Formulation and Evaluation of Mucoadhesive Vaginal Films of Ketoconazole

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ABSTRACT

Purpose: The aim of the present study was to formulate and evaluate mucoadhesive films containing Ketoconazole (KT) for the treatment of vaginal infections. Method: The vaginal films were made by solvent casting technique with different ratios of chitosan as mucoadhesive polymer. The prepared films were evaluated for their weight uniformity, thickness uniformity, drug content, folding endurance, in vitro diffusion, in vitro mucoadhesion study, in vitro dissolution and accelerated stability studies at 40°C ± 2°C and 75% ± 5% for three months. Results: Compatibility studies by FTIR showed that there was no significant interaction between drug and polymer used. The weight of films was found to be between 1.03 g to 2.47 g. The thickness of films ranged between 0.15 mm to 0.32 mm. The drug content was found to be between 80.5% to 93.7% w/w. The folding endurance of films was found to be between 72.6 and 175.66. Mucoadhesive strength of films ranged between 17.45 g to 26.72 g. F4 was selected as the best formulation based on the evaluation studies and it showed zero order release. Conclusion: Based on the in vitro results it was concluded that the mucoadhesive films prepared with chitosan can be used as controlled drug delivery system and frequency of drug administration can be minimized leading to better patient compliance.

Key words: Chitosan, Ketoconazole, Mucoadhesive films, Mucoadhesive polymers, Vaginal films.

INTRODUCTION

Administering drugs through the vaginal route has been an area of great promise and curiosity to the scientists and pharmaceutical industry.1 The chief benefits of vaginal drug delivery compared to conventional routes of drug delivery are the evasion of the hepatic first-pass metabolism and relatively high permeability for diverse drugs. Additionally, other important factors, such as accessible surface area, rich vasculature and fewer side effects, make it a prospective route for both local and systemic delivery of drugs.2 On the other hand, conventional vaginal dosage forms, such as creams, foams, pessaries and jellies, are considered to dwell at the targeted site for a relatively less period of time due to the self-cleansing action of the vaginal tract resulting in diminished therapeutic effect.3 Consequently, there is a demand to develop effective drug delivery systems that would lengthen the contact of the drug with mucosal surface and enable sustained release of the included drug to gain better drug therapy. The function and potential of mucoadhesion in drug delivery as a technique for prolonging residence time for several drug formulations, has been of interest in pharmaceutical industry since the early 1980s.4 Mucoadhesion is frequently defined as the adhesion between two materials, of these materials one should be a mucosal surface, like vaginal mucosa in this particular study.5 A number of factors contribute to mucoadhesion, such as molecular weight, pH, charge, hydrogen bonding capacity and concentration of polymer.6,7
Key advantages concerning vaginal administration are the possibility of prolonged in situ residence and intimate contact with mucus, hence superior and prolonged drug delivery. Among various polymers, chitosan is favored with respect to safety and mucoadhesiveness. It is a common, natural-origin mucoadhesive polymer, suitable as a stable vehicle for the vaginal administration of drugs. Films have been extensively used as drug carriers for the treatment of both local and systemic diseases. They are able to incorporate a range of both hydrophilic drugs and due to their ability to sustain and/or control the release of entrapped drug. Films are also apt for vaginal application.

Vaginal candidiasis can be treated with topical antifungal drugs, such as, ketoconazole, miconazole, itraconazole, clotrimazole. Ketoconazole is an anti-fungal drug which is a synthetic imidazole analogue. It is effective for superficial fungal infections, genital candidiasis and chronic mucocutaneous candidiasis. It is used in immuno-compromised patients and advanced prostatic carcinoma. It can be used in the treatment of vaginal infections and as a suitable agent against vaginitis.

The objective of this study was to prepare a mucoadhesive vaginal films that could control the release of a sufficient amount of drug for a long time in vaginal mucosa to treat the fungal infections. In this study the pharmaceutical characteristics of film including the weight, thickness, folding endurance, rate of drug release, in vitro diffusion, mucoadhesive strength has been evaluated.

**MATERIALS AND METHODS**

The materials used included Ketoconazole powder (Fine Chem, Peenya, Bangalore), chitosan, polyvinylpyrrolidone (PVP K-30), propylene glycol (S.D. Fine Chem. Ltd., Mumbai), and acetone (Qualigens Fine Chemicals, Mumbai).

The instruments used in this study included UV/VIS Spectrophotometer UV 2301 (Shimadzu Corporation, Japan), FTIR Spectrophotometer (Shimadzu 8400 series, Japan), pH meter (Digisun Electronics, Mumbai), Magnetic stirrer (Remi equipments, Mumbai), Dissolution apparatus (Labindia Analytical Instruments Pvt. Ltd. Bangalore) and Hot air oven (Labline Instruments, Kochi).

**Preparation of Ketoconazole films**

Solvent casting was employed to formulate Ketoconazole films. Varying concentrations of chitosan were used to prepare batches coded F1-F8. Table 1 contains the composition of prepared mucoadhesive films. Chitosan solution was prepared in 1.5% (v/v) acetic acid in distilled water, by stirring for 48 h, utilizing a magnetic stirrer. The weighed quantity of drug was added to the above solution, while stirring and put aside for 2 h to yield a uniform solution devoid of air-bubbles. Polyvinylpyrrolidone (PVP K-30) was stirred into the chitosan solution to augment the release of drug. Subsequently, propylene glycol (5% v/v) was incorporated as a plasticizer with constant stirring. The ensuing solution was set aside at room temperature to produce a clear solution, free from bubbles. Then, this solution was carefully introduced into a glass Petri dish of 6 cm diameter. The dummy patch devoid of drug was also made. The Petri dishes were kept on flat surface and covered by inverted funnel to permit controlled evaporation of solvent at room temperature. Dried films were carefully detached and inspected for any defects or air bubbles. The patch composed of Ketoconazole drug was packed in aluminum foil and stored in an airtight glass container to maintain the integrity and elasticity of the patches. The formula for Ketoconazole mucoadhesive films is represented in Table 1.

**Evaluation of Mucoadhesive Films**

**Physical appearance**

Patches were physically inspected for color, clarity and surface texture.
Vernier caliper was utilized to measure the thickness with a least count of 0.01 mm. Mean and standard deviation values were calculated.

**Uniformity of weight**

The film was cut into portions of size 2×2 cm² and weight of each portion was taken individually, mean and standard deviation values were calculated.

**Folding endurance**

A ribbon of film (2×2 cm²) was cut and repetitively folded at the same place till it split. The number of times the film could be folded at the same place without splitting yielded the value of folding endurance.

**Drug content uniformity**

A square piece of film measuring 4 cm² was cut and drenched in a beaker containing 100 ml of SVF. The contents were stirred by ultrasonicator for 24 h to dissolve the patch. Suitable aliquots were made and filtered. The absorbance of the filtered solution was found out by using UV-visible spectrophotometer at 277 nm.

**In vitro release study**

The drug release from mucoadhesive films was determined using the USP dissolution apparatus by basket method. A strip of mucoadhesive film (2×2 cm²) was cut and attached over the basket by using tap in such a way that drug can be released without obstruction. 900 ml of Simulated Vaginal Fluid (SVF) was used as dissolution medium and the study was continued up to 12 h. Stirring rate was maintained at 50 rpm and temperature at 37 ± 0.5°C. The aliquots (1 ml) were withdrawn at predetermined time intervals and replenished with same volume of SVF of pH 4.2. The samples were analyzed for drug content using UV spectrophotometer at 277 nm.

**In vitro diffusion study**

A section of dialysis membrane was positioned between donor and receptor compartment of Franz diffusion cell. A strip of mucoadhesive film (2×2 cm²) was placed on it which was wetted with 1 ml of SVF. The receptor compartment was filled with 50 ml of SVF, which was magnetically stirred. The aliquots (1 ml) were withdrawn at predetermined time intervals and replaced with same volume of SVF of pH 4.2. The samples were analyzed for drug content using UV spectrophotometer at 277 nm.

**Mucoadhesive strength**

A strip of mucoadhesive film (2×2 cm²) was cut and smeared onto the surface of flat faced disk attached to the top pan balance. The disk was then placed to the dialysis membrane wetted with SVF pH 4.2 attached to a flat immovable surface. After a contact time of 2 min, weights were gradually added on the other side of the top pan balance and the weight required for detaching the mucoadhesive film from mucosa was calculated as the strength.

**Mucoadhesion time**

A section of dialysis membrane was tied onto a glass slide. A 4 cm² mucoadhesive film was placed on the membrane, which was previously wetted with SVF. The complete assembly was then attached to basket rack assembly of USP tablet disintegrating test apparatus.
Table 3: The drug content, mucoadhesive strength, mucoadhesion time of formulated films

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug content (%)</th>
<th>Mucoadhesive strength ± SD (g)</th>
<th>Mucoadhesion time ± SD (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.03 ± 0.015</td>
<td>0.15 ± 0.015</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>F2</td>
<td>1.04 ± 0.037</td>
<td>0.17 ± 0.015</td>
<td>72.6 ± 4.93</td>
</tr>
<tr>
<td>F3</td>
<td>1.33 ± 0.020</td>
<td>0.22 ± 0.015</td>
<td>121.6 ± 8.08</td>
</tr>
<tr>
<td>F4</td>
<td>1.51 ± 0.005</td>
<td>0.27 ± 0.005</td>
<td>175.66 ± 3.78</td>
</tr>
<tr>
<td>F5</td>
<td>1.74 ± 0.030</td>
<td>0.29 ± 0.015</td>
<td>89.33 ± 3.51</td>
</tr>
<tr>
<td>F6</td>
<td>2.02 ± 0.020</td>
<td>0.30 ± 0.015</td>
<td>97.66 ± 6.02</td>
</tr>
<tr>
<td>F7</td>
<td>2.25 ± 0.005</td>
<td>0.31 ± 0.01</td>
<td>96.66 ± 2.51</td>
</tr>
<tr>
<td>F8</td>
<td>2.47 ± 0.011</td>
<td>0.32 ± 0.01</td>
<td>87.33 ± 1.33</td>
</tr>
</tbody>
</table>

Table 4: Characteristic IR spectrum of Ketoconazole

<table>
<thead>
<tr>
<th>Band position (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3032</td>
<td>Aromatic C-H</td>
</tr>
<tr>
<td>2966, 2926</td>
<td>Sp³ CH stretching</td>
</tr>
<tr>
<td>1572</td>
<td>Carbonyl group (C=O)</td>
</tr>
<tr>
<td>1504, 1454</td>
<td>Aromatic unsaturation</td>
</tr>
<tr>
<td>1294</td>
<td>C-O stretch</td>
</tr>
<tr>
<td>1178</td>
<td>C-N stretch</td>
</tr>
<tr>
<td>555</td>
<td>C-Cl</td>
</tr>
<tr>
<td>1404</td>
<td>C=N</td>
</tr>
</tbody>
</table>

Table 5: Characteristic IR spectrum of Ketoconazole and Chitosan

<table>
<thead>
<tr>
<th>Band position (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3429, 3399</td>
<td>-NH₂ Group</td>
</tr>
<tr>
<td>2924</td>
<td>Sp³ CH₃</td>
</tr>
<tr>
<td>1575</td>
<td>C=O</td>
</tr>
<tr>
<td>1502, 1454</td>
<td>Aromatic substitution</td>
</tr>
<tr>
<td>1294</td>
<td>C-O stretch</td>
</tr>
<tr>
<td>1178</td>
<td>C-N stretch</td>
</tr>
<tr>
<td>607,561</td>
<td>C-Cl</td>
</tr>
</tbody>
</table>

Figure 1: In vitro dissolution graph of Ketoconazole films (F1 to F8)

The disintegrating test apparatus was operated such that membrane was given regular up and down oscillations in a beaker containing SVF which was maintained at 37° ± 2°. The time taken for the mucoadhesive film to completely detach from membrane was noted.

**Accelerated stability studies**

The accelerated stability studies were conducted on the best formulation to check the influence of environmental factors like temperature and humidity. The best formulation was selected on the basis of physicochemical characteristics, in vitro drug release of the formulations. The formulation was subjected to accelerated stability studies as per ICH guidelines (40 °C ± 2°, 75 ± 5% RH). The most satisfactory formulation was sealed in an aluminum foil and stored at 40 ± 2°, 75 ± 5% RH for 3 months.

**RESULTS**

The results of physical evaluation of vaginal films are shown in Table 2, the drug content, mucoadhesive strength, mucoadhesion time are shown in Table 3, the characteristic bands of Ketoconazole spectra are shown in Table 4, the characteristic bands of Ketoconazole mixed with chitosan is shown in Table 5. The in vitro dissolution, in vitro diffusion, drug and drug-polymer FTIR spectra are shown in Figure 1-4 respectively.
Figure 2: Graphical representation of *in vitro* diffusion of the films (F1 to F8)

Figure 3: FTIR spectra of physical mixture of Ketoconazole

Figure 4: FTIR spectra of physical mixture of KT and Chitosan
Figure 5: Zero order release of the best formulation (F4)

\[ y = 8.230x + 0.203 \]
\[ R^2 = 0.996 \]

Figure 6: First order release of the best formulation (F4)

\[ y = 0.124x + 2.210 \]
\[ R^2 = 0.971 \]

Figure 7: Korsmeyer-Peppas release of the best formulation (F4)

\[ y = 5.385x + 0.421 \]
\[ R^2 = 0.767 \]
**Kinetic study of the best formulation**

The kinetic study of the best formulation is shown in Figure 5-8.

**Stability data**

**Physical appearance**

There was no change in color of the films. They were transparent and smooth as before the stability studies.

**Drug content**

The drug content before the stability study was 93.7% and after the study it was 93%.

**Folding endurance**

The folding endurance before the stability study was 175.66 ± 3.78 and after the study it was 175.66 ± 2.51. FTIR spectrum of best formulation after stability studies is shown in Figure 9.

**DISCUSSION**

The results indicate that there was only a little difference in the thickness of film within the formulations. The thickness of films was found to increase with the increase in polymer ratio. The weight of patches was found to increase with the increase in polymer ratio. The folding endurance of the films was in the following order F2>F1>F8>F5>F7>F6>F3>F4. It depicts that all formulations had good film properties. Drug content of the film was carried out to ascertain that the drug is uniformly distributed into the formulation. From the results obtained, it is clear that there is proper distribution of Ketoconazole in the films. Hence it can be concluded that drug was uniformly distributed in all the formulation. The mucoadhesive strength of the vaginal films was in the following order F1>F2>F3>F4>F5>F6>F7>F8. Though the increase in polymer concentration increased the mucoadhesive...
strength the difference was not significant enough. The in vitro diffusion studies of films done by USP dissolution apparatus I showed that formulations F2, and F6 released drug up to 6 h; formulations F3, and F5 released drug up to 8 h; formulations F7, and F8 released drug up to 10 h and formulation F4 released drug up to 12 h. Based on the evaluation, formulations F4 was selected as the best formulation.

The best formulation was subjected to accelerated stability studies as per ICH guidelines at 40 ± 2°C and 75% ± 5% RH for three months. The patch was assessed for colour, appearance and flexibility. There was no change in the physical appearance, folding endurance and drug content. Furthermore, the FTIR revealed that polymer has suppressed some of the functional groups of the drug, and hence no sharp peaks could be identified. Previous literature reports formulation of ketoconazole bioadhesive films based on sodium carboxymethylcellulose and hydroxypropyl methylcellulose (HPMC K-100) polymers. The results of the previous work showed that the vaginal films released the drug for a maximum period of 7 h, whereas, the present research shows that the drug was released for a period of 12 h. Hence, more sustained release is observed in the present research work. This effect may be due to the use of chitosan which is less susceptible to erosion when compared to HPMC.13

CONCLUSION
From the present work it may be concluded that the formulation F4 prepared by using chitosan (2.5%) is better for sustained release of drug up to 12 h. The kinetic study showed that formulation F4 followed zero order kinetics (R²=0.996). Results of the present study demonstrate that Ketoconazole vaginal films with mucoadhesive polymer chitosan can be used as controlled drug delivery system and frequency of drug administration can be minimized. Finally delivering KT in form of mucoadhesive films may provide better patient compliance. The vaginal delivery via mucoadhesion phenomena can also improve therapeutic profile by improved local availability of the drug, compared to oral route.

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CONFLICTS OF INTEREST
The authors declare that there is no conflict of interest.

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