FORMULATION AND EVALUATION OF BIOADHESIVE MICONAZOLE NITRATE GEL FOR VAGINAL CANDIDIASIS

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Abstract

Miconazole nitrate is an imidazole derivative and has higher efficacy in the treatment of the protozoal and anaerobic bacterial infection of the vagina. The purpose of the present study was to formulate miconazole nitrate bioadhesive vaginal gel using different bioadhesive polymers such as Carbopol 934P, Hydroxypropylmethyl cellulose (HPMC), and Sodium carboxy methyl cellulose (NaCMC). Further, the bioadhesive gels were evaluated for determination of drug content, pH, viscosity, bioadhesion strength, in vitro release through artificial diffusion barrier and effect of flow rate on release of drug from bioadhesive gels. The results obtained revealed that NaCMC was found to have the highest bioadhesive strength. Viscosity studies revealed that as polymer concentration increased, viscosity also increased. The in vitro drug release was found to be diffusion controlled and the process followed first order kinetics. These findings indicate that the prolong release of bioadhesive vaginal gel formulation of miconazole nitrate is effective dosage form for treating vaginal candidiasis.

Keywords: Bioadhesive gels, Carbopol, HPMC, Miconazole nitrate, NaCMC, Vaginal candidiasis.

Introduction

Vaginal candidiasis (VC) is a fungal infection of the female lower genital tract-the vulva and the vagina caused by Candida species (Sobel, 2007; Nyirjesy et al., 2003; Marrazzo, 2002). Vaginal candidiasis is a condition which is associated with severe inflammation of vagina, itching and thick milky discharges. It is one of the most frequent
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gynecological diseases. Literature shows that 30-50% of vaginitis is due to Candida infection and two third of women experience acute episodes of vaginal candidiasis at least once during in their lifetime.

The vagina, as a site of drug delivery, offers certain unique features that can be exploited in order to achieve desirable effects. The vaginal route of administration is a favorable site for local and systemic delivery of drugs that can be used specifically for female related conditions. The vaginal cavity has been used for local delivery of drugs such as prostaglandins, steroids, antibiotics, antifungal, antiviral, antiprotozoal and spermicidal agents.

Conventional formulations for intravaginal delivery comprises of creams, foams, pessaries and jellies that are designed to disperse drug throughout the vaginal tract. Long residence time is often required for the activity of vaginal administration of antimicrobial drugs. Vaginal formulations are prone to leakage and their efficacy is limited by a poor retention at the site of action. Patients discontinued the treatment because of inconvenience for usage. This is also a major reason for poor acceptability and usefulness of intravaginal medications. These vaginal formulations are associated with limitations such as poor retention, leakage and messiness, thereby causing inconvenience for users. To overcome these limitations, formulations that adhere to vaginal mucosa for a sufficient period of time need to be developed.(Robinson and Bolonga 1994, Vermani and Garg 2000) Bioadhesion and prolonged retention are desirable characteristics can be built in vaginal formulation by the use of bioadhesive polymers. Hence, the vaginal route of administration offers a promising option for local and systemic delivery of drugs with the use of bioadhesive polymers. Vaginal gels has advantages wide acceptability, feasibility and low cost (J. Das Neves 2006)

The objective of the present study includes novel approach to formulate miconazole nitrate in the form of different gels using various bioadhesive polymers Carbopol 934 P, cellulose derivative Hydroxypropylmethylcellulose (HPMC) and Sodium CMC (NaCMC) and evaluating them for different parameters including bioadhesive strength and in vitro release of miconazole nitrate.

Materials and Methods

Materials:
Miconazole nitrate was kindly supplied by Bhavani Pharmaceuticals, Kanpur. Hydroxypropylmethylcellulose (HPMC) was obtained by Banner Pharma caps. Bangalore. Carbopol 934 P and Methanol were obtained from Loba...
Chemie, Mumbai. Sodium CMC (NaCMC) was obtained from Baker, Mumbai. Triethanolamine, tween 80 and glycerol were obtained from Ranbaxy Fine Chemicals, New Delhi. Dialysis membrane was obtained from Hi media labs, Mumbai.

All chemicals used in the current study were of analytical grade.

Methods:

Spectrophotometric scanning of Miconazole Nitrate

A specified concentration of miconazole nitrate in mixture of methanol and water, and drug solution in all prepared gel bases in the same medium were scanned spectrophotometrically at 200 – 400 nm to determine the wavelength of maximum absorption (Shimadzu-1601, Japan). The absorption maxima of Miconazole nitrate were found to be 272 nm and this wavelength was used for further studies.

Preparation of bioadhesive Vaginal Gels:

Formulations of bioadhesive vaginal gels containing miconazole nitrate and with excipients are listed in Table (1). The formulated gels were prepared according to the following procedures:

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug (in mg)</th>
<th>Gel Base</th>
<th>Concentration of the Polymer</th>
<th>Methylparaben</th>
<th>Triethanolamine</th>
<th>Glycerol</th>
<th>Water in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1000</td>
<td>Carbopol 1</td>
<td>0.2</td>
<td>0.1 ml</td>
<td>----</td>
<td>----</td>
<td>Q.S.</td>
</tr>
<tr>
<td>F2</td>
<td>1000</td>
<td>Carbopol 1.5</td>
<td>0.2</td>
<td>0.1 ml</td>
<td>----</td>
<td>----</td>
<td>Q.S.</td>
</tr>
<tr>
<td>F3</td>
<td>1000</td>
<td>Carbopol 2</td>
<td>0.2</td>
<td>0.1 ml</td>
<td>----</td>
<td>----</td>
<td>Q.S.</td>
</tr>
<tr>
<td>F4</td>
<td>1000</td>
<td>NaCMC 2</td>
<td>0.2</td>
<td>----</td>
<td>2</td>
<td>Q.S.</td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>1000</td>
<td>NaCMC 2.5</td>
<td>0.2</td>
<td>----</td>
<td>2</td>
<td>Q.S.</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>1000</td>
<td>NaCMC 3</td>
<td>0.2</td>
<td>----</td>
<td>2</td>
<td>Q.S.</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>1000</td>
<td>HPMC 3</td>
<td>0.2</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>Q.S.</td>
</tr>
<tr>
<td>F8</td>
<td>1000</td>
<td>HPMC 3.5</td>
<td>0.2</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>Q.S.</td>
</tr>
<tr>
<td>F9</td>
<td>1000</td>
<td>HPMC 4</td>
<td>0.2</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>Q.S.</td>
</tr>
</tbody>
</table>
Preparation of Carbopol 934 Gel: For the preparation, 1% w/w of Carbopol 934 P was dispersed in distilled water by vigorous stirring. A 0.1 ml of triethanolamine was added with stirring till a transparent clear gel was formed. The part of plain gel was added to the powdered drug with gentle stirring to produce smooth layer of gel. The rest of the gel is added gradually with constant stirring till homogeneous dispersion is obtained. Different other concentration of carbopol gels was also prepared in the same procedure.

Preparation of Sodium CMC Gels: 3% w/w of Sodium carboxy methyl cellulose (Na.CMC) was dissolved with 2 gm of glycerol in a beaker and the mixture was poured in small amount into the vortex of prepared drug solution and stirred until a clear gel as formed. Different other concentration of HPMC gels was also prepared in the same procedure

Preparation of Hydroxypropylmethylcellulose Gel: 3% w/w of Hydroxypropylmethylcellulose (HPMC) was dispersed in purified water; the dispersion was mixed using a magnetic stirrer until a clear transparent gel free from air bubbles was obtained. The resultant gel mass was left overnight for complete swelling. Part of the plain gel was added the powdered drug with gentle stirring to produce a smooth layer of the gel. The incorporation of the drug into the prepared gel was followed the steps carried out in the preparation of other gels. Different other concentration of HPMC gels was also prepared in the same procedure.

Drug excipients compatibility studies
The FTIR spectra of the sample were obtained using FT-infrared spectrophotometer (Spectra 1000, Perkin Elmer Japan.) by KBr pellet method. The position of the peak in FT-IR spectra of pure Miconazole nitrate is compared with those in FT-IR spectra of Miconazole nitrate plus excipients.

Determination of Miconazole nitrate in prepared gels
The prepared formulations were analyzed for drug content, by taking 2g of formulation in 100 ml volumetric flask. 20 ml of Methanol and water mixture (1:1) was added to volumetric flask and shaken well. Further, the volume was made upto the mark with the same solution and then diluted to 100 ml with water. The supernatant was filtered and measured. The drug content was determined by measuring the absorbance at 272 nm using UV-spectrophotometer (Shimadzu-1601, Japan).
Determination of viscosity

The viscosity of all the formulations was determined by using Brookfield digital viscometer. The carbopol gel viscosity measurement was carried out using spindle No.10 at 100 rpm, which was maintained at 30° ± 1°C. The HPMC and Sodium CMC gel viscosity measurement was carried out using spindle No.93 (T-bar spindle) at 6 rpm.

In vitro bioadhesion testing

The vaginal gels were tested for bioadhesion properties using modified two-armed balance by Gupta A et al. The apparatus used for in vitro bioadhesion studies is shown in Figure 1. In vitro bioadhesion studies were carried out using sheep vaginal mucosa and modified two-armed balance. The beaker on one side of the balance was counter balanced by using suitable weights on the other side. A circular piece of sheep vaginal mucosa was fixed to the tissue holder with cyanoacrylate adhesive and was immersed in water and temperature was maintained at 37 ± 1°C. Then the gel was placed on the vaginal mucosa by using a preload of 50 gm and kept it aside for 5 min to facilitate adhesion bonding. After preloading time, the preload was removed and the water was allowed to flow into the beaker kept on the other side of the balance at the flow rate of 1 drop / sec until the gel, detaches from the vaginal mucosa. The weight required to detach gel from vaginal mucosa was noted.

Figure 1: Modified two-armed balance for in vitro bioadhesive test.

In Vitro Drug Release Studies

The in vitro release study data was quantified by using PCP-DISSO-V2.08 software developed by Industry Institute Partnership Cell (IIPC) of Poona College of Pharmacy, Poona. This software was used to determine the
percentage drug release and also to determine the release mechanism. It was also utilized to fit various mathematical models and to determine the best model. The in vitro release of Miconazole nitrate from different gel base was determined by dialysis method. The dialysis tube having average flat width of 24.26 mm average diameter of 14.33 mm and capacity 1.61 ml /cm was utilized for diffusion studies. Prior to diffusion studies, the dialysis tube was soaked over night in water. The hydrated membrane was used for diffusion study. 1 gm of gel was kept in a dialysis membrane, which was sealed on both sides; the dialysis tube was then placed in glass beaker containing 20 ml of water. The release studies were performed at 37\(^\circ\)C for different time interval (from 1-7 hr.)

5 ml of recipient solution was withdrawn at 1 hr interval and replaced with an equal amount of fresh water to maintain sink condition. Samples were analyzed for Miconazole nitrate by UV-spectrophotometrically at 272 nm after appropriate dilution against blank.

**Effects of flow rate on release of drug from gels**

The apparatus used for this is shown in Figure 2. The effect of flow rate on release of drug from gels has been carried out. The dialysis membrane of 2.5 cm diameter was used as simulated vaginal mucosa in cylindrical form. The dialysis membrane was soaked in the diffusion medium overnight. The pre-soaked dialysis membrane (open end on both sides) was inserted into a glass test tube. The known weight of gel was loaded in the dialysis membrane, by the help of syringe. The flow rate of water was adjusted with the help of infusion set. The flow rates were chosen based on the reported human cervical secretion rates. The temperature was maintained at 37\(^\circ\)C \pm 1\(^\circ\)C in a temperature controlled water bath.

**Figure 2: Diagram of effect of flow rate on release of the drug.**
Results and Discussion

Drug – Excipients compatibility studies

The compatibility between the drug and polymer was compared by FT-IR spectra. The position of peak in FT-IR spectra of pure Miconazole nitrate is compared with those in FT-IR spectra of Miconazole nitrate plus excipients in (1:1) ratio. It was observed that, there was no disappearance or shift in peak position of Miconazole nitrate in any spectra of drug and excipients, which proved that drug and excipients were compatible. Hence, the drug was in free state and can release easily from polymeric network.

Determination of drug content of gel formulation

The prepared gel formulations were analysed for drug content and the data is reported in Table 2. The result of the drug content uniformity shows that the drug is uniformly distributed in the prepared gels.

Determination of Viscosity

The viscosity of semisolid preparations plays an important role in drug release from the vehicles and greatly affects drug bioavailability. In addition, it is reported that, the bioadhesive properties of a range of well defined polymers are greatly influenced by their viscosities and molecular weights. The result of the viscosity studies shows that with increase in concentration of carbopol 934P, Na.CMC and HPMC, the viscosity of the gels also increased.

Table 2: Different Evaluation parameters of bioadhesive Gels.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug content in mg</th>
<th>Viscosity in cps</th>
<th>Bioadhesive strength (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>19.82±0.24</td>
<td>11479±15.93</td>
<td>60.33±2.08</td>
</tr>
<tr>
<td>F2</td>
<td>20.20±0.12</td>
<td>16901±54.92</td>
<td>63.67±1.52</td>
</tr>
<tr>
<td>F3</td>
<td>20.00±0.18</td>
<td>22152±17.59</td>
<td>70.00±1.00</td>
</tr>
<tr>
<td>F4</td>
<td>19.95±0.20</td>
<td>5637±10.62</td>
<td>68.17±1.25</td>
</tr>
<tr>
<td>F5</td>
<td>20.03±0.18</td>
<td>7679±20.83</td>
<td>75.83±1.75</td>
</tr>
<tr>
<td>F6</td>
<td>19.75±0.12</td>
<td>9479±37.86</td>
<td>87.67±1.52</td>
</tr>
<tr>
<td>F7</td>
<td>19.98±0.24</td>
<td>4174±21.92</td>
<td>37.00±1.00</td>
</tr>
<tr>
<td>F8</td>
<td>19.83±0.22</td>
<td>6561±25.95</td>
<td>43.67±1.15</td>
</tr>
<tr>
<td>F9</td>
<td>20.08±0.23</td>
<td>8924±57.3</td>
<td>48.33±1.52</td>
</tr>
</tbody>
</table>
**In vitro bioadhesive testing**

*In vitro* bioadhesion testing for both blank as well as drug loaded gel was evaluated by detachment force measurements of gel from sheep vaginal mucosa. The strong interaction between both blank and drug-loaded formulation with the mucous lining of the tissue helped to increase contact time and permit localization.

Increasing the polymer concentration caused an increase in the bioadhesive strength. Adhesion was reported to be affected by hydration. Hydration of the bioadhesive polymer is essential to initiate the bioadhesive bonding process. The cohesive force arises when water from the space between the mucosa and the polymer is taken up; this plays a vital role in the establishment of an effective bioadhesive bond.

The bioadhesive strength of carbopol gels (F1-F3) was found to be 60-70 gm. The Na CMC gel formulations (F4-F6) showed maximum strength of 87 gm where as HPMC gel formulations (F7-F9) showed bioadhesive strength of 68-87 gm.

The bioadhesive strength of the selected formulations followed the pattern of Na CMC gel > carbopol gel > HPMC gel. These results also explained that with the increase in the polymer concentration, the bioadhesive strength was also increased.

**In Vitro Drug Release**

The polymers like Carbopol 934 P, Na CMC and HPMC were selected owing to their excellent bioadhesive strength, release rate, non toxic and stable in vaginal pH (Yamsani et al 2007). The polymers Na CMC and HPMC are known to provide the formulation with control drug release along with desired bioadhesive properties (El-Kamel et al 2002). The results indicated that the drug release was found to be first order in all the formulations. The results are represented in figure 3. The release data of various formulations showed that; the concentration of polymer significantly affected the percentage drug release. With increase in polymer concentration, the drug diffusion was decreased accordingly. The gel containing 2 % of Carbopol showed release upto 64.32 % at the end of 8 Hrs which indicates that the drug release from gels can be controlled by polymer concentration, where as the gel containing Na
CMC showed release upto 70.37%, and gel containing HPMC showed release upto 73.12%. As the concentration of polymers increased in formulations, the Miconazole Nitrate release rate from the gels decreased.

**Figure 3: Force of Adhesion of Miconazole gels. Each data point represents as n=3; mean ± standard deviation.**

![Force of Adhesion of Miconazole gels](image)

**Effect of flow rate on release pattern from gels**

Many factors affects the release of drug from vagina like vaginal pH, cyclic changes in thickness of vaginal epithelium, fluid volume and composition pH and sexual arousal could potentially affect drug release from intravaginal delivery systems (K Carlstrom Et Al 1988). In the present work, the effect of flow rate on release of drug from vaginal gels was performed for selected formulations F3, F6 and F9. The effect of flow rate on gel was studied to know about the pattern of drug release from the gel. The formulations F3, F6 and F9 were selected for the study based on diffusion studies data. The drug release from carbopol gels was found to be 53 and 57 % when the flow rate was adjusted to 3 and 5 ml/hr respectively. The results were found to follow similar pattern for the other two bioadhesive polymers viz., gels containing Na. CMC the release was upto 65% at 3ml/hr and 70% at 5ml/hr and gels containing HPMC the release was upto 63% at 3ml/hr and 68% at 5ml/hr. From the results it was evident that, as the flow rate increased the drug release from the gels was also increased.
Conclusions:
The preformulation studies of Miconazole nitrate showed that the drug had optimum solubility and pH to be developed into a gel formulation. From the FT-IR studies, it was observed that the characteristic peaks were similar for drug and polymers with minor differences. Hence, it can be concluded that there was no chemical interaction between drug and polymer. From the results of content uniformity studies, it can be inferred that there was proper distribution of drug in gel. Viscosity studies revealed that as polymer concentration increased, viscosity also increased. The *in vitro* drug release was found to be diffusion controlled and the process followed first order kinetics.

As the flow rate increased the drug release from the gels was also increased. The vaginal gels containing Na CMC exhibited good bioadhesive property, optimum viscosity and good sustained release up to 70% at the end of 8th hour. Thus, the bioadhesive vaginal gels have good bioadhesive property that would enhance the retention & sustained drug release in the vagina.

**Figure 4: In Vitro Drug Release of the formulation F1 to F3.**
Figure 5: *In Vitro* Drug Release of the formulation F4 to F6.

![Graph showing In Vitro Drug Release of formuations F4 to F6.](image1)

Figure 6: *In Vitro* Drug Release of the formulation F7 to F9.

![Graph showing In Vitro Drug Release of formuations F7 to F9.](image2)
Figure 7: Effect of flow rate on release of the drug of formulation F3.

Figure 8: Effect of flow rate on release of the drug of formulation F6.
Figure 9: Effect of flow rate on release of the drug of formulation F9.

References


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