pH change, the presence of ions and ultraviolet irradiation, electrical sensitivity, enzyme sensitive from which the drug gets released in a sustained and controlled manner. In situ gels are presently under investigation as a delivery system for bioactive molecules, because of their similar physical properties as that of living tissue, which is due to their high water content, soft and rubbery consistency, and low interfacial tension with water or biological fluids.3

From a manufacturing point of view, the production of such devices is less complex and thus lowers the investment and manufacturing cost. The primary requirement of a successful controlled release product focuses on increasing patient compliance, good stability and biocompatibility characteristics make the in-situ gel dosage forms very reliable. Use of biodegradable and water-soluble polymers for the in situ gel formulations can make them more acceptable and excellent drug delivery systems.3

Clotrimazole is chemically C₂₂H₁₇CN₂, antifungal agents. IUPAC (the International Union of Pure and Applied Chemistry) Name is 1-[(2-chlorophenyl) diphenylmethyl]-1H-imidazole. It has a molecular weight of 344.837 g/mol, 14–34% oral bioavailability, metabolized via the liver, excreted in the urine and faeces, BCS Class II, water solubility 0.49 µG/mL, Pka (strongest basic) 6.62, volume of distribution 1.1 L/kg in mice and 0.7 L/kg in humans, half-life 2 h, log p 6.1, protein binding 90%. It interacts with 14-α demethylase, a cytochrome P-450 enzyme that converts lanosterol to ergosterol, an essential component of the yeast membrane. It may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms and the uptake of purine, impair triglyceride and phospholipid biosynthesis. It also inhibits the movement of calcium and potassium ions across the cell membrane by blocking the ion transport pathway known as the Gardos channel.4

AIM
The aim of the present study was to investigate the feasibility of developing and characterization of pH and temperature dependent in situ vaginal gel of clotrimazole.

MATERIALS AND METHODS

DRUG AND REAGENTS
Clotrimazole was purchased from Chethana pharmaceuticals, Kerala, India. Poloxamer-407 was purchased from Sigma-Aldrich Chemie USA. Chitosan, HPMC (Hydroxypropyl methylcellulose)-K-100M, HPMC-E-50, acetic acid, methanol, hematoxylin, diethyl ether, and eosin were purchased from Astron Chemicals, Ahmedabad, India. Glycerol and formalin were purchased from Acme Chemicals, Mumbai, India. Candida albicans (ATCC 10231) was occupied from the school of science, RK University, India.

PRELIMINARY STUDY
Solutions (100 µG/mL) of clotrimazole in methanol and in methanolate phosphate buffer (pH 5.4) were scanned in UV (LT-2900, Double Beam UV visible Spectrophotometer, Labtronics (I) Pvt. Ltd., Ambala, India) between 200 and 400 nm range. Phosphate Buffer pH 5.4 was prepared by dissolving 40 g of sodium dihydrogen phosphate and 8.0 g of sodium hydroxide in sufficient water to produce 1000 mL. The pH was adjusted to 5.4 with 1 M H₂SO₄ or 1 M NaOH (sodium hydroxide).

A weighed quantity of clotrimazole (20 mg) was placed in 250 mL of the standard volumetric flask (Borosil®) and made up the volume with phosphate buffer pH 5.4. The stock solution obtained was 100 µG/mL solution. Aliquots of 2, 4, 6, 8, 10, 20, and 30 mL of stock solution were pipetted into 100 mL standard volumetric flasks (Borosil®) and final volume was adjusted up to 100 mL with phosphate buffer (pH 5.4) to give the concentration of 2, 4,
6, 8, 10, 20, and 30 µG/mL. The absorbances were measured at wavelength maximum in UV spectrophotometer against reagent blank phosphate buffer pH 5.4.5

METHOD FOR PREPARATION OF TEMPERATURE-DEPENDENT IN SITU VAGINAL GEL

In the present work, the in situ gel was prepared on a weight basis using the cold method. An amount of Pluronic F-127 (Poloxamer 407) sufficient to yield 20% gel was slowly added to freezing water (5°C) with constant stirring. The prepared dispersion was refrigerated until a clear solution was formed (for 5 h). To control the release of drug from the gel, the different concentration of sustained release polymers like HPMC E-50, and HPMC K-100M were added to the Pluronic gel. Chitosan was dissolved in 1% acetic acid then add glycerol in solution and this solution was mixed with the pluronic gel. The concentration of clotrimazole (10 mg/mL) was kept constant in all the batches.6

A PRELIMINARY TRIAL BATCH OF IN SITU VAGINAL GEL

From the results of preliminary screening for optimization of Poloxamer 407 amount in situ gel formulation, it was found that 20% Poloxamer 407 was giving in situ gelling characteristics near to desirable property of in situ gel. Therefore, 20% Poloxamer was fixed and other variables were changed for further study. Preliminary trial batches were prepared by trial and error method.

COMPATIBILITY STUDY

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Sample containing clotrimazole and mixture of clotrimazole with different polymers in equal proportion were mixed geometrically and analyzed for DSC study (DCS 60, TA-60WS, and Shimadzu, Japan) at a heating rate of 5°C/min.5

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Clotrimazole-excipients interactions play a vital role in the release of it from the formulation. FTIR was used to study the physical and chemical interactions between clotrimazole and the excipients used. The sample consisted of clotrimazole and physical mixture of in situ gel components was analyzed by FTIR spectroscopy. FTIR spectra were obtained on Shimadzu FTIR-8400 spectrophotometer, Japan using the KBr disk method (2 mg sample in 200 mg KBr). The scanning range was in between 400–4000 cm⁻¹ and the resolution was 1 l/(cm).5

THE COMPOSITION OF FINAL BATCH

In the final batch, the estimated dose of the clotrimazole was added in methanol. These clotrimazole solutions directly added to the solution of 20% poloxamer at a cool temperature. The constant stirring was maintained. The prepared dispersion was refrigerated until a clear solution was formed (5 h).

CHARACTERIZATION OF FORMULATION

VISUAL APPEARANCE

The appearance was checked visually. The appearance of the formulations before and after gelling was determined by visual examination of the formulations against white and black backgrounds.7

TEXTURE ANALYSIS

The consistency, firmness, and cohesiveness of in situ gel were assessed by manual checking which mainly indicated gel strength and easiness in administration, in vivo higher value of adhesiveness of gel was needed to maintain an intimate contact with the mucous surface.7

SYRINGEABILITY

The syringeability of clotrimazole in situ gel was carried out by using a 1 mL plastic syringe fitted with a 22-gauge needle (Dispovan, India). A specified amount of formulation stored at 4°C (which was preserved it as sol) was filled in the syringe and gentle force was applied by pressing the injector part of the syringe. The ease of ejection was assessed qualitatively.8

SURFACE pH MEASUREMENT

The pH of the thermo-reversible vaginal gel of clotrimazole was determined by a digital pH meter (Labtronics, India). The formulation was taken in a beaker and pH was checked using pH meter.9

GELING TEMPERATURE

The sol-gel transition temperature of the vaginal sol was determined using test tube investing method. 5 mL of sol transferred to test tubes, immersed in a thermostat controlled electric water bath (Today tech Scientific solution, Ambala Cant, India) at an initial temperature of 4°C, and sealed with aluminum foil (Krisha Scientific, India). The temperature of the water bath was increased in increments of 0.5°C and the sol was left to equilibrate for 1 min at each new setting. The sample was then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting at 90° angles. The gelling temperature was recorded.9
The gelling time
To find out the gelling time of 5 mL of the sol was taken in a test tube and placed in a water bath maintained at 37°C and the time is taken to gel was recorded.9

Rheology
The viscosity of vaginal gel formulation was a crucial factor in determining the residence time of clotrimazole in the vaginal cavity. The viscosity of vaginal sol was determined using a programmable viscometer (DV III ULTRA and LV, Brookfield Engineering Laboratory Inc, USA) and T-bar spindle (S-64). There were 30 g of sol filled in a 50 mL beaker and the spindle was lowered perpendicularly taking care that the spindle did not touch the bottom of the beaker. The spindle was rotated at a speed of 20 rpm to generate torque greater than 10% and the viscosity readings were recorded after 60 s. Viscosity parameters were collected at different rpm with 1-min equilibration time at every rpm. The samples were applied to the lower plate using a spatula (Krishna Scientific, India) to ensure that formulation shearing did not occur. To test the effect of temperature, the measurements were made at 25°C and 37°C.8

Percentage clotrimazole content
Formulations were tested for percentage clotrimazole content. Bottles containing the formulation were properly shaken for 2–3 min. 1 mL of the formulation was transferred into a 100 mL volumetric flask. Fifty mL of simulated vaginal fluid (pH 5.4±0.02) was added. The formed gel was completely crushed with the help of a glass rod, followed by vigorous shaking until the formed gel got completely dispersed to give a clear solution. The volume was adjusted to 100 mL with simulated vaginal fluid. 2 mL solution was taken and diluted to 10 mL with simulated vaginal fluid. The solution was filtered through an 11-µm filter membrane (Angle Marketing, India) and the clotrimazole concentration was determined with a UV-visible spectrophotometer at 260 nm.9

In vitro clotrimazole release studies
The dissolution studies were performed using a modified dialysis tube (Nepro India) method. In this study, Cellophane paper (Angle Marketing, India) was used as a membrane. Before dissolution Cellophane paper was soaked with dilute NaOH for 30 min. After that this soaked Cellophane paper was attached to the open part of the test tube and another part was cut off. Typically, 1 g of Pluronic gel was placed in a dialysis tube. The test tube was then placed in a vessel containing 100 mL of phosphate buffer pH 5.4, maintained at 37.5°C, and stirred at 30 rpm (with magnetic beads, Remi equipment, India). The aliquots were collected periodically after 1-h interval up to 10 h and replaced with fresh dissolution medium. After filtration through Whatman filter paper # 41 (Angle Marketing, India), the concentration of clotrimazole was determined spectrophotometrically at 260 nm respectively.6

In vitro antifungal activity
The test was carried out by the disc diffusion method. Candida albicans (ATCC 10231) was used as reference microorganism in present work. The marketed vaginal gel of clotrimazole, clotrimazole pure drug, and the optimized formulation was poured into sterile nutrient agar previously seeded with Candida albicans. After allowing diffusion of the solutions for 2 h, the agar plates were incubated at 37±0.5°C for 24 h. The zone of inhibitions was measured. The entire operation except the incubation was carried out in a laminar flow unit.6

Gel persistent capacity (GPC) and spreadability
GPC was determined by placing a drop off prepared formulation in a vial containing 2 mL of simulated vaginal fluid and observed till it completely erodes. The spreadability was determined by using two transparent scales. About 20 g was added on the upper scale and the time was noted for upper slide (movable) to separate completely from the fixed slides.5

Bioadhesion measurement
The method was based on the measurements of the tensile strength or shear stress required to break the adhesive bond between the model membrane and the test formulation. The test formulation was placed in 1 cm² area as a sandwiched between two slide membranes and fixed on the flexible support in the assemblies for 10 sec. after adhesive bond was formed, the weight required to separate slide from sandwiched between two slides is noted and calculated in dyne/cm² (1 dyne/cm² = 0.00101972 g/cm²) as bioadhesive force.5

Vaginal irritation test
The study had been approved by the School of Pharmacy, RK University animal ethics committee, Rajkot, India. The study adhered to the ARRIVE guidelines for reporting animal research10 and the
law of India. Total 18 female rats were randomized for the study. Rats of the negative control group received water injection 1 mL (n = 6), the positive control group received 1 g of Candid-V gel (n = 6), and treatment group received 1 g of the optimized gel formulation (n = 6). Formulations were applied by a blunt applicator (20 gauges, Dispovan®, India) once daily for two weeks. After two weeks, rats were anesthetized (diethyl ether anesthesia) and vaginal tissues were collected and fixed in formalin, then stained with hematoxylin and eosin stain. The tissue was observed under microscope (Olympus India) and graded as 0: no irritation, 1: minimum irritation, 2: mild irritation, 3: moderate irritation, 4: severe irritation.11

STATISTICAL ANALYSIS
Data were presented as mean±SD of five individual experiments. One-way ANOVA following Dunnett Multiple Comparisons Test were performed for in vitro antifungal activity (considering critical value [q] > 2.5 as significant) and grading of histopathology (considering q > 3.651 as significant). Results were considered significant at 95% of confidence level. InStat, GraphPad, USA was used for statistical analysis.

RESULTS
Clotrimazole shows maximum absorbance at 260 nm. Formulation A1 and A2 gel had poor gel strength, showed complete diffusion within little duration of time, more gelling time, and soft with less viscosity. Formulation A4, A5, A6, A7, A10, A11, A12, A13, A16, A17, and A18 had poor gel strength, showed more diffusion, less viscosity, very soft, and low adhesion (thumb tack test). Formulation A25, A26, A27, A28, A29, and A30 were more viscous gel formulation, difficult to handle and store, and poor drinkability. A13 and A22 batches possessed a medium viscosity, medium diffusion, and less adhesion. A3, A8, A9, A14, A15, A19, A20, A21, A23, and A24 gel had good gel strength, near to desirable viscosity in situ gelling system and in gelling system, and good gel bioadhesive (thumb tack) in situ gelling system may provide sustained release and bioadhesive action, looks like near to expected formulation (Table 1).

The DSC thermogram of the physical mixture of clotrimazole with polymers was exhibited the sharp endothermic peaks at 145.56°C and sharp a single peak of the Poloxamer-407 at 54.39°C and clotrimazole 144.20°C only and no peak of other polymers could be traced in thermogram (Fig. 1). FTIR spectroscopy of the physical mixture of clotrimazole with polymers showed the bands at 3170 cm⁻¹ (for C-H aromatic stretch of clotrimazole), 1590 cm⁻¹ and 1490 cm⁻¹ (for benzene ring stretching of clotrimazole), and 740 cm⁻¹ (for C-H stretching of clotrimazole) (Fig. 2).

The compositions of the final batch of in situ vaginal gel formulation of clotrimazole are shown in Table 2. All batches were syringeable at cold temperature, had transparent clear solution appearance, smooth texture, and showed % clotrimazole content in between 95.50±0.20–99.60±0.26% (Table 3). Table 4 shows in vitro clotrimazole release of formulation F1 to F10. Kinetic analysis of the release data of clotrimazole from gel formulations is shown in Table 5. The zone of inhibitions for clotrimazole pure drug, the marketed vaginal gel of clotrimazole, and optimized gel formulation were 9.15±0.75 mm, 14.35±1.12 mm, and 18.85±1.56 mm, respectively (p < 0.0001, q = 5.98) (Fig. 3).

Candid-V gel (0.71±0.095 vs 0.85±0.1, p =0.032, q = 3.546) and optimized gel formulation (0.71±0.095 vs 0.91±0.2, p = 0.051, q = 3.522) were not irritant to vaginal tissue (Fig. 4).

DISCUSSION
The standard curve of clotrimazole had shown λmax value at 260 nm. Clotrimazole shows λmax value at 260 nm in the literature.4 A representative standard calibration curve was found to be linear in the range of 2–30 µG/mL at λmax 260 nm.

Primary trial batch (A1–A30) was formulated by using polymer concentration of (0.2% to 20.0%) of all polymers. Gelation occurred in the case of all trial batch formulation, but gelling capacity was very less with HPMC E-50 polymer in most of the formulation. As the concentration of all 3 polymers (chitosan, HPMC E-50, and HPMC K-100M) increase from (0.2%) to (2.0%) the viscosity of all formulation increases which increases the residence time of gel in vagina up to 8 to 10 h. Viscosities of HPMC and chitosan gels are directly proportional to their concentration.12 In respect to results of primary trial batches, it was found that polymer concentration below (0.2%) did not give effective gel formation and above (2.0%) formed a more viscous solution form.

DSC and FTIR had suggested clotrimazole with polymers had no chemical interaction13 and suggesting compatibility of the clotrimazole with excipients.

The appearance of all the formulations was found
Table 1. Preliminary trial batch by trial and error method

| Formula-   | Chitosan | HPMC- K-100 M | HPMC-E-50 | Formula-   | Chitosan | HPMC- K-100 M | HPMC-E-50 |
|------------|----------|---------------|-----------|------------|----------|---------------|-----------|
| A1         | 0.2%     | 0.2%          | 0%        | A16        | 1.0%     | 0%            | 0.2%      |
| A2         | 0.2%     | 0.5%          | 0%        | A17        | 1.0%     | 0%            | 0.5%      |
| A3         | 0.2%     | 1.0%          | 0%        | A18        | 1.0%     | 0%            | 1.0%      |
| A4         | 0.2%     | 0%            | 0.2%      | A19        | 1.5%     | 0.2%          | 0%        |
| A5         | 0.2%     | 0%            | 0.5%      | A20        | 1.5%     | 0.5%          | 0%        |
| A6         | 0.2%     | 0%            | 1.0%      | A21        | 1.5%     | 1.0%          | 0%        |
| A7         | 0.5%     | 0.2%          | 0%        | A22        | 1.5%     | 0%            | 0.2%      |
| A8         | 0.5%     | 0.5%          | 0%        | A23        | 1.5%     | 0%            | 0.5%      |
| A9         | 0.5%     | 1.0%          | 0%        | A24        | 1.5%     | 0%            | 1.0%      |
| A10        | 0.5%     | 0%            | 0.2%      | A25        | 2.0%     | 0.2%          | 0%        |
| A11        | 0.5%     | 0%            | 0.5%      | A26        | 2.0%     | 0.5%          | 0%        |
| A12        | 0.5%     | 0%            | 1.0%      | A27        | 2.0%     | 1.0%          | 0%        |
| A13        | 1.0%     | 0.2%          | 0%        | A28        | 2.0%     | 0%            | 0.2%      |
| A14        | 1.0%     | 0.5%          | 0%        | A29        | 2.0%     | 0%            | 0.5%      |
| A15        | 1.0%     | 1.0%          | 0%        | A30        | 2.0%     | 0%            | 1.0%      |

The concentration of Poloxamer 407 (Pluronic gel) was constant 20% in all batches.

Figure 1. The DSC thermogram of the physical mixture of clotrimazole with polymers.
to be the transparent clear solution. The texture of the formulations after gelling was determined by manual checking. The texture of all formulations was found to be smooth and it did not show any kind of grittiness. Results revealed that all the formulations from F1 to F10 were syringeable at cold temperature. The pH of the formulations was found to be satisfactory in the range of 4.55–4.76. Any formulation with more than 4.8 pH may cause vaginal irritation. The prepared formulation did not cause any irritation in the vaginal cavity.

All the developed formulations had gelling time in a range of 28 to 34 sec. It was concluded that increasing the concentration of mucoadhesive polymers, gelling temperature decreases, due to this gelling time also decreases.

Viscosity was varied with the concentration of polymer. As the concentration of polymer increases, viscosity increases proportionally. There was 20% gel offers a good syringe ability and optimum viscosity characteristics. Therefore, 20% gel was selected for this study. Viscosity primarily affects the release of clotrimazole from the gel, as viscosity increases the clotrimazole release decreased from the gel. It was seen that as the concentration of mucoadhesive polymer increases, viscosity increases proportionally. The pluronic gel showed the thermoreversible property. The Pluronic gel at cold temperature converts into liquid form. As the temperature of the system increases the liquid form converts into the gel at room temperature. Thus, Pluronic gel showed the temperature dependent thixotropic behavior. This sol-gel transition characteristic plays a key role in the development of in situ gel formulations. The viscosities of all the developed gel formulations were in the range 16.4 to 20,756 cp for gel form respectively.

The clotrimazole content ensured delivery of clotrimazole to the site after administration of the gel formulation. The prepared in situ gel was intended for place-

### Table 2. Composition of the final batches of an in-situ vaginal gel formulation of clotrimazole

| Formula- | Chitosan | HPMC K-100 M | HPMC E-50 |
|----------|----------|--------------|-----------|
| F1       | 0.2%     | 1.0%         | 0%        |
| F2       | 0.5%     | 0.5%         | 0%        |
| F3       | 0.5%     | 1.0%         | 0%        |
| F4       | 1.0%     | 0.5%         | 0%        |
| F5       | 1.0%     | 1.0%         | 0%        |
| F6       | 1.5%     | 0.2%         | 0%        |
| F7       | 1.5%     | 0.5%         | 0%        |
| F8       | 1.5%     | 1.0%         | 0%        |
| F9       | 1.5%     | 0%           | 0.5%      |
| F10      | 1.5%     | 0%           | 1.0%      |

Clotrimazole was 1.0% w/w in all batches. Poloxamer 407 (Pluronic gel) was 20% in all batches.

Figure 2. FTIR Spectroscopy CLO: clotrimazole, CLO + EXP: The physical mixture of clotrimazole with polymers.
Table 3. Physicochemical parameters of *in situ* vaginal gel formulation of clotrimazole

| Formulation Code | pH       | Gelling Temp (°C) | Gelling Time (Sec) | Viscosity (cp) in solution form | Viscosity (cp) in gel form | Gel Persistent capacity (h) | Spreadability in distance (cm) | Bioadhesive strength (dyne/cm²) |
|------------------|----------|-------------------|--------------------|---------------------------------|-----------------------------|-----------------------------|--------------------------------|---------------------------------|
| F1               | 4.72±0.026 | 33.53±0.70        | 32.86±0.60         | 31.2                            | 20.396                      | 4.9                         | 5.2                            | 2991.01                         |
| F2               | 4.64±0.02  | 27.26±0.23        | 28.26±0.83         | 16.4                            | 10.500                      | 4.1                         | 6.2                            | 2255.52                         |
| F3               | 4.67±0.02  | 29.60±1.44        | 30.26±0.55         | 18.8                            | 16.516                      | 4.6                         | 6.1                            | 2549.71                         |
| F4               | 4.55±0.02  | 28.40±0.52        | 29.06±1.00         | 30.3                            | 17.216                      | 4.8                         | 5.8                            | 2598.75                         |
| F5               | 4.60±0.05  | 30.66±1.25        | 31.33±0.90         | 49.6                            | 20.576                      | 5.1                         | 5.1                            | 3040.05                         |
| F6               | 4.63±0.055 | 31.0±0.91         | 32.20±0.79         | 40.0                            | 16.872                      | 4.9                         | 6.0                            | 2559.52                         |
| F7               | 4.60±0.036 | 30.26±0.68        | 31.23±0.55         | 63.8                            | 20.576                      | 5.5                         | 5.1                            | 3040.05                         |
| F8               | 4.76±0.020 | 32.40±0.60        | 30.60±0.75         | 54.5                            | 18.656                      | 5.3                         | 5.6                            | 2657.59                         |
| F9               | 4.63±0.065 | 33.06±0.66        | 30.86±0.86         | 115.4                           | 20.576                      | 5.9                         | 5.0                            | 3089.05                         |
| F10              | 4.69±0.02  | 35.26±0.83        | 33.26±0.41         | 182.6                           | 20.756                      | 6                           | 4.9                            | 3167.53                         |

Data were presented as mean ± SD; n = 3

The simulated vaginal fluid, inflammatory exudate flows continuously in the vagina. The pH of the simulated vaginal fluid is 5.4 and pH of a vaginal fluid is pH 4.2–7.0 but *Candida albicans* have optimal growth medium with pH ~ 5.4. Hence in this study, phosphate buffer pH 5.4 was used for the *in vitro* clotrimazole release studies of the gel formulations. The release studies were performed using a dialysis membrane. On addition of various mucoadhesive polymers like Chitosan, HPMC K-100, and HPMC-E50, the release decreases to an extent level. F1 to F10 batches release clotrimazole completely within 8 to 10 h. Based on control release characteristics, F2, F4, F6, and F8 batches were selected for further study.

From the R² value, it was concluded that F8 batch released clotrimazole at first order rate. There was n value found to be 0.403. It indicated that the F8 batch gave release of clotrimazole by a Fickian diffusion mechanism.

Antibiotic assay of clotrimazole gel was performed by disc diffusion method on *Candida albicans*. Clotrimazole specifically acts on gram-negative anaerobic, facultative bacteria which are responsible for the vaginal disease. The optimized batch of gel formulation had a significant higher zone of inhibition than marketed formulation (Candid-V Gel 2% Clotrimazole cream).

Figure 3. **A**: Zone of inhibitions of clotrimazole pure drug, the marketed vaginal gel of clotrimazole, and optimized gel formulation against *Candida albicans*. **A**: Clotrimazole pure drug, **B**: Candid-V Gel (marketed formulation of 2% clotrimazole), and **C**: optimized gel formulation.
Table 4. *In vitro* drug release of *in situ* vaginal gel formulation of clotrimazole

| Time (h) | F1         | F2         | F3         | F4         | F5         | F6         | F7         | F8         | F9         | F10        |
|----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 1        | 19.96±1.32 | 19.65±1.32 | 18.68±1.30 | 22.28±1.40 | 16.40±1.12 | 19.96±1.1  | 18.06±1.32 | 19.30±1.38 | 18.68±1.26 | 20.63±1.4  |
| 2        | 28.30±1.45 | 27.55±1.42 | 26.53±1.20 | 31.85±1.58 | 30.43±1.16 | 32.92±1.40 | 33.08±1.28 | 26.22±1.16 | 26.53±1.35 | 32.89±1.32 |
| 3        | 37.53±1.29 | 39.86±1.56 | 33.63±0.85 | 38.60±1.36 | 36.40±0.78 | 37.56±1.36 | 51.64±1.35 | 32.89±1.20 | 43.63±1.62 | 40.89±1.18 |
| 4        | 50.98±0.78 | 51.07±1.12 | 46.06±1.28 | 49.87±1.20 | 48.83±1.2  | 57.01±0.95 | 58.07±1.46 | 40.22±0.98 | 52.06±1.57 | 50.22±0.87 |
| 5        | 68.89±0.90 | 71.82±1.18 | 61.66±1.54 | 62.84±0.91 | 63.82±1.35 | 68.92±1.15 | 68.54±0.96 | 48.69±0.80 | 67.40±1.38 | 68.38±0.93 |
| 6        | 72.43±1.47 | 73.57±1.54 | 72.80±1.36 | 78.09±0.82 | 68.17±1.25 | 75.41±0.89 | 78.70±0.84 | 56.24±1.14 | 82.08±0.83 | 72.24±1.23 |
| 7        | 79.69±1.31 | 73.84±1.10 | 73.05±0.50 | 78.20±1.48 | 72.55±0.92 | 82.20±1.40 | 82.10±1.52 | 67.40±1.38 | 84.02±1.46 | 89.40±1.49 |
| 8        | 79.72±1.56 | 73.92±1.16 | 73.18±0.88 | 78.24±1.28 | 73.21±0.98 | 82.24±1.60 | 82.15±0.90 | 74.39±1.23 | 84.12±0.97 | 90.42±1.60 |
| 9        | 79.74±1.42 | 73.98±1.21 | 73.21±0.90 | 78.26±1.30 | 73.26±1.02 | 82.26±1.62 | 82.15±0.98 | 84.15±1.15 | 84.15±1.27 | 90.46±1.28 |
| 10       | 79.74±1.30 | 73.98±1.25 | 73.21±0.91 | 78.26±1.32 | 73.26±1.05 | 82.26±1.65 | 82.15±1.26 | 90.46±1.18 | 84.15±1.27 | 90.46±1.42 |

Data were represented as mean ± SD; n = 3
In Situ Vaginal Gel of Clotrimazole

Table 5. Kinetic analysis of the release data of clotrimazole from gel formulations

| Batch  | Higuchi R Square Value | Zero Order | First Order | Hixon Crowell | Korsmeyer-Peppers | Best fit model |
|--------|------------------------|------------|-------------|---------------|-------------------|---------------|
| F8     | 0.8187                 | 0.9021     | 0.9599      | 0.8319        | 0.8515            | First order   |

Figure 4. Histopathology of vaginal irritation test. A: water for injection, B: optimized gel formulation, C: Candid-V gel.

The local drug delivery system in the present study was simple and easy to use. Its syringe ability allows easy insertion of gel formulation into the vagina. The developed formulation can release the clotrimazole at a controlled rate for a prolonged duration. The results indicate that these targeted devices for the treatment of vaginal diseases show significant advantages over the conventional therapy.

Although the study was quite innovative and addressed a critical drug delivery related issues or mechanistic, it has some limitations, for example, lack of in vivo efficacy data in a suitable model.

CONCLUSIONS

The finding was concluded that it was possible to formulate clotrimazole in situ vaginal gel which can be targeted in treating vaginal diseases and reduce dosing frequency, increase the bioavailability, better patient compliance with acceptable adverse effects.

REFERENCES

1. Gunther LS, Martins HP, Gimenes F, et al. Prevalence of Candida albicans and non-albicans isolates from vaginal secretions: comparative evaluation of colonization, vaginal candidiasis and recurrent vaginal candidiasis in diabetic and non-diabetic women. Sao Paulo Med J 2014; 132(2): 116-20.
2. Sun MG, Huang Y, Hong Xu YH, et al. Efficacy of vitamin B complex as an adjuvant therapy for the treatment of complicated vulvovaginal candidiasis: An in vivo and in-vitro study. Biomed Pharmaco 2017; 88: 770-7
3. Dash AK, Ganguly S. A novel in situ gel for sustained drug delivery and targeting. Int J Pharm 2004; 276(1–2): 83-92.
4. Clotrimazole. Available from: https://www.drugs.com/pro/clotrimazole.html. (Access on 10/2013)
5. Patel P, Patel P. Formulation and evaluation of clindamycin HCL in situ gel for vaginal application. Int J Pharm Investig 2015; 5(1): 50-6.
6. Swati R, Sandeep W, Swaroop L. In situ gel formulation of ornidazole for the treatment of periodontal disease. Curr Pharm Res 2010; 1(1): 60-9.
7. Patel K, Vadalia KR, Patel JK. Development and evaluation of in situ gelling system for treatment of periodontitis. Am J Pharm Tech Res 2012; 2(4): 2102-12.
8. Nasra MM, Khiri HM, Hazzah HA, et al. Formulation, in vitro characterization and clinical evaluation of curcumin in situ gel for treatment of periodontitis. Drug Deliv 2017; 24(1): 133-42.
9. Neeraj K, Kamla P. Dual controlled release, in situ gelling periodontal sol of metronidazole benzoate and serratipeptidase: statistical optimization and mechanistic evaluation. Curr Drug Del 2012; 9(1):74-84.
10. Kilkenny C, Browne WJ, Cuthi I, et al. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Vet Clin Pathol 2012; 41(1): 27-31.
11. Podaralla S, Alt C, Shankar GN. Formulation development and evaluation of innovative two-polymer (SR-2P) bioadhesive vaginal gel. AAPS PharmSci-
12. Alam MA, Al-Jenoobi FI, Al-Mohizea AM, et al. Effervescence assisted fusion technique to enhance the solubility of drugs. AAPS Pharm SciTech 2014; 15(4): 928-38.
13. Gupta NV, Natasha S, Getyala A, et al. Biodhesive vaginal tablets containing spray-dried microspheres loaded with clotrimazole for treatment of vaginal candidiasis. Acta Pharm 2013; 63(3): 359-72.
14. Forbes CJ, Lowry D, Geer L, et al. Non-aqueous silicone elastomer gels as a vaginal microbicide delivery system for the HIV-1 entry inhibitor maraviroc. J Control Release 2011; 156(2): 161-9.
15. Spear GT, McKenna M, Landay AL, et al. Effect of pH on cleavage of glycogen by vaginal enzymes. PLoS One 2015; 10(7). DOI: 10.1371/journal.pone.0132646.
16. Meng-Lund E, Muff-Westergaard C, Sander C, et al. A mechanistic-based approach for enhancing buccal mucoadhesion of Chitosan. Int J Pharm 2014; 461(1-2): 280-5.
17. Esposito E, Sguizzato M, Bories C, et al. Production and characterization of a clotrimazole liposphere gel for Candidiasis treatment. Polymers 2018; 10(160). doi: 10.3390/polym10020160.
18. Faidallah HM, Rostom SA, Khan KA, et al. Synthesis and characterization of some hydroxypyridone derivatives and their evaluation as antimicrobial agents. J Enzyme Inhib Med Chem 2013; 28(5): 926-35.

**In situ** температурно-зависимый гель клотримазола: экспериментальное исследование

Випул П. Пател, Харшад М. Дамасия, Панкадж Капупара, Калпеш К. Ашара
Фармацевтический факультет, Университет Раджкот, Раджкот, Гуджарат, Индия

**Адрес для корреспонденции:**
Випул П. Пател, Фармацевтический факультет, Университет Раджкот, Трамбая, Раджкот-360020, Гуджарат, Индия
E-mail: vipul.patel@rku.ac.in
Tel: +919712902310
**Дата получения:** 30 июля 2018
**Дата приемки:** 27 ноября 2018
**Дата онлайн публикации:** 27 декабря 2018
**Дата публикации:** 30 июня 2019

**Ключевые слова:** кандидоз, клотримазол, in situ гель, Полоксамер-407, вагинальный гель

**Образец цитирования:** Patel VP, Damasiya HM, Kapupara P, Ashara KC. Temperature-dependent in situ gel of clotrimazole: an experimental study. Folia Med (Plovdiv) 2019;61(2):266-76.
doi: 10.2478/folmed-2018-0073

**Введение:** Полимерные гелеобразующие композиции in situ обеспечивают устойчивое и длительное действие по сравнению с конвенциональными системами доставки лекарств.

**Цель:** Изготовить и оценить формулу вагинального геля клотримазола in situ.

**Материалы и методы:** Полоксамер-407 (20%) медленно добавляли в ледяную воду (5 °C) при постоянном перемешивании. Приготовленную дисперсию замораживали в течение 5 часов, добавляя различные концентрации полимеров для приготовления предварительных партий. Дифференциальная сканирующая калориметрия (ДСК) и инфракрасная спектроскопия с Фурье-преобразованием (FTIR) были проведены для изучения совместимости адъювантов с клотримазолом. Последняя партия была подготовлена и оценена на предмет физико-химических параметров, экскреции клотримазола in vitro, противогрибковой активности in vitro и был проведён тест на раздражение влагалищной ткани in vivo.

**Результаты:** Исследование совместимости не показало химического взаимодействия между клотримазолом и используемыми адъювантами. Параметры оценки показали, что экскреция клотримазола проходила в диапазоне от 8 до 10 часов, температура образования геля была в диапазоне 27-35 °C, время образования геля было в диапазоне 28-34 секунд, рН был между 4,4-4,8, и вязкость была между 16,4-182,6 cП (раствор) и 10500-20,756 cП (гель). Зона ингибирования клотримазола в чистом виде, вагинального геля клотримазола и оптимизированной гелевой формулы составляли 9,15 ± 0,75 мм, 14,35 ± 1,12 мм и 18,85 ± 1,56 мм соответственно (р <0,0001, q = 5,98). Оптимизированная формула геля не вызывает раздражения влагалищной ткани.

**Заключение:** Возможно изготовление формулы эффективного in situ вагинального геля клотримазола с контролируемой экскрецией.