Design of mucoadhesive vaginal metronidazole films

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Abstract

A mucoadhesive drug delivery system for local availability of Metronidazole through vaginal route was formulated. Metronidazole films were prepared by solvent evaporation method using various compositions of Carbopol, HPMC and plasticizers like Sorbitol, Glycerine, PEG 400 and PG. The films were evaluated for their weight, thickness, surface pH, folding endurance, drug content uniformity, in vitro drug release and mucoadhesion. Films containing 10% PG and Carbopol-HPMC in the ratio of 1:1 and 1:2 represented optimal composition as they showed acceptable, physical, mechanical, drug release and mucoadhesion characteristic for vaginal delivery of Metronidazole proposed for local contraception.

Keywords: Metronidazole, vaginal films, carbopol, HPMC, mucoadhesion, contraception.

Introduction

Although the vaginal contraceptive formulations have been available since the early 1960s, worldwide there is urgent need for safe, effective, acceptable and affordable contraceptive formulation. The presently marketed vaginal contraceptive available over the counter contains Nonoxanol-9 (N-9) as the active ingredient. However, studies have shown that frequent use of N-9 leads to vaginal irritation and lesions on the epithelium, which increase the risk of STDs transmission including HIV (Niruthisard et al. 1991, Weir et al. 1995).

Metronidazole has proved to be very effective for the therapy of amoebiasis, trichomoniasis, lambliasis and anaerobic infections (Tracy and Webster 1996, Freeman et al. 1997). It has been found to be mutagenic in bacterial assay (Voogd 1981) and murine spermatozoa (Mudry 2007) when administered orally. Intravaginal Metronidazole is safe, effective, well tolerated and already established. Many studies have been conducted concerning the efficacy of Metronidazole as a local spermicide (El-Gizawy and Aglan 2003).

Various vaginal dosage forms available in the market are solutions, suppositories, creams, ointments, gels, foams, sprays, tablets, capsules, etc (Garg et al. 2001). Conventional vaginal dosage forms are associated with disadvantages of poor retention, leakage and messiness causing inconvenience to users, leading to poor patient compliance and loss of therapeutic efficacy.

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This limitation can be overcome by novel bioadhesive vaginal drug delivery system (Robinson and Bologna 1994, Varmanini and Garg 2000). Vaginal films are advantageous because of portability, convenient application, long retention time, easy storage and handling and improved stability (Garg et al. 2005). Various bioadhesive and film forming polymers like polycarbophil, carbopol, sodium alginate, xanthan and guar gums, cellulose derivatives, polyvinyl alcohol, polyvinyl pyrrolidone etc have been tried for the film formation (Garg et al. 2001).

Films are mostly manufactured by melt fabrication, in-situ polymerization and solvent evaporation method (Heller 1987). They are evaluated for their appearance, thickness, weight, uniformity of content, surface pH, mechanical properties, in-vitro, ex-vivo and in-vivo drug release and mucoadhesion, etc.

Hence, the present work was envisaged to develop novel and aesthetic mucoadhesive vaginal films of Metronidazole.

**Materials and Methods**

Metronidazole from Novartis Mumbai, India, Carbopol 974P from Lubrizol Advanced Materials India Pvt. Ltd. Mumbai, India and Hydroxypropylmethyl cellulose (HPMC, Methocel K4M) from Colorcon Asia Pvt. Ltd. Goa, India were received as gift samples. Sorbitol (70 % solution) from Loba Chemie (Mumbai, India), Propylene Glycol (PG) from Fisher Scientific (Mumbai, India), Glycerol and Polyethylene Glycol 400 (PEG 400) from Alpha Chemical Lab (Mumbai, India) were procured through local market. Water prepared by reverse-osmosis system (Milipore, USA) was used as solvent for film preparation. All other reagents and solvents were of analytical grade.

**Preparation of films:** Films were cast by solvent evaporation method on glass substrate. Aqueous solutions containing Carbopol, HPMC and a plasticizer was prepared and Metronidazole dissolved in Glacial acetic acid was loaded in each polymeric solution by stirring mechanically at room temperature, followed by centrifugation (3000 RPM for 15 min.) to remove entrapped air. Solution was then poured into Anumbra petriplates followed by drying in oven at 45 °C. Drying time of films varied from 24 to 48 hrs depending upon formula and depth poured. The films obtained on drying were removed from the petriplates, placed on butter paper backing and were cut into individual circular films of size 3 cm in diameter using a sharp scalpel. The films were packed by carefully wrapping in aluminium foil and stored at room temperature. They were studied for the physical, aesthetic and mechanical properties. Peclability of films which gives ease of production was assessed on the basis of comfortness of removal from casting surface.

**Film weight and thickness:** For the evaluation of weight, five films of every composition were taken and weighed individually on digital balance (Mettler Toledo, USA) and average weights were calculated. Similarly, thickness of each film was measured at five different locations (centre and four peripheral locations) using a micrometer screw gauge (Hi-Tech Metrology Pvt. Ltd., India.) and a mean value of five measurements was used as the film thickness.

**Folding endurance:** Three 2x2 cm square shaped films of each formulation of size were cut by using a sharp scissors. Folding endurance was determined by repeatedly folding a strip of the film at the same place by 90° and 180° at the rate of 30-35 folds/min till broke. The number of times, the film could be folded at the same place without breaking gave the value of folding endurance.

**Mechanical properties:** The polymer film was cut into rectangle (3x10 cm). A rectangular strip was placed between the upper and the lower grip (HW10 Grips, Benchtop Tester, Tinius Olsen, USA) which
were controlled by a pneumatic grip controller (HT400, Benchtop Tester, Tinius Olsen, USA) mounted on test stand (HSKS, Benchtop Tester, Tinius Olsen, USA) aligning the long axis of the specimen and the grip with an imaginary line joining the points of attachment of the grip to the machine. The two grips were kept at a distance of 100 mm in a same plane. Lower grip was stationary and the upper one was moved linearly away from the former at a rate of 1 mm/s. The stress strain curve was recorded and tensile strength (force/unit unit area required to break the film), percentage elongation at break were determined.

**Surface pH:** For determination of surface pH three films of each formulation were allowed to swell for 2 h on the surface of an agar plate. The pH was measured by placing pH indicator paper on surface of swollen patch. A mean of three readings was recorded.

**Swelling study:** For the swelling study three films of each formulation were allowed to swell on surface of agar plate kept in an incubator maintained at 37 ± 0.2 °C. Increase in weight of the film was determined at the predetermined time interval (1-3 h). The swelling index, S was calculated using the following equation:

\[
\text{Swelling Index (S)} = \frac{(S_t - S_0)}{S_0}
\]

Where, \( S_0 \) is the initial weight of film at zero time, \( S_t \) is the weight of swollen film after time \( t \).

**Method of analysis:** Metronidazole was analysed by validated high performance liquid chromatography (HPLC) (Jasco, USA) method (Venkateshwaran and Stewart 1995). Metronidazole was eluted on an octadecylsilane HPLC column (Brownlee RP-18, 220 mm x 4.6 mm I.D., 10 μm), with a mobile phase of 0.01 M aqueous monobasic potassium phosphate (pH 4.0) and absolute methanol (85:15, v/v) at a flow rate of 1 ml/min and detected with UV detector at 318 nm. Metronidazole was eluted at 9.16 min under the above mentioned experimental conditions.

**Drug content uniformity:** To ensure the uniformity of distribution of Metronidazole in a film, a content uniformity test was performed. One square centimetre of samples representing five different regions (centre and four corners) within a film were cut off, weighed and dissolved in 100 ml of water and stirred for 2 h, filtered through 0.45- μm nylon membrane, and filtrate was analyzed for Metronidazole content. Filtrate (20 μl) was injected into column and Metronidazole content was determined from a calibration curve prepared using different concentrations (1- 50 μg/ml) under the same above mentioned chromatographic conditions.

**In vitro release study:** Rate and extent of release of Metronidazole from films was studied in 100 ml of simulated vaginal fluid (VSF), maintained at 37°C and stirred on a magnetic stirrer at 100 rpm. The samples (2 ml each) were withdrawn periodically with replacements at predetermined intervals of time and analyzed by HPLC.

**In vitro mucoadhesion:** In vitro mucoadhesion of the vaginal film was studied by a modified procedure reported by Agrawal and Mishra (1999) Figure 1 using the rat vaginal mucosa.

The excised vaginal mucosa was washed with saline solution and kept in VSF prior to use. A square piece (surface area 1 cm²) of the mucosa (F) was cut and glued with cyanoacrylate adhesive on the ground surface of tissue holder (D) made of polyethylene with mucosal side exposed. Similarly, the Metronidazole film (G) (surface area 1 cm²) was glued to another tissue holder (E) of the same size. Thereafter the tissue holders with the mucosa and the film were kept in contact with each other with uniform and constant pressure produced by placing a 5 gm weight for 3 min (preload time) to facilitate adhesion bonding. The tissue holder with the vaginal mucosa was allowed to hang on stand (A) with the help of clamp (B) copper wire (C) fastened with a hook provided on the back side of the upper holder. A
light-weight preweighed polypropylene bottle (H) was attached to the hook on the back side of the lower holder. Water was added to the propylene bottle through a burette (I) which was mounted on stand (K) with the help of clamp (J). Addition was set at a rate of 1 ml/min till detachment of the film and vaginal mucosa. The water collected in the bottle was measured, weights of lower hook, bottle and water put together expressed as force (g) required for the detachment, which in turn denoted the force of adhesion.

![Diagram of apparatus](image)

**Figure 1.** Diagrammatic representation of apparatus for *In-vitro* mucoadhesion study

### Result and Discussion

Compositions of Metronidazole films prepared using different polymers and plasticizers along with their physical characteristics are mentioned in Table 1. Four different water-soluble plasticizers viz Sorbitol, Glycerol, PEG400 and PG were used for preparation of the films. Only PG gave colorless and transparent films. The superior behaviour of the PG might be due to its aid in the dispersion of the drug in the film.

The effect of plasticizer concentration on Metronidazole films containing Carbopol, HPMC and PG on mechanical properties was also studied and results are shown in Table 2. To be effective, plasticizer must distribute itself between the polymer chains and interact with functional groups, thereby reducing the interaction between the polymer chains (Swarbrick and Boylan 1997).

Inter formulation variations in the weight and thickness was reported. This difference in the weight and thickness was because of the effect of difference in molecular weight and proportion of polymers used in the films. Carbopol and HPMC are high molecular weight network polymer and PG is low molecular weight compound (Rowe et al. 2003). So decrease in the Carbopol-HPMC concentration decreased weight and thickness of the film.
Table 1. Composition of metronidazole films containing different polymers and plasticizer and their physical characteristics.

<table>
<thead>
<tr>
<th>Films</th>
<th>Metronidazole (%)</th>
<th>HPMC</th>
<th>Carbopol</th>
<th>Sorbitol</th>
<th>Glycerol</th>
<th>PEG400</th>
<th>PG</th>
<th>Physical characteristics of film</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>10</td>
<td>42.50</td>
<td>42.50</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>White, opaque, could not be removed</td>
</tr>
<tr>
<td>F2</td>
<td>10</td>
<td>42.50</td>
<td>42.50</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>White, opaque, brittle</td>
</tr>
<tr>
<td>F3</td>
<td>10</td>
<td>42.50</td>
<td>42.50</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>White, opaque, soft</td>
</tr>
<tr>
<td>F4</td>
<td>10</td>
<td>42.50</td>
<td>42.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>Colorless, transparent, brittle</td>
</tr>
<tr>
<td>F5</td>
<td>10</td>
<td>40.00</td>
<td>40.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>Colorless, transparent, soft</td>
</tr>
<tr>
<td>F6</td>
<td>10</td>
<td>37.50</td>
<td>37.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>Colorless, transparent, too soft</td>
</tr>
<tr>
<td>F7</td>
<td>10</td>
<td>56.70</td>
<td>28.30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>Colorless, transparent, brittle</td>
</tr>
<tr>
<td>F8</td>
<td>10</td>
<td>53.30</td>
<td>26.70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>Colorless, transparent, soft</td>
</tr>
<tr>
<td>F9</td>
<td>10</td>
<td>50.00</td>
<td>25.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>Colorless, transparent, too soft</td>
</tr>
<tr>
<td>F10</td>
<td>10</td>
<td>63.75</td>
<td>21.25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>Colorless, transparent, brittle</td>
</tr>
<tr>
<td>F11</td>
<td>10</td>
<td>60.00</td>
<td>20.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>Colorless, transparent, soft</td>
</tr>
<tr>
<td>F12</td>
<td>10</td>
<td>56.25</td>
<td>18.75</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>Colorless, transparent, too soft</td>
</tr>
</tbody>
</table>

The folding endurance of a film is frequently used to estimate the ability of the film to withstand repeated bending, folding, and creasing and may be encountered as a measure of the quality of films. The folding endurance was observed to be varying among the batches which were found to be increasing along with the increase in the plasticizer concentration up to 15% which was used in the formulation. Composition of Carbopol and HPMC also showed effect on it. Increase in HPMC concentration showed increase in the folding endurance. This might be because of film forming property of the HPMC. Further the tensile strength got decreased and elongation at break increased as there was increase in the PG concentration. This is because; plasticizers increase the ease of film deformation, which is manifested by a decrease in the tensile strength and an increase in the elongation at break (Swarbrick and Boylan 1997). Carbopol and HPMC concentration had similar effect as that was found for folding endurance i.e. tensile strength was increased and elongation at break was decreased as there was increase in the Carbopol concentration among the batches.

Considering the fact that acidic pH of the vagina (4 - 5) causes the immobilization of sperm (Suarez and Pacey 2006) an attempt was made to maintain the pH in the vaginal range. Addition of glacial acetic acid solved the problem of solubility of the Metronidazole (El-Gizawy and Aghan 2003) as well as surface pH of the films. It was found that the increase in the Carbopol content also caused decrease in the surface pH of the films. This could be attributed to the intrinsic properties of Carbopol, such as acidic pKa and polyelectrolyte nature of the ionized form of the polymer, which altered the pH (Riley et al. 2001).

Drug content in the film among all the batches was found to be uniform within range of 3.37 ± 0.03 mg. On the basis, it was considered that the drug was dispersed uniformly throughout the film, substantiating the formulation and casting method.
Table 2. Physical and mechanical evaluation of polymeric films.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Weight (mg)b</th>
<th>Thickness (μm)b</th>
<th>Folding Enduranceb</th>
<th>Tensile Strength (N/mm²)b</th>
<th>Elongation at break (%)b</th>
<th>Surface pHb</th>
<th>Metronidazole Content (mg/cm²)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4</td>
<td>304.15 ± 16</td>
<td>305.10 ± 12</td>
<td>175.68 ± 4.50</td>
<td>107.50 ± 4.7</td>
<td>53.87 ± 5.6</td>
<td>4.57 ± 0.3</td>
<td>3.39 ± 0.03</td>
</tr>
<tr>
<td>F5</td>
<td>284.10 ± 15</td>
<td>270.45 ± 13</td>
<td>250.71 ± 7.80</td>
<td>85.45 ± 6.0</td>
<td>60.67 ± 13.0</td>
<td>4.60 ± 0.2</td>
<td>3.40 ± 0.01</td>
</tr>
<tr>
<td>F6</td>
<td>250.19 ± 12</td>
<td>230.11 ± 12</td>
<td>310.71 ± 1.89</td>
<td>52.25 ± 8.9</td>
<td>74.23 ± 18.2</td>
<td>4.59 ± 0.1</td>
<td>3.39 ± 0.03</td>
</tr>
<tr>
<td>F7</td>
<td>257.81 ± 10</td>
<td>241.78 ± 18</td>
<td>189.12 ± 5.40</td>
<td>101.75 ± 1.9</td>
<td>52.41 ± 4.2</td>
<td>4.68 ± 0.3</td>
<td>3.39 ± 0.03</td>
</tr>
<tr>
<td>F8</td>
<td>220.19 ± 19</td>
<td>215.23 ± 14</td>
<td>269.19 ± 4.70</td>
<td>81.08 ± 6.7</td>
<td>59.21 ± 12.1</td>
<td>4.67 ± 0.4</td>
<td>3.37 ± 0.03</td>
</tr>
<tr>
<td>F9</td>
<td>199.67 ± 17</td>
<td>180.98 ± 22</td>
<td>315.23 ± 3.80</td>
<td>48.70 ± 15.2</td>
<td>71.89 ± 20.1</td>
<td>4.66 ± 0.2</td>
<td>3.37 ± 0.04</td>
</tr>
<tr>
<td>F10</td>
<td>219.34 ± 15</td>
<td>215.11 ± 18</td>
<td>194.47 ± 5.70</td>
<td>97.11 ± 2.8</td>
<td>53.21 ± 6.2</td>
<td>4.79 ± 0.1</td>
<td>3.38 ± 0.01</td>
</tr>
<tr>
<td>F11</td>
<td>197.76 ± 17</td>
<td>187.99 ± 19</td>
<td>277.29 ± 4.10</td>
<td>77.89 ± 7.6</td>
<td>60.19 ± 14.1</td>
<td>4.76 ± 0.4</td>
<td>3.39 ± 0.05</td>
</tr>
<tr>
<td>F12</td>
<td>185.88 ± 20</td>
<td>180.11 ± 12</td>
<td>320.74 ± 4.50</td>
<td>45.23 ± 11.2</td>
<td>73.43 ± 19.7</td>
<td>4.78 ± 0.3</td>
<td>3.39 ± 0.02</td>
</tr>
</tbody>
</table>

a, Values are expressed as mean ± S.D.; n=5; b, Values are expressed as mean ± S.D.; n=3.

Swelling study: Hydration is required for the mucoadhesive polymer to expand and create a proper bond between the polymer and the mucin. The capability of polymer to swell governs the release rate of incorporated drug and also bioadhesiveness of the formulation (Chen and Cyr 1970, Mortazavi and Smart 1993). Polymer swelling permits the mechanical entanglement by exposing the bioadhesive site for hydrogen bonding and/or electrostatic interaction between the polymer and mucin network of mucus (Gu et al. 1998). These various physicochemical interactions occurs to consolidate and strength the adhesive joints, leading to prolonged adhesion (Smart 1999). The effect of various compositions on the swelling index of Metronidazole films are shown in Figure 2.

![Figure 2. Swelling index of metronidazole films](image)

Data represented as mean; n=3.

The films were rapidly swelled within 30 - 45 min and thereafter gradually reach a plateau. The high initial uptake of water was due to the faster hydration rate of HPMC (Agarwal and Mishra 1999). It was found that the swelling index was increased as Carbopol concentration increases. This is attributed to the property of Carbopol to retain water and increase the swelling degree to form thick swollen mass (Agarwal and Mishra 1999).

In-vitro release study: The release study was performed in VSF to mimic the conditions of vagina. Figure 3 shows the profile of release of Metronidazole from film. In all formulations, the burst release was observed within first 15 min. This burst release observed because of HPMC. Formulation F5 and F8 showed the maximum release of drug upto 2 h. It was observed that, as the Carbopol concentration in the formulation increased, the Metronidazole release rate
from film decreased, which indicates that the Carbopol to HPMC concentration ratio is important factor to decide the release pattern.

**Figure 3. Release profile of metronidazole films**

![Graph showing release profile](image)

Data represented as mean; n=3.

*In vitro Mucoadhesion:* The Mucoadhesive property of Metronidazole films containing varying proportion of Carbopol and HPMC was determined with a view to develop film with good adhesiveness without any difficulty of administration and any other handling problem. In case of dry or partially hydrated dosage forms in contact with thin or discontinuous mucus layers, the affinity for water shown by mucoadhesive polymers seems to be greater an almost "suction-like" effect and it leads to formation of straighter bond between them (Smart 1999). *In vitro* mucoadhesion study was performed as determination of detachment force. Results of *in vitro* mucoadhesion are shown in Figure 4. The values for force of adhesion were between 28 to 45 g/cm². The behaviour seemed to be dependent on the composition of the polymeric films. The maximum mucoadhesive strength was observed in all the formulation made of Carbopol:HPMC = 1:1. Among them film F4 showed highest mucoadhesion that because of higher composition of the carbopol as compared to other batches. It was found that the degree of adhesion increase as there was increase in the Carbopol content of the film.

**Figure 4. Force of adhesion of metronidazole films**

![Bar chart showing force of adhesion](image)

Data represented as mean; n=3.

**Conclusion**

The Mucoadhesive vaginal films of Metronidazole composed of optimized ratios of Carbopol, HPMC and PG were formulated by solvent casting method. The films made up of
Carbopol:HPMC as 1:1 and 1:2 with 10 % PG were found to be suitable. They possessed acceptable aesthetic, physico-mechanical properties and mucoadhesion. The drug release profile indicated the suitability of the proposal for single use spermicidal contraception.

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References


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