Effect of Formulation Factors on In Vitro Corneal Permeation of Fluconazole through Excised Sheep Cornea

Sunil Thakral¹, Munish Ahuja²

¹Gurukul College of Pharmacy, Suratgarh, Rajasthan, India  
²Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India  
*Corresponding author’s email: sunil.thakral@gmail.com

ABSTRACT

Fungal keratitis is a corneal inflammation with blurred vision and painful eye, which can be treated with fluconazole eye drops. The aim of the study was to find out effect of various formulation parameters on in vitro corneal permeation of fluconazole eye drops. The corneal permeation studies were conducted using freshly excised sheep cornea, mounted between donor and receptor chambers of an all glass modified Franz diffusion cell, containing 11 ml of ringer bicarbonate (pH 7.4, 34°±1°C). At the end of the experiment, each cornea (freed from sclera) was weighed, soaked in 1 m methanol, dried overnight at 90 °C and reweighed. From the differences in weights corneal hydration was calculated. The formulation with pH 6 and concentration 2% containing phosphate buffer and mannitol as tonicity modifier, butyl alcohol as preservative, and hydroxypropyl methyl cellulose (HPMC) as viscosity modifier showed maximum permeation.

KEYWORDS: Fungal keratitis, Fluconazole, In Vitro Permeation, Corneal hydration, Preservative, Viscosity modifier
INTRODUCTION

Pharmaceutical preparations are applied topically to the eye to treat surface or intraocular conditions. These may include conditions such as infections of the eye or eyelid, secondary to bacterial, fungal or viral pathogens, allergic or infectious conjunctivitis, inflammations, elevated intraocular pressure and glaucoma, as well as dry-eye due to an inadequate production of fluids bathing the eye (1).

Upon topical administration of ophthalmic drugs to the eye, most of the administered amount is rapidly eliminated from the pre-corneal area due to drainage via the nasolacrimal duct and dilution by tear turnover. The cornea, considered a major pathway for ocular penetration of topically applied drugs, is an effective barrier to drug penetration because the corneal epithelium has annular tight junctions (zonula occludens), which completely surround and effectively seal the superficial epithelial cells. Thus topical drug administration to the eye results in low bioavailability due to the short residence time as well as the limited corneal absorption of drugs (2). Improvement of ocular bioavailability can permit reduction of the instillation frequency or of the dose, with a consequent decrease in undesired side effects. As a result of this, various studies have been carried out with the aim of improving the ocular bioavailability (3).

Fungal infections of the cornea (mycotic or fungal keratitis, keratomycosis) present as suppurative, usually ulcerative, lesions. Fungal keratitis is recognizable by the presence of a coarse granular infiltration of the corneal epithelium and the anterior stroma. Such a corneal infection poses a challenge to the ophthalmologist because of its tendency to mimic other types of stromal inflammation, and because its management is restricted by the availability of effective antifungal agents and the extent to which they can penetrate into corneal tissue (4).

The synthetic bistriazole antifungal compound fluconazole exhibits outstanding physical and pharmacokinetic properties. Fluconazole is a stable, water-soluble, bis-triazole antifungal that has low molecular weight, high bioavailability, good ocular penetration when used either systemically or topically, and low toxicity. It is potentially useful as a topical ocular agent. It is quite effective against *candida* species (5).

Azoles bind to a cytochrome P-450 fungal enzyme involved in the 14α demethylation of either lanosterol or 25-methylenedihydrolanosterol, resulting in a decrease in ergosterol synthesis and an accumulation of 14α-methylated sterols. This leads to increased permeability of the fungal cell membrane, alteration of membrane enzymes, inhibition of growth, and ultimate death of the fungal cell. All azoles, except fluconazole, appear to compromise the function of immune system cells, especially lymphocytes. This lessens the degree of tissue damage occurring with the inflammatory reaction but also affects the efficacy of the azoles *in vivo*. Since azoles, with the exception of fluconazole, achieve only limited concentrations in the eye, they are to be considered fungistatic when used in ocular fungal infections (6).

Thus the aim of the study was determine the effect of various formulation parameters on *in vitro* corneal permeation of fluconazole eye drops.
MATERIALS AND METHODS

Fluconazole was obtained as a gift sample from APL research Center, Mandal (A.P.), India. Hydroxypropyl cellulose–high viscosity grade (HPC-H) was obtained as a gift sample from Jubilant Organosys Ltd., Noida, India. Hydroxypropyl methyl cellulose (HPMC E50LV Premium) was purchased from Loba Chemie Pvt. Ltd., Bombay, India. Sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sodium bicarbonate, sodium dioxgrogen orthophosphate and dextrose were purchased from Qualigens fine chemicals (Mumbai, India). All other chemicals purchased were of analytical grade and were used as received. Fresh whole eye ball of sheep were obtained from local butcher shop (Hisar, India), within one-half hour of the animal being slaughtered. The apparatus used in permeation studies was same as published elsewhere (7).

PREPARATIONS OF TEST FORMULATIONS:

Fluconazole ophthalmic solutions of different pH

Fluconazole (0.3% w/v) ophthalmic solutions, pH 4.0, 5.0, 6.0 and 7.2 were prepared in distilled water and made isotonic with sodium chloride (0.9% w/v). The pH was adjusted with 0.01N HCl and sodium bicarbonate.

Fluconazole ophthalmic solutions of increasing concentration of pH 6.0

Required quantity of fluconazole was dissolved in 25 ml distilled water to achieve a concentration of 0.2, 0.3 and 0.5% w/v and pH, adjusted to 6.0 and made isotonic with sodium chloride.

Fluconazole ophthalmic solution (0.2%w/v, pH-6.0) of different buffers

Fluconazole (0.2g) was dissolved in 100 ml of Sorenson phosphate buffer (pH 6.0, 0.0667M USP) and citrate buffer (pH 6.0, 0.0667M USP), and the solution were made isotonic by adding 0.5 g and 0.346 g of sodium chloride respectively.

Fluconazole ophthalmic solutions (0.2% w/v, pH 6.0) containing different tonicity modifiers

Fluconazole 0.2% w/v ophthalmic solution in 0.0667M phosphate buffer (pH-6.0), made isotonic with either sodium chloride, glucose or mannitol were prepared.

Fluconazole ophthalmic solutions (0.2%w/v, pH 6.0) containing preservatives

Fluconazole 0.2% w/v solutions in isotonic phosphate buffer (0.0667 M, pH 6.0), containing benzalkonium chloride (BAC 0.01% w/v), phenyl mercuric nitrate (PMN 0.001%w/v), phenyl mercuric acetate (PMA 0.001% w/v), benzyl alcohol (BA 0. 5%v/v) or thiomersal (TM 0.05%w/v) were prepared.

Fluconazole Ophthalmic solutions (0.2% w/v, pH 6.0) containing different viscolizing agent

Fluconazole 0.2% w/v solution in 0.0667M isotonic phosphate buffer (pH 6.0), containing either hydroxypropyl methylcellulose (HPMC) or hydroxypropyl cellulose-high viscosity grade (HPC-H) or polyvinyl alcohol (PVA 1.4% w/v) were prepared.

In Vitro transcorneal permeation study (8-10)

Corneal preparation

Whole eye balls of the sheep were obtained from the local butcher’s shop within half one-half of the animal being slaughtered, then immediately
transported to the laboratory in cold (4°C) normal saline (0.9%). The cornea were carefully excised along with 2-4 mm of surrounding sclera tissues and washed with cold normal saline till free from proteins.

**PERMEATION EXPERIMENT**

Fresh cornea was mounted by sandwiching the surrounding scleral tissue between clamped donor and receptor cells of modified version of Franz diffusion cell in such a way that its epithelial surface (apical) faced the donor compartment and endothelial surface faced to receptor compartment. Cell was placed on magnetic stirrer in holding position. The receptor compartment was filled with 11 ml of freshly prepared bicarbonate ringer solution (pH 7.4) and stirred using Teflon coated magnetic stir bar. Drug solution (1 ml) was placed to the epithelial side of cornea in donor cell and stirring of the receptor fluid (jacketed with water at 34±1°C) was started. At appropriate intervals, 2 ml samples were withdrawn from the receptor compartment and withdrawn sample volume was replaced with equal volume of fresh bicarbonate ringer solution to ensure sink conditions. Withdrawn samples were analyzed spectrophotometrically (Varian-Cary 5000 UV-VIS-NIR) by measuring absorbance at λ_max of 260 nm. Each experiment was continued for about 2.0 hours and was performed at least in triplicate.

At the end of the experiment, each cornea (freed from sclera) was weighed, soaked in 1 ml methanol, dried overnight at 90 °C and reweighed. From the difference in weights, corneal hydration was calculated.

**Calculation of Apparent permeability coefficient**

The apparent permeability coefficient was calculated using following equation:

\[ P_{\text{app}} = \frac{\Delta Q}{\Delta t} \times \frac{1}{(A.C_0.60)} \]

Where \( \Delta Q/\Delta t \) (μg/min.) is the flux across corneal tissue, A is the exposed surface area of corneal tissue (0.786 cm²), \( C_0 \) is the initial drug concentration (μg/ml) in the donor compartment and 60 is included to convert minutes to seconds. The flux across the cornea was determined from the slope of the regression line obtained from the linear part of the curve, between the cumulative amount permeated (Q) vs. time (t) plot.

**Statistical analysis**

Statistical calculation were done by one-way analysis of variance (ANOVA) followed by Dunnett’s test. A p value < 0.05 was considered significant.

**RESULTS AND DISCUSSION**

**Effect of pH on In Vitro Transcorneal Permeation of Fluconazole**

*Table 1 and Figure 1* compare the effect of pH on corneal permeation of fluconazole. Fluconazole is a weak acidic with a pKa of 2.0 (11). Thus, reducing the pH of the formulation should theoretically increase the corneal permeability by providing the higher unionized lipid soluble fraction of the drug. The results show a similar trend, as the pH of the formulation was reduced from 7.2 to 4.0, there was an increase in apparent corneal permeability of fluconazole. The highest apparent corneal permeability of fluconazole was obtained at pH 6.0. The unusually higher permeation of fluconazole at pH 6.0 indicates that apart from passive diffusion, some other pH-specific transport mechanisms are also involved in corneal permeation of
fluconazole and needs to be investigated further. The normal pH of lacrimal fluid is 7.2 and eyes can tolerate pH between 6.0 to 8.5 without much discomfort (12). Upon instillation of eye drops of different pH into the eye, the lachrymal secretion owing to its buffering action will bring back the pH to normal value of lacrimal fluid i.e. 7.2. The results of titration of ophthalmic formulations with 0.01N NaOH to pH 7.2 indicate that during in vivo use 1.7 fold and 4.7 fold greater amount of lacrimal function would be required to bring the pH of tears to normal. The titre value also reveals that the irritation potential of formulations will be in the order of pH 6.0 < pH 5.0 < pH 4.0. Taking corneal permeability and irritation potential into account, fluconazole ophthalmic solutions of pH 6.0 were found to be optimum and thus selected for further study.

**TABLE 1:** Comparative corneal permeation of fluconazole from formulations of different pH

<table>
<thead>
<tr>
<th>pH</th>
<th>% Cumulative Permeation*</th>
<th>$P_{app} \times 10^6$ (cm./sec)</th>
<th>Relative $P_{app}$</th>
<th>Titre Value#</th>
<th>% Corneal Hydration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>90</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>15.32±1.12</td>
<td>17.26±0.78</td>
<td>19.95±0.62</td>
<td>1.19±0.01†</td>
<td>1.25</td>
</tr>
<tr>
<td>5.0</td>
<td>10.63±1.79</td>
<td>13.54±2.51</td>
<td>15.74±2.83</td>
<td>0.95±0.17</td>
<td>1.40</td>
</tr>
<tr>
<td>6.0</td>
<td>16.23±4.25</td>
<td>19.17±4.02</td>
<td>21.26±3.05</td>
<td>1.29±0.19†</td>
<td>1.89</td>
</tr>
<tr>
<td>7.2</td>
<td>7.84±2.16</td>
<td>10.08±2.07</td>
<td>11.24±2.83</td>
<td>0.68±0.16</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Values are mean±S.D. (n=3), # Vol. of 0.01N NaOH required to titrate 1.0 ml of the formulation. † Significantly different (p < 0.05) as compared to formulation of pH 7.2 as determined by one-way ANOVA followed by Dunnett’s test.

**FIGURE 1:** Effect of pH on *In Vitro* Transcorneal Permeation of Fluconazole
Effect of Concentration on In Vitro Transcorneal Permeation of Fluconazole

Table 2 and Figure 2 compare the effect of concentration on corneal permeation of fluconazole. Fluconazole ophthalmic solutions are commercially available as 0.3% w/v solution. In some studies 0.5% w/v (13) and 0.2% w/v (14) solutions of fluconazole have been used successfully in therapeutic management of fungal keratitis.

The results show that there was a significantly decrease in apparent corneal permeability (P_{app}) of fluconazole as its concentration was increased from 0.2% to 0.5%. Similar results of decrease in corneal permeability of ketorlac and diclofenac (15) with increase in concentration of drug have been observed. Thus, keeping in view the higher loss of drug at higher conc. (0.5%) and therapeutic effectiveness of fluconazole at lower concentrations (0.2%), a fluconazole ophthalmic solution of 0.2% w/v concentration was selected for further studies.

**Table 2:** Comparative corneal permeation of fluconazole from formulations of different concentrations.

<table>
<thead>
<tr>
<th>Conc. (% w/v)</th>
<th>% Cumulative Permeation*</th>
<th>( P_{app}\times10^6 ) (cm./sec)</th>
<th>Relative ( P_{app} )</th>
<th>% Corneal Hydration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>90</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>0.2%</td>
<td>22.18±1.27</td>
<td>26.51±2.91</td>
<td>29.44±3.56</td>
<td>2.68±0.33</td>
</tr>
<tr>
<td>0.3%</td>
<td>16.23±4.25</td>
<td>19.17±4.02</td>
<td>21.26±3.05</td>
<td>1.29±0.19</td>
</tr>
<tr>
<td>0.5%</td>
<td>9.52±3.19</td>
<td>10.72±3.75</td>
<td>12.02±3.90</td>
<td>0.44±0.14†</td>
</tr>
</tbody>
</table>

*Values are mean±S.D. (n=3). † Significantly different (p < 0.05) as compared to formulation of 0.2% w/v conc. as determined by one-way ANOVA followed by Dunnett’s test.

**FIGURE 2:** Effect of Concentration on In Vitro Transcorneal Permeation of Fluconazole.
Figure 3 and table 3 compare the effect of different buffers on corneal permeation of fluconazole. The results show that buffering the formulation with either citrate or phosphate caused a significant decrease in corneal permeation of fluconazole. There was no significant difference between the apparent corneal permeability ($P_{\text{app}}$) of fluconazole from citrate or phosphate buffered formulation. Since permeation of fluconazole is pH dependent, if buffered vehicle is not used, the pH shift during storage of the product may have adverse impact on the corneal permeation.

The results of titre value of fluconazole ophthalmic solution show that phosphate buffer has slightly more buffer capacity than citrate buffer and thus would be more able to resist change in pH during storage. So, it will also have somewhat more irritation potential as compared to citrate buffer. Thus, phosphate buffer was selected as the vehicle for further study.

**TABLE 3:** Comparative corneal permeation of fluconazole from 0.2% ophthalmic formulations of pH 6.0 containing different buffer

<table>
<thead>
<tr>
<th>Buffer</th>
<th>% Cumulative Permeation*</th>
<th>$P_{\text{app}} \times 10^6$ (cm./sec)</th>
<th>Relative $P_{\text{app}}$</th>
<th>Titre Value (ml)</th>
<th>% Corneal Hydration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>90</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbuffered</td>
<td>22.18±1.27</td>
<td>26.51±2.91</td>
<td>29.44±3.56</td>
<td>2.68±0.33</td>
<td>1.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>14.54±0.63</td>
<td>18.59±1.82</td>
<td>19.76±2.64</td>
<td>1.83±0.24†</td>
<td>0.68</td>
</tr>
<tr>
<td>Citrate</td>
<td>19.33±3.41</td>
<td>20.14±1.60</td>
<td>21.98±2.22</td>
<td>1.99±0.19†</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*Values are mean±S.D. (n=3). † Significantly different (p < 0.05) as compared to unbuffered formulation as determined by one-way ANOVA followed by Dunnett’s test.

**FIGURE 3:** Effect of buffer on *In Vitro* Transcorneal Permeation of Fluconazole.
EFFECT OF TONICITY MODIFIERS ON IN VITRO TRANSCORNEAL PERMEATION OF FLUCONAZOLE

Table 4 and Figure 4 compare the effect of different tonicity modifiers on corneal permeation of fluconazole. Ophthamlic solution containing mannitol as tonicity modifier provided maximum permeation followed by glucose containing formulation and the least corneal permeation was provided by fluconazole ophthalmic solutions containing sodium chloride as tonicity modifier. There was a significant increase in apparent corneal permeability of fluconazole when mannitol was employed as tonicity modifier as compared to sodium chloride. Thus, mannitol was selected for further study.

**TABLE 4:** Comparative corneal permeation of fluconazole from 0.2% ophthalmic formulations of pH 6.0 containing different tonicity modifier

<table>
<thead>
<tr>
<th>pH</th>
<th>% Cumulative Permeation</th>
<th>P$_{app}$×10$^6$ (cm/sec)</th>
<th>Relative P$_{app.}$</th>
<th>% Corneal Hydration</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>14.54±0.63</td>
<td>18.59±1.82</td>
<td>19.76±2.64</td>
<td>1.83±0.24†</td>
</tr>
<tr>
<td>90</td>
<td>16.41±1.45</td>
<td>17.86±0.58</td>
<td>19.89±1.60</td>
<td>1.80±0.09†</td>
</tr>
<tr>
<td>120</td>
<td>21.11±0.45</td>
<td>24.03±0.95</td>
<td>25.95±1.05</td>
<td>2.37±0.09</td>
</tr>
</tbody>
</table>

*Values are mean±S.D. (n=3). † Significantly different (p<0.05) as compared to formulation containing mannitol as determined by one-way ANOVA followed by Dunnett’s test.

**FIGURE 4:** Effect of Tonicity Modifier on In Vitro Transcorneal Permeation of Fluconazole
EFFECT OF PRESERVATIVES ON IN VITRO TRANSCORNEAL PERMEATION OF FLUCONAZOLE

Table 5 and Figure 5 compare the effect of different antimicrobial preservatives on corneal permeation of fluconazole. The corneal permeation of fluconazole was found to be in the order of BA > PMN > BAC > PMA > TM. The maximum corneal permeability of fluconazole was provided by benzyl alcohol. The results show a significant increase in corneal permeation of fluconazole when BA was employed as preservative. So, no significant difference in apparent corneal permeability was observed when BAC or TM or PMN or PMA were employed as preservatives in fluconazole ophthalmic formulations. Thus BA was selected to be used as preservatives in model formulation.

TABLE 5: Comparative corneal permeation of fluconazole from 0.2% fluconazole formulations containing different preservative in phosphate buffer (pH-6.0, 0.0667M)

<table>
<thead>
<tr>
<th>pH</th>
<th>% Cumulative Permeation*</th>
<th>P&lt;sub&gt;app&lt;/sub&gt; × 10&lt;sup&gt;-6&lt;/sup&gt; (cm/sec)</th>
<th>Relative P&lt;sub&gt;app&lt;/sub&gt;</th>
<th>% Corneal Hydration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>90</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>BAC</td>
<td>17.63±3.83</td>
<td>23.99±5.05</td>
<td>31.93±5.12</td>
<td>2.82±0.48</td>
</tr>
<tr>
<td>TM</td>
<td>17.68±2.64</td>
<td>21.89±3.74</td>
<td>29.01±2.98</td>
<td>2.55±0.30</td>
</tr>
<tr>
<td>PMN</td>
<td>28.95±1.69</td>
<td>34.22±2.02</td>
<td>40.41±0.61</td>
<td>3.62±0.09</td>
</tr>
<tr>
<td>PMA</td>
<td>19.54±13.03</td>
<td>23.86±13.59</td>
<td>31.14±11.73</td>
<td>2.74±1.11</td>
</tr>
<tr>
<td>BA</td>
<td>43.02±4.20</td>
<td>61.12±5.14</td>
<td>74.29±4.27</td>
<td>6.71±0.42&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (Unpreserved)</td>
<td>21.11±0.45</td>
<td>24.03±0.95</td>
<td>25.95±1.05</td>
<td>2.37±0.09</td>
</tr>
</tbody>
</table>

* Values are mean±S.D. (n=3). † Significantly different (p<0.05) as compared to control formulation as determined by one-way ANOVA followed by Dunnett’s test.
**FIGURE 5:** Effect of Preservative on *In Vitro* Transcorneal Permeation of Fluconazole

![Graph showing effect of preservative on in vitro transcorneal permeation of fluconazole](image)

**Effect of Viscosity modifiers on *in vitro* transcorneal permeation of Fluconazole**

*Table 6* and *Figure 6* compare the effect of different viscolizers on corneal permeation of fluconazole with the control formulation. The results reveal that there was no significant difference between the apparent corneal permeability ($P_{\text{app.}}$) of viscolizer containing preparation as compared to control formulation containing no viscolizer. Viscolizers are employed to increase the pre-corneal residence time of the drug so that a higher and prolonged ocular availability is achieved. It is expected that during *in vivo* use HPMC containing formulation having viscosity of 8.24 cps will provide a greater pre-corneal residence and thus greater ocular availability. Thus, HPMC was selected to be used as viscosity modifier in model formulation.

**Table 6:** Comparative corneal permeation of fluconazole from 0.2% ophthalmic formulations containing different viscolizer in phosphate buffer (pH-6.0, 0.0667M)

<table>
<thead>
<tr>
<th>Viscosity Modifier</th>
<th>Viscosity (cps)</th>
<th>% Cumulative Permeation*</th>
<th>$P_{\text{app.}} \times 10^6$ (cm./sec)</th>
<th>Relative $P_{\text{app.}}$</th>
<th>% Corneal Hydration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>90</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>PVA</td>
<td>3.46±0.03</td>
<td>35.11±12.60</td>
<td>45.63±16.99</td>
<td>52.76±18.32</td>
<td>4.79±1.69</td>
</tr>
<tr>
<td>HPMC</td>
<td>8.24±0.20</td>
<td>23.91±3.34</td>
<td>29.82±5.04</td>
<td>37.89±10.90</td>
<td>3.36±0.89</td>
</tr>
<tr>
<td>HPC-H</td>
<td>5.77±0.12</td>
<td>37.81±8.83</td>
<td>42.69±9.58</td>
<td>48.34±11.18</td>
<td>4.36±1.01</td>
</tr>
<tr>
<td>Control</td>
<td>1.09±0.24</td>
<td>21.11±0.45</td>
<td>24.03±0.95</td>
<td>25.95±1.05</td>
<td>2.37±0.09</td>
</tr>
</tbody>
</table>

*Values are mean±S.D. (n=3)
**CONCLUSION**

In the present investigation, the effect of formulation parameters, on fluconazole corneal permeation, were characterized. Transcorneal permeation studies were conducted using isolated sheep cornea. On the basis of these studies following conclusions can be drawn:

1). The use of Sorenson phosphate buffer as a vehicle (0.0667 M, pH 6.0) favors the permeation of fluconazole. 2). Tonicity adjustments with mannitol provide better permeation than glucose, followed by NaCl.

Fluconazole (0.2% w/v), ophthalmic solution (pH 6.0) containing benzyl alcohol (0.5% w/v) provided maximum in vitro ocular availability through sheep cornea followed in turn by ophthalmic solution containing phenyl mercuric nitrate, benzalkonium chloride, phenyl mercuric acetate and thiomersal. Polyvinyl alcohol (1.4% w/v) or hydroxypropyl cellulose-high viscosity grade (0.25% w/v) provided an almost equivalent decrease in in vitro diffusion of fluconazole across the cornea.

In *in vivo* use HPMC (1.0% w/v) having higher viscosity of all the tested formulations is expected to provide maximum pre-corneal residence and thus would be most suitable. Fluconazole solution demonstrates the maximum corneal permeation at pH 6.0. Further investigations in *in vivo* models are warranted in order to adequately assess the ocular bioavailability of *in vitro* optimized model formulations.

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