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Original Research

Development of a xanthan gum film for the possible treatment of vaginal infections

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Abstract: Bacterial vaginosis is a vaginal infection that affects 60% of women of reproductive age worldwide. It is mainly caused by the bacterium *Gardnerella vaginalis* and is a factor that increases the probability of getting sexually transmitted diseases. We aimed to develop a new pharmaceutical form for the treatment of vaginal infections. We employed the solving-casting method to fabricate a polymeric film with Xanthan gum, a natural polymer produced by the bacterium *Xanthomonas campestris*, and metronidazole, one of the most commonly used drugs for vaginal infections. In order to characterize the film, we measured pH, dose uniformity, dissolution profile, and the percentage of swelling. Moreover, we performed a thermogravimetric analysis and scanning electron microscopy. The results demonstrated a pH suitable for vaginal application and uniform distribution of the drug in the film. Also, the formulation exhibited a high percentage of swelling and a slow release of the drug in a simulated vaginal fluid medium. All these attributes indicated that the manufactured film has ideal characteristics to be used and administered vaginally. It could be an excellent alternative to treat bacterial vaginosis and also improve user adherence.

Key words: Natural polymers; Xanthan gum; Metronidazole; Polymeric film; Vaginal infection; Bacterial vaginosis; Solving-casting.

Introduction

The vagina is a membranous muscular tube that extends from the cervix to the vestibule of the vulva. The vagina of healthy women possesses an abundance of gram-positive bacilli (generally *Lactobacillus* spp), low pH (4-4.5), and absence of facultative and obligatory anaerobic gram-negative bacteria (1). The vaginal cavity is a moist anatomical region, very susceptible to changes in pH and, therefore, vaginal infections. Some risk factors are vaginal showers, multiple sexual partners, use of an intrauterine devices, and very tight underwear (2,3). Globally, one of the most common infections is bacterial vaginosis (BV); it is frequent in women of 15-67 years old and is estimated to occur in 60% of women (2). BV is characterized by an increase in whitish or yellowish vaginal fluid and a characteristic odor that usually intensifies after intercourse. BV is caused by a decrease in ordinary hydrogen peroxide-producing lactobacilli, with an overgrowth of anaerobic bacteria (4). The main bacteria associated with this infection is *Gardnerella vaginalis* (2). Nonetheless, other microorganisms have also been associated, including *Atopobium* spp, *Prevotella* spp, *Bacteroides* spp, *Mobiluncus* spp, *Sneathia* spp, *Leptotrichia* spp, and *Mycoplasma* spp, which create a biofilm that subsequently allows other opportunistic bacteria to grow within the vagina (2). Additionally, those women who suffer from BV are more susceptible to acquire other sexually transmitted infections, including the human immunodeficiency virus, gonorrhea, chlamydia, and the herpes simplex virus (2).

Recommended treatments for BV are oral metronidazole (MTZ; 500 mg twice daily for seven days), MTZ 0.75 % gel (intravaginally at bedtime for five days), and clindamycin 2% cream (intravaginally at bedtime for seven days) (4). MTZ is an azomycin-derivative synthetic antibiotic (5). MTZ is believed to cross the target cell membrane by passive diffusion, where its nitro group is reduced to nitro radicals by ferredoxin or flavodoxin. The reaction generates toxic metabolites such as N-(2-hydroxyethyl) oxamide and acetamide that can react with DNA and form adducts with proteins and nucleic acids (6). MTZ is available in oral, intravenous, vaginal, and rectal presentations. The oral presentation is the most clinically applied although new dosage forms have been sought (6).

New dosage forms through bioadhesive mucosa include adhesive tablets, gels, and patches. However, the use of polymeric films has exhibited great potential in recent years (7). Several advantages have been found in their possible vaginal application, such as efficient drug release, improved bioadhesive properties, negligible vaginal discharge, discreet use, and low cost. Likewise, the films are easy to insert (without applicator) and have longer contact time with the mucosa; thus, there is a reduction in treatment time and the frequency of applications (7,8). Moreover, vaginal films provide an additional female-controlled dosage form option, which might improve users' adherence (7).

These films are usually thin and disintegrate quickly, although they can be adapted for slower disintegration. Films are typically formulated with a drug, hydrophilic polymers, plasticizers, and surfactants, among others (7). Some of the most common methods for producing polymeric films are solving-casting, hot-melt extrusion, solid dispersion, and lamination methods (7). Among the most used polymers are hydroxypropyl methylcellulose, polyvinyl acetate, and polyvinylpyrrolidone. However, in recent years, natural polymers such as xanthan gum (XG), pullulan, carrageenan, guar gum, and alginate have been employed (9).

It is noteworthy that there are studies of films with MTZ and some natural polymers such as chitosan (10,11) and pectin (12). However, to our knowledge, no previous film studies with XG and any other nitroimidazole exist. Hence, our objective was to develop a new pharmaceutical form based on XG and MTZ (XG-MTZ film) for the treatment of BV using the solving-casting method (13). We characterized the films by tests of pH, dose uniformity, percentage of swelling, dissolution profile, Thermogravimetric Analysis (TGA), and Scanning Electron Microscopy (SEM). Our results indicated that the films have a homogeneous distribution of the drug, surface free of pores with the presence of MTZ crystals, a high percentage of swelling, and a prolonged release of the drug. Therefore, this vaginal film could be a new alternative for the treatment against BV.

Materials and Methods

Materials

XG, glycerin, urea, glucose, calcium hydroxide, sodium chloride, and lactic acid were purchased from Cosmopolita Drugstore (Mexico City, Mexico). Bovine serum albumin, potassium hydroxide, acetic acid, and MTZ were acquired from Sigma-Aldrich (Darmstadt, Germany).

Solubility of MTZ

Since the common solvent between XG and MTZ was water, 50 mg of MTZ was added to 30 mL of distilled water under magnetic stirring at 130 rpm and 50 °C. The addition of more drug was stopped at the moment when the solution was saturated.

Polymeric films

The films were fabricated using the solving-casting technique (13). The corresponding amount of MTZ (300 mg) was mixed with XG (10% p/v) and glycerin (1% p/v) in 40 mL of water with heating (50 °C), under constant stirring at 130 rpm. After mixing, the film was allowed to cool and cure for 24 h, then transferred to a plastic Petri dish or Teflon molds and dried in an oven at 70 °C for 12 h (OAKTON Stable Temp Oven, Illinois, USA). These conditions were established after optimization to slowly evaporate the solvent and obtain a thin and uniform polymeric film with the minimum amount of bubbles or pores possible (XG-MTZ film). Storage was carried out in a plastic Petri dish, taking care of sealing to avoid excessive dryness of the films. Samples without drug were prepared following the same procedure (XG film).

Preparation of the simulated vaginal fluid medium

The simulated vaginal fluid medium was prepared by mixing the following reagents: glycerin (0.16 g/L), sodium chloride (3.51 g/L), urea (0.4 g/L), glucose (5 g/L), potassium hydroxide (1.4 g/L), calcium hydroxide (0.22 g/L), acetic acid (1 g/L), lactic acid (2 g/L) and bovine serum albumin (0.018 g/L) (14,15). Distilled water was used as the solvent.

Validation of the quantification of MTZ in the film

The validation of the analytical method was carried out with the evaluation of the following parameters: selectivity, linearity, repeatability, and accuracy. The validation was performed following the FDA Guide: Analytical procedures and methods validation: chemistry, manufacturing, and controls documentation (16). All samples were analyzed in triplicate by spectrophotometry (DLAB SP-UV 1000, Beijing, China), at a wavelength of 277 nm.

Selectivity

In order to determine the selectivity, the following physical solutions and mixtures were prepared in 40 mL of distilled water: XG, glycerin, and MTZ (individually). Also, XG with glycerin (physical mixture), and XG with glycerin and MTZ (formulation). A physical mixture was used as a blank. Furthermore, the recovery percentage for MTZ was obtained.

Linearity

Three calibration curves were made with different concentrations of MTZ (15, 24, 33, 42, 51, and 60 µg/mL, using distilled water as solvent) to obtain a single calibration curve. The standard deviation (SD), the coefficient of variation (CV), and the correlation coefficient (r^2) were obtained.

Repeatability

In this case, the same procedure was used to determine linearity, but now for three different days. A single curve was obtained with its corresponding SD and CV.

Accuracy

The precision test was performed by taking three concentrations of MTZ (at random) within the calibration curve. Their absorbances were analyzed in triplicate, and the following data were obtained: SD, CV, and their recovery percentage.

Validation of the quantification of MTZ in the simulated vaginal fluid medium

The validation of the analytical method was carried out with the evaluation of the following parameters: selectivity, linearity, repeatability, and accuracy. The validation was performed following the FDA Guide: Analytical procedures and methods validation chemistry, manufacturing, and controls documentation (16). All the samples were analyzed in triplicate by spectrophotometry at a wavelength of 277 nm.

Selectivity

The selectivity analysis was performed with the following individual solutions (in 100 mL of distilled water): NaCl, KOH, $Ca(OH)_2$, bovine serum albumin, lactic acid, glycerin, urea, glucose, acetic acid, and MTZ. Their absorbances were recorded, and the dilutions corresponding to those that obtained values greater than zero were made to calculate the concentration corresponding only to MTZ in the following tests.

The physical mixture was carried out with the reagents mentioned in the previous paragraph, except for MTZ. All reagents and MTZ were mixed, the dilution obtained was analyzed directly, and the theoretical and real MTZ concentration was calculated, as well as its percentage of recovery. In the latter procedure, the physical mixture was used as a blank.

Linearity

The linearity test was performed with three calibration curves in the simulated vaginal fluid medium, as well as with different concentrations of MTZ (15, 24, 33, 42, 51, and 60 µg/mL) to obtain a single calibration curve. The SD, the CV, and the correlation coefficient (r^2) were obtained.

Repeatability

In this case, the same procedure as linearity was done, but now for three different days. A single curve was obtained with its corresponding SD and CV.

Accuracy

In order to obtain the test of precision, the simulated vaginal fluid medium was tested at three different concentrations of MTZ (at random), which were within the calibration curve. Their absorbances were analyzed in triplicate, and the following data were obtained: SD, CV, and their recovery percentage.

Dose uniformity

In order to determine the dose uniformity, XG-MTZ films were cut in three square pieces of 1 cm^2 from different zones of the film and individually introduced into 150 mL of simulated vaginal fluid medium with constant stirring (130 rpm) and a temperature of 37±2 °C. After 48 h, aliquots of 10 mL were extracted from each dissolved sample. Absorbances were analyzed in triplicate at a wavelength of 277 nm and determined the mg of MTZ in the film.

Dissolution profile

The MTZ release test was performed with three MTZ samples (control), three squares of 1 cm^2 of XG-MTZ (samples), and two blanks (one for the control and one for the sample). It should be mentioned that 1 cm^2 of the polymeric film is equivalent to approximately 4 mg of MTZ. The system was assembled as follows: a container was placed on a multi-rack (DLAB MS-M-S16, CA, USA), where a water bath was fixed at 37±2 °C. Once the beakers with their corresponding volume of 200 mL of the simulated vaginal fluid medium had been introduced, the containers were equilibrated at 37±2 °C before introduce the MTZ (3 samples of 300 mg) or the XG-MTZ samples.

The samples were introduced at different times to take the 10 mL aliquots with the least possible error. Finally, the absorbances of samples were analyzed by spectrophotometry at a wavelength of 277 nm, with their corresponding dilutions if necessary. The test was carried out under sink conditions (17).

TGA

The thermal analysis was carried out to determine the change in the physical properties of chemical compounds and materials as a function of temperature and time. The TGA measured the loss or gain of a sample mass when exposed to specific temperature changes, such as fusion, crystallization, boiling, sublimation, glass transitions, and polymorphic transformations.

This analysis was performed at a heating rate of 10 °C/min with a temperature range of 0-700 °C, under atmosphere nitrogen in a Q5000 calorimeter (TA Instruments, Delaware, USA). The individual analysis of the following excipients was made: XG, glycerin, and MTZ, with a sample mass of 3.160, 1.269, and 6.980 mg, respectively. The thermal analysis of the polymeric films was also carried out (XG and XG-MTZ) with a sample mass of 16.020 and 10.970 mg, respectively. It should be mentioned that the treatment of any of the samples was not required.

SEM

XG and XG-MTZ samples were analyzed through SEM to observe and characterize the surface of the films. It was carried out in an SEM equipment (CROSSBEAM 550, ZEISS) with a magnification of 38, 55, and 65 X, and a voltage of 3.89 and 4 kV. The samples were coated with a thin layer of gold by plasma-assisted deposition using the JEOL Fine Coat Ion JFC-1100 equipment.

pH

The pH of the polymeric film and the simulated vaginal fluid medium was registered with a pH potentiometer (Potentiometer PHS 3-C). For the XG-MTZ film, the pH was recorded when the material was fabricated.

Swelling test

The swelling test (13) was performed by cutting six pieces of 1 cm^2 from the XG film and six from the XG-MTZ film. Their masses (approximately 40 and 50 mg, respectively) were weighed on an analytical balance (Precisa Gravimetrics AG, Switzerland) and were introduced into test tubes (size: 10x100 mm, capacity: 5 mL) with the aid of a ribbed spatula. The test tubes contained 2 mL of the simulated vaginal fluid medium. The tubes were placed in a rack, covered with parafilm, and left at room temperature (25 °C). The samples were extracted from the medium at 3 and 7 days, and their masses were weighed again. The percentage of swelling was obtained with the following equation:

$$\%\ Swelling = \left[\frac{Wet\ sample\ mass - Dry\ sample\ mass}{Dry\ sample\ mass}\right] x100$$

Results

Solubility of MTZ

The maximum amount of MTZ that could be solubilized in the 30 mL of the selected solvent was 300 mg.

Polymeric films

The films obtained after drying had the following visual and texture characteristics:

XG (Figure 1A): Film with a circular shape, smooth, without the presence of bubbles or pores, odorless and translucent, elastic, and with bioadhesive properties. XG film had an area of approximately 79 cm^2 and a thickness of 0.30 mm.

XG-MTZ (Figure 1B): Film with a circular shape, smooth, without the presence of bubbles or pores, odorless, whitish in color, elastic, and with bioadhesive properties. XG-MTZ film had an area of approximately 79 cm^2, a thickness of 0.35 mm, and 300 mg of MTZ.

Validation of the quantification of MTZ in the film

In order to verify that the quantification method of MTZ in the formulation has the requirements for the desired analytical application, it was validated (amount of the MTZ in the film). In this case, the following parameters were evaluated: selectivity, linearity, repeatability, precision, and accuracy. These results are shown in Tables 1 and 2, as well as in Figure 2.

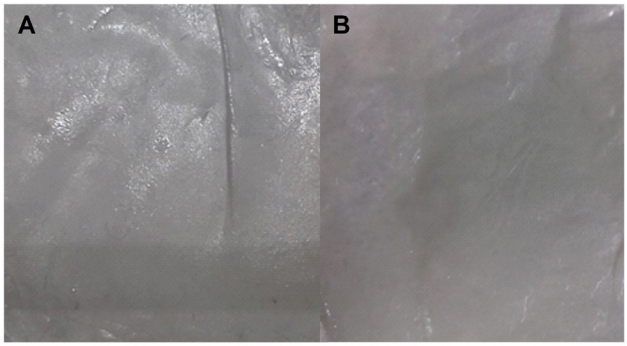

Figure 1. Macroscopic appearance of (A) XG and (B) XG-MTZ films.

Validation of the quantification of MTZ in the simulated vaginal fluid medium

The validation of MTZ in the simulated vaginal fluid medium was also performed. Different aspects were evaluated, which included selectivity, linearity, repeatability, precision, and accuracy. These results are detailed in Tables 3 and 4, as well as in Figure 3.

Dose uniformity

In this study carried out the evaluation of the dose uniformity of XG-MTZ film to verify the homogeneity of the drug in the pharmaceutical dosage. Dose uniformity fulfills with the validation requirements. The results are depicted in Table 5.

Dissolution profile

The MTZ release profile is represented in Figure 4.

Table 1. Absorbance values obtained during the MTZ selectivity test in the film.

	Absorbance without dilution	Absorbances with dilution		
Excipient	Individual	Individual	Physical mixture	Formulation
XG	1.895	0.004	0.008	0.45
Glycerin	0.052	0.007		
MTZ	3	0.560	---	

The dilution applied to the individual samples, physical mixture, and formulation was 1:200

Table 2. Parameters estimated during the validation of MTZ in the film.

Parameter	Acceptance requirements	Result	Qualify for requirements
Linearity	$r^2 > 0.98$	r^2 - 0.9994	Yes
Repeatability (Precision)	CV< 5 %	15 µg/mL = 0.37 %	Yes
		24 µg/mL = 2.15 %	Yes
		33 µg/mL = 0.45 %	Yes
		42 µg/mL = 1.04 %	Yes
		51 µg/mL = 2.27 %	Yes
		60 µg/mL = 1.49 %	Yes
Accuracy	97-103 % recovery	30 µg/mL = 101.32 %	Yes
		40 µg/mL = 102.65 %	Yes
		50 µg/mL = 105.50 %	No
Detection limit	-----	15 µg/mL	Yes
Limit of quantification	-----	5 µg/mL	Yes

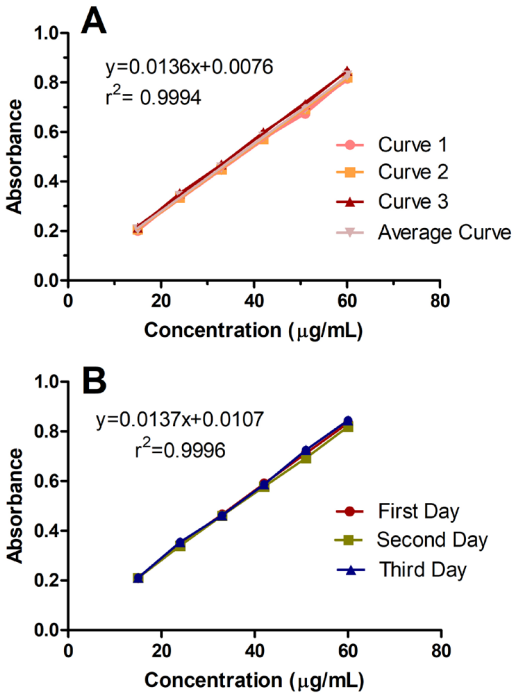

Figure 2. Graphs corresponding to (A) linearity and (B) repeatability of the validation of MTZ in the film.

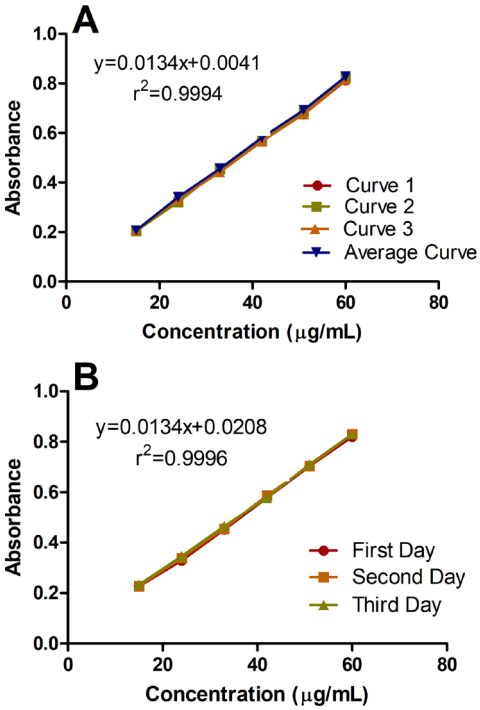

Figure 3. Validation of MTZ in the simulated vaginal fluid medium. Graphs correspond to (A) the linearity and (B) repeatability.

The release of MTZ from XG-MTZ began before 1 min with a release percentage of 47%, which increased rapidly until 5 min, where 89% release was reached. At 6 h, it was released almost 93% of MTZ, while MTZ (control) solubilized in 95% in just 15 min.

TGA

The thermograms obtained in the TGA analysis of the films and excipients used are shown in Figure 5. XG (Figure 5A) exhibited a weight loss of 42.47 % from 250 °C to 350 °C, while glycerin (Figure 5B) showed a weight loss of 85.81 % from 215 °C to 275 °C. °C. In the case of XG film (Figure 5C), a weight loss of 78.13% was found from 150 °C to 275 °C, while XG-MTZ had a weight loss of 76.69% from 150 °C to 275 °C. Finally, the MTZ thermogram (Figure 5E) revealed a weight loss of 95.44% in the temperature range of 155 °C to 225°C.

SEM

The morphology of the polymeric films was analyzed using SEM. The images for XG, which had a thickness of 267.8 µm, are shown in Figures 6A and 6B. In Figure 6C and 6D, we observed the images for XG-MTZ, which possessed a thickness of 255.3 µm. In this case, the MTZ is perceived on the surface as rectangular crystals with approximately 138 µm long and 24 µm wide.

pH

The pH obtained for the simulated vaginal fluid medium was 4.53. On the other hand, the pH of the polymeric film was 5.1.

Swelling test

The percentage of film swelling at 3 and 7 days, with and without the drug, is represented in Figure 7. In the case of XG, a percentage of swelling greater than 200% was achieved in 7 days, while only 185% was reached

Table 3. Absorbance values obtained during the MTZ selectivity test in the simulated vaginal fluid medium.

	Absorbance without dilution	**Absorbances with dilution**		
Excipient	Individual	Individual	Physical mixture	Physical mixture + MTZ
NaCl	0.002	0		
KOH	0.003	0		
$Ca(OH)_2$	0.052	0.002		
Bovine Serum Albumin	0.022	0		
Glycerin	0	0	0	0.632
Urea	0.0	0		
Glucose	0.0	0		
Lactic acid	0.012	0		
Acetic acid	0.0	0		
MTZ	3.000	0.682	----	

The dilution applied to the individual samples, physical mixture, and formulation was 1:150.

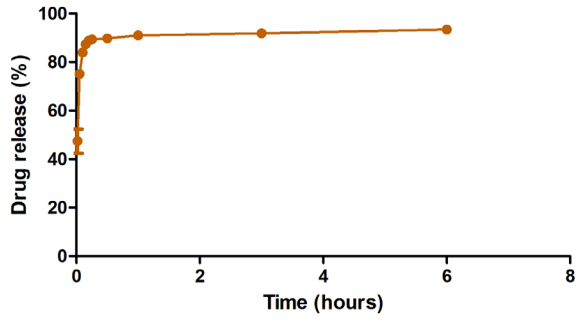

Figure 4. MTZ release profile from XG-MTZ polymeric film in a simulated vaginal fluid medium.

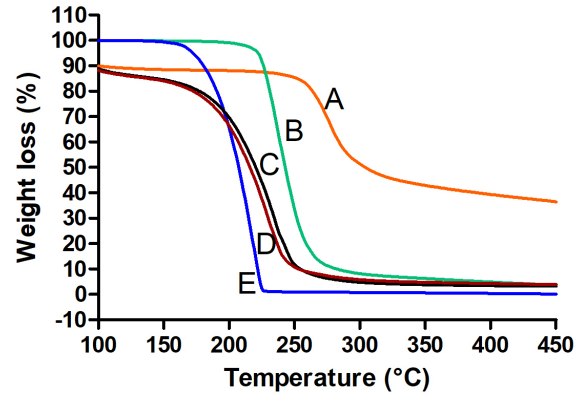

Figure 5. TGA of the excipients of the polymeric film and TGA of the polymeric films: (A) XG, (B) Glycerin, (C) XG film, (D) XG-MTZ film, and (E) MTZ.

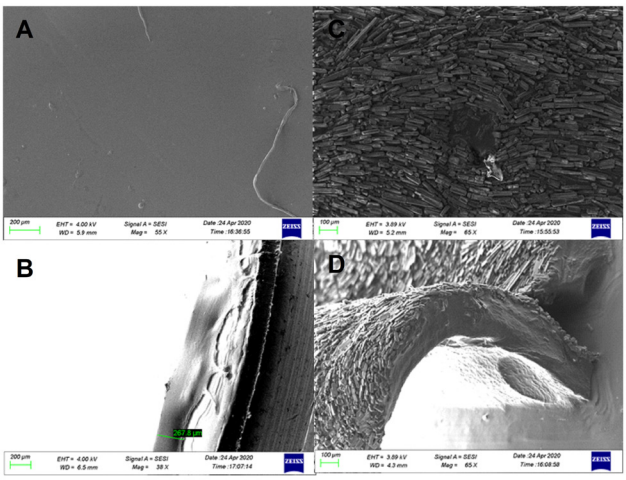

Figure 6. Scanning Electron Microscopy (SEM) images obtained from XG with a measurement bar scale equivalent to 200 µm and a magnification of 55X (A) and 38 X (B), respectively. XG-MTZ film is exhibited with a magnification of 65X (C) and (D), the scale of the measuring bar is equivalent to 100 µm.

with XG-MTZ. In both films, more than 100% of the swelling was achieved after three days.

Discussion

The films for the release of antiparasitic drugs in the pharmaceutical area must have select physical and chemical properties to ensure the release of the drug at the site of action. Thus we performed a variety of tests to characterize the XG-MTZ film.

The maximum amount of MTZ that we could solubilize in 30 mL of the selected solvent for film fabrication was 300 mg, which was consistent with reported MTZ solubility (10,000 mg/L at 25 °C) (18). It is essential to point out that commercial MTZ presentations are of dosages between 250-500 mg (6). Therefore, the amount of MTZ solubilized was according to commercial dosages.

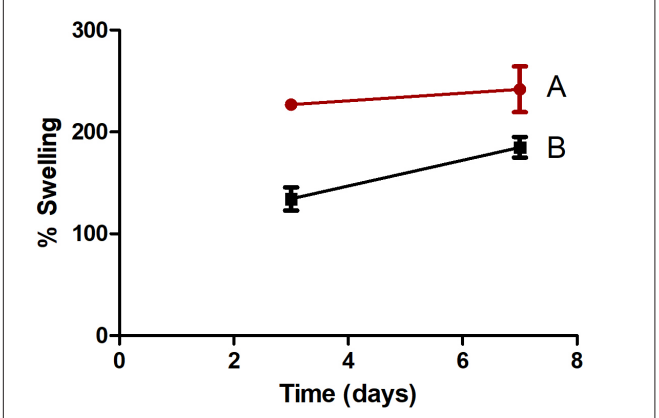

Figure 7. Percentage of swelling of (A) XG and (B) XG-MTZ films as a function of time in the simulated vaginal fluid medium.

Our XG-MTZ films were homogeneous in thickness, smooth surface, elastic, bioadhesive, without bubbles or pores (Figure 1), with the capacity for controlled drug release, and the potential to offer a longer retention time at the application site. These are some of the characteristics sought in the pharmaceutical area for this type of formulations since the vaginal tract has a self-cleaning action (19). That self-cleaning mechanism causes some difficulties, including the expulsion of many pharmaceutical formulations, the fast dissolution of the dosage form, and reduced contact time between the formulations and the vaginal mucosa, which leads to a decreased therapeutic effect (8). For this reason, our XG-MTZ film may be an option for the treatment of BV because it is aesthetically pleasing, thin, and easy to insert without the need for an applicator (7).

Validation is the process by which the capability of a method to satisfy the requirements for any analytical application is demonstrated (17). In this research, we evaluated selectivity, linearity, repeatability, precision, and accuracy. The validation was performed for both MTZ in the film and MTZ in the simulated vaginal fluid medium for the dissolution profile experiment. In the experiment of selectivity, we obtained the absorbance analysis corresponding only to the analyte of interest within the range of reliable analyses (absorbances 0.2-0.8), until a 1:200 dilution (Table 1). This result indicated that the formulation's excipients would not interfere with the MTZ signal in the subsequent tests. Moreover, the quantification of MTZ in the film fulfilled the established criteria in the experiments of linearity, repeatability, and precision (Figure 2 and Table 2). Similarly, during selectivity analysis for the simulated vaginal fluid medium, we only recorded the absorbance corresponding to the analyte of interest (Table 3). The result was within the range of reliable analyses (absorbances 0.2-0.8). However, we utilized a dilution of 1:150. We found the excipients did not interfere in the quantification of MTZ in the simulated vaginal fluid medium, complying with the established criteria for linearity, re-

Table 4. Parameters estimated during the validation of MTZ in the simulated vaginal fluid medium.

Parameter	Acceptance requirements	Result	Qualify for requirements
Linearity	$r^2 > 0.98$	r^2 - 0.9994	Yes
Repeatability (Precision)	CV< 5 %	15 µg/mL = 1.80 %	Yes
		24 µg/mL = 2.69 %	Yes
		33 µg/mL = 1.56 %	Yes
		42 µg/mL = 0.98 %	Yes
		51 µg/mL = 0.62 %	Yes
		60 µg/mL = 0.71 %	Yes
Accuracy	97-103 % recovery	25 µg/mL = 98.78 %	Yes
		37 µg/mL = 97.13 %	Yes
		49 µg/mL = 98.93 %	Yes
Detection limit	-----	15 µg/mL	Yes
Limit of quantification	-----	5 µg/mL	Yes

Table 5. Result and evaluation of dose uniformity of the polymeric film.

Sample	Acceptance requirements	Result	Qualify for requirements
1	85 %-115 %	85.46 %	Yes
2		85.17 %	Yes
3		85.03 %	Yes

peatability, and precision analyses (Figure 3 and Table 4). Therefore, the method employed fulfilled the desired requirements and guaranteed reliable results when proceeding with experimental tests

On the other hand, a dose uniformity test is performed to verify that the pharmaceutical dosage form contains a single dose or part of a drug dose in one unit (20). In this study, we proposed a polymeric film that satisfies the classification of a transdermal system. Thus, the acceptance criterion is that the result must range from 85 to 115% (20). Table 5 exhibits the acceptance criteria for our polymeric film, which indicates that it could be appropriate and effective in the treatment against BV since there is a homogeneous distribution of the drug throughout the area of the XG-MTZ film.

In order to determine the amount of MTZ released from the XG-MTZ film, we performed an *in vitro* release profile in a simulated vaginal fluid medium (Figure 4). The release of the drug began before 1 min and increased rapidly until 5 min. After 5 min and up to 6 h, the release of MTZ was slow and in a lower percentage. At six h, we found that 93% of MTZ was released from the polymeric film. On the other hand, MTZ as control, solubilized by 95% in just 15 min, indicating a reduction of half in the time of disposition of the drug in the biological environment. This finding suggests that the excipients' interaction with the drug helped its retention in the polymeric film to obtain a prolonged release. This property is highly desirable in our XG-MTZ film since, together with the mucoadhesiveness, it will cause a longer retention time at the application site, a greater bioavailability, and a controlled release. With the above, there would be a reduction in the frequency of applications and treatment time, which would undoubtedly improve user adherence (7,8). In this context, a previous study developed different polymeric films with pectin, glycerin, and MTZ for periodontal applications. The authors demonstrated the release profiles of three different formulations HM-G-MZ, LM-G-MZ, and LM-G-MZ in Tris Buffer with a pH of 6.6 (where HM is high methoxyl pectin, LM is low methoxy pectin, G is glycerin, and MZ is MTZ) (12). For the LM-G-MZ formulation, the authors described a release profile very similar that the obtained by our XG-MTZ film (Figure 4), since they found a release of 60% from the first 5 min, and it increased until reaching up to 80% in 60 min, and 92% released in approximately seven days. In the same way, the increase was slow from 12 h (12).

In order to characterize the interaction of excipients utilized for the XG-MTZ fabrication, we determined their thermal properties by TGA. We observed a substantial weight loss (42.47%) in the XG profile, approximately from 250 °C to 350 °C. This result agrees with previous research, where the characterization of XG obtained from sugar cane underwent a 40% weight loss at a temperature of 220-320 °C (21). This loss may be due to the breakdown of bonds of this polymer, including hydrogen bonds. Likewise, we observed that glycerin had a mass loss of 85.81% from 215 °C to 275 °C, a finding that is consistent with the literature (22). This mass loss could correspond to the loss of humidity (since glycerin is a hygroscopic component) combined with a pyrolysis process (where methane would be released) (22,23). Furthermore, we found a weight loss of 95.44 % in the record of MTZ in the temperature range of 155 °C to 225 °C. This is due to the melting of the drug because the melting temperature is reported at 160 °C (24). Other studies determined that MTZ suf-

fers a weight loss of 94.1% between 200 °C and 250 °C, similar to our result (25). Finally, we observed that the XG and XG-MTZ films had a very similar weight loss (76.69 % and 78.13%, respectively), both with the same temperature range from 150 °C to 275 °C (Figure 5C and 5D). Therefore, the addition of the MTZ did not alter the thermal stability of the film.

We also analyzed the morphology of the polymeric film by SEM (Figure 6). The XG-MTZ film presented a flat and smooth surface. We detected the presence of MTZ as rectangular shaped crystals of approximately 138 µm long and 24 µm wide. Other investigations that have prepared films with MTZ also reported drug crystals on their surface (19,26), which is desirable because it creates the first front of drug dissolution against *Gardnerella vaginalis* and other microorganisms that cause BV.

The pH obtained for the artificial vaginal fluid medium was 4.53, which is within the reported range of 4.2-4.5 (15,27), while the pH of the polymeric film was 5.1. These pH values are adequate because the normal vaginal pH is usually within the range of 4-4.5. This low pH value is caused by the breakdown of glycogen present in the vaginal epithelium, followed by the fermentation of carbohydrates and the production of lactic acid (1). Since the XG-MTZ film's pH is very close to that of the normal vaginal pH, it may be safe to insert and use as a treatment against BV, without causing any damage to this cavity by the difference in pH.

The swelling process in polymeric films usually controls the drug's release and is related to the degree of bioadhesion. A swelling greater than 200% was reached in seven days for XG (Figure 7), while XG-MTZ only reached 185%. This may be because, in the latter case, there is not only an interaction between polymer-plasticizer, there is also an interaction between polymer-plasticizer-drug, which is found on the surface of the film in a crystalline form and is moderately soluble in water. The swelling experiment in the simulated vaginal fluid medium also showed that the XG-MTZ film remained undissolved for at least seven days in vaginal fluid conditions, releasing the drug for a long time.

In summary, we developed an XG-MTZ film with beneficial characteristics for its vaginal administration. Our formulation could offer less frequency of applications with continuous drug release to provide effective treatment and higher adherence. Although further experiments will be needed to corroborate this assumption, it is expected that this film can be considered as an alternative for the treatment of BV (Figure 8).

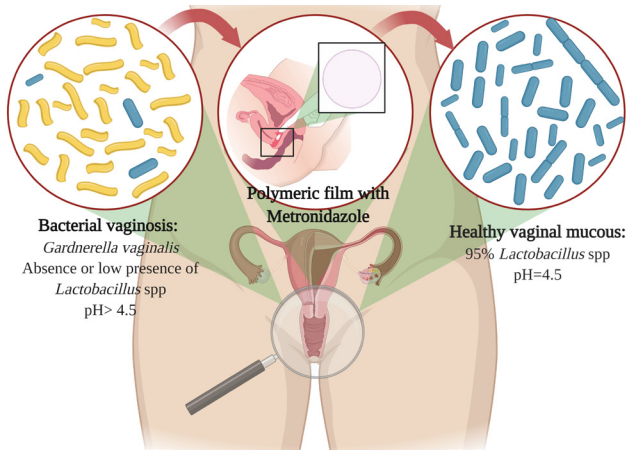

Figure 8. Expected conditions of the vaginal cavity before and after treatment with XG-MTZ, present in the pharmaceutical form proposed in this experimental work.

This study proposed a novel polymeric film as an alternative for the treatment of BV. The film offers characteristics as a slow-release, high percentage of swelling, and uniform drug distribution. These attributes would increase the contact time, thereby decreasing the treatment time and the frequency of application. Also, the film offers an adequate pH that would not alter or cause discomfort when inserting into the vaginal cavity, in addition to having a pleasant visual appearance and being easy to insert. The film composition is a natural polymer proven in the cosmetic and food industry; therefore, it offers wide biosecurity.

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Conflicts of Interest

The authors declare no conflict of interest.

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