Formulation and In-vitro evaluation of topical transferosomol gel of bifonazole for fungal infections

Sumaiya Parveen and Sirisha Mittapally

Abstract
Human fungal diseases pose a significant danger, but often overlooked, burden on public health, affecting over 1 billion people worldwide. Superficial fungal infections (e.g., nail, skin, and urogenital) affect most people at some point in their lifetimes, but are usually curable when treated with antifungal drugs. Nevertheless, they affect quality of life and burden health services. Transdermal drug delivery is one of the promising drug delivery system for administration of drug across the skin but it has drawback of less permeability due to the stratum corneum, the outer most protective layer which prohibit the entry of xenobiotics. Therefore several vesicular drug delivery systems have been developed. The present aim of the study is to increase the permeability of Bifonazole which is a broad spectrum antifungal imidazole drug. It is a class IV drug which has less permeability and low solubility, so as to increase the permeability and solubility, it has been loaded into one of the best vesicular system i.e. transferosomes which has ultradeformable flexibility. In this study 12 formulations were prepared by reverse phase evaporation method using soya lecithin, span 60, span 80 and tween 80 and evaluated for in vitro drug release. Five formulations for each surfactant were prepared by keeping the drug concentration constant. Among three surfactants span 60 showed better results. Among the five formulations of span 60, F1 and F2 with ratio of 2:1 and 4:1 of soya lecithin and span 60 respectively showed highest drug release of 75.905% and 94.8% respectively. F2 showed highest drug content and entrapment efficiency of 91.25 and 93.13%. But zeta potential report showed very low stability; hence to increase the stability of formulation; cholesterol was added in the ratio of 2:1 and 4:1 of phospholipid and cholesterol respectively. Then the zeta potential results of formulation were in the acceptable range. Hence it can be concluded that Bifonazole can be loaded in to transferosomal carrier which was found to be effective for increasing the permeability and can be proceeded for further future studies.

Keywords: Transdermal drug delivery system, transferosomes, bifonazole

1. Introduction
Transdermal drug delivery is preferable over other routes of administration due to its potential benefits compare to other routes but it has major drawback of less permeability due to the stratum corneum, the outer most protective layer which prohibit the entry of xenobiotics. Several methods had been developed to increase the permeability of the drug molecules through different techniques like skin permeation enhancers (surfactants, sulfoxides etc), microneedles (vaccines), iontophoresis (peptides and oligonucleotides), electroporation (vaccines, oligonucleotides etc), sonophoresis (hormones, proteins, vaccines etc). Recent advances developed a vesicular carrier called transferosomes which is a second generation liposomes. Transferosomes are ultra-deformable vesicles possessing an aqueous core surrounded by the complex lipid bilayer (similar to liposomes). The size and shape of these vesicles is self-coordinating and self-optimizing based on the composition [1-6]. Bifonazole is a broad spectrum antifungal imidazole drug which is active against dermophytes, moulds, yeast, dimorphic fungi etc. It is used for topical fungal infections like athletes foot, Tinea corporis and it is a class IV drug which has less permeability and solubility issues. The aim of the present study is to increase the permeability and solubility by incorporating drug in transferosomal gel which is prepared by reverse phase evaporation method using soya lecithin, span60, span 80, tween 80 and cholesterol. Formulations were evaluated for in vitro drug release and it shows effective diffusion compare to 1% creams available in market [7].

2. Materials and Methods
2.1 Materials
Bifonazole was obtained as a gift sample from vital Laboratories Pvt. Ltd, soya lecithin was...
purchased from Himedia, chloroform was purchased from Fischer Scientifics, span 60, 80 and tween 80 was procured from S.D fine chemicals, cholesterol was procured from Fischer Scientifics and Carbopol 934 was procured from S.D fine chemicals.

### 2.2 Preparation of Transfersomes

Transfersomes were prepared by reverse phase evaporation method. Drug, surfactant, soya lecithin was taken in different proportions in solvent mixture of chloroform and methanol which was taken in ratio of 2:1 and then it was kept for 24 hours until the formation of thin film. This thin film was hydrated using phosphate buffer 6.8 at 55 °C and sonicated by ultrasonic probe sonicator at a frequency of 20 KHz for 2 min. Then unentrapped drug was removed by centrifugation and were transferred to 1 % w/v carbopol gel and stored at 4°C. [8, 9]

### 3. Evaluation

#### 3.1 Drug- Excipient Compatibility

Compatibility studies were done using Shimadzu Fourier-transform infrared spectrophotometer. FTIR spectrum was taken for pure drug and for physical mixture of excipients with drug by potassium bromide pellet method [9, 10].

#### 3.2 Melting Point

The melting point of bifonazole was determined using capillary method [9, 10].

#### 3.3 Surface Morphology of Transfersomes

Scanning electron microscopy is used to determine the shape and size of formulated bifonazole loaded transfersomes [11, 12].

#### 3.4 Particle Size Distribution and Zeta Potential Determination

Particle size distribution and zeta potential was determined by Dynamic light scattering method by particle size analyzer (nanopartica- Horiba Scientifics) [11, 12].

#### 3.5 Drug Content

1ml of bifonazole loaded transfersomal suspension was taken and diluted with 6.8 phosphate buffer. It was ultracentrifuge (Remi-CM12plus) for 4000 rpm for 45min at 4°C and after centrifugation, disruption of vesicles were done using appropriate quantity of methanol then 1ml of supernatant liquid was taken and suitable dilutions were made and analyzed by UV spectrophotometer at 255nm and % drug content can be calculated using following formula [11-13]

\[
\% \text{ drug content} = \frac{m.l}{t} \times 100
\]

Where, \( S \) - Spreadability in g.cm / sec
m - Weight tied to upper slide
l - Length of glass slide
t - Time in seconds

#### 3.6 Entrapment Efficiency

1ml of bifonazole transfersomal suspension was taken and diluted with 6.8 phosphate buffer and ultracentrifuge (Remi-CM12plus) for 4000 rpm for 45min at 4°C, from that 1ml of supernatant liquid is taken and appropriate dilutions were made and analyzed using UV spectrophotometer at 255nm. % entrapment efficiency can calculate using following formula [11-13]

\[
\text{Entrapment efficiency} = \frac{\text{Amount entrapped}}{\text{Total amount added}} \times 100
\]

### Table 1: Formulation chart of bifonazole loaded transfersomal gel

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
<th>F12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Drug (mg)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2.</td>
<td>Lecithin (mg)</td>
<td>100</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>100</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>100</td>
<td>200</td>
<td>400</td>
<td>800</td>
</tr>
<tr>
<td>3.</td>
<td>Span 60</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Span 80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Tween 80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>6.</td>
<td>Chloroform: methanol</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
<td>2:1</td>
</tr>
<tr>
<td>7.</td>
<td>Carbopol 934 (%W/V)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8.</td>
<td>Sodium Benzoate(%W/V)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

#### 3.7 Homogeneity

All developed gels were tested by visual inspection after the gels have been set in the container. They were tested for their color, appearance and presence of any aggregates [9].

#### 3.8 Viscosity

Viscosity of all the formulated gels was measured using Brookfield DV-E viscometer. The samples were taken in 100ml beaker and viscosity was measured at the rotation of 2 rpm shear rate at roomtemperature [9].

#### 3.9 pH Determination

The pH of formulated transfersomal gels was determined using pH meter. Required amount of gel was added to 10ml of distilled water to get a uniform suspension. The electrode was immersed in suspensions and readings were recorded on pH meter [9].

#### 3.10 Spreadability

Spreadability can be determined by taking two glass slides. 500mg of prepared gel is placed on one of the glass slide and the next slide is kept on the top of first slide which is tied to 10g weight then it is allowed to spread for 1min and length of the spreaded gel on the glass slide was measured using scale. The time (seconds) required to separate the two slides was taken as a measure of spreadability. It was calculated using the formula,

\[
S = \frac{m.l}{t}
\]

Where, \( S \) - Spreadability in g.cm / sec
m - Weight tied to upper slide
l - Length of glass slide
t - Time in seconds

#### 3.11 In-Vitro Diffusion Studies

A diffusion study of 12 formulations was carried out using Franz diffusion cell through dialysis membrane from Himedia. Dialysis membrane was soaked in distilled water for 24 hours. Franz diffusion cell contain two compartments upper donor and lower receptor compartment. The receptor compartment was filled with 6.8 phosphate buffer and donor compartment contain 200mg of transfersomal gel on dialysis membrane with exposure area of 2cm² to receptor medium and whole assembly was kept on magnetic stirrer at 600rpm.
for a period of 360 minutes and samples were withdrawn at an interval of 30 minutes for first 3 hours and then 1 hour interval was given for another 3 hours and replaced with equal volume of buffer. Samples were appropriately diluted with buffer and analyzed using UV spectrophotometer at 255nm. 

4. Results and Discussion
4.1 Drug Excipient Compatibility Studies
Compatibility studies were done using Shimadzu Fourier-transform infrared spectrophotometer. The characteristic absorption for drug and for physical mixture of drug with excipients was found at peaks mention below:

The major peaks for bifonazole were found at 1598 cm\(^{-1}\), 1581 cm\(^{-1}\), 1650 cm\(^{-1}\) and 1075 cm\(^{-1}\) for C=C (imidazole ring stretching), C=N (imidazole ring), C=C (aromatic) and C-N respectively. The major peaks of span 60 were found at 3815 cm\(^{-1}\), 1707 cm\(^{-1}\). The major peaks for span 80 were found at 3834 cm\(^{-1}\) and 1566 cm\(^{-1}\). The major peaks for tween 80 were found to be at 3030 cm\(^{-1}\), 3590 cm\(^{-1}\), and 2960 cm\(^{-1}\) the major peaks for lecithin were found at 1735 cm\(^{-1}\) (C=O), 1350 cm\(^{-1}\) (P-O) and 1072 cm\(^{-1}\) (3\(^{rd}\) amines). FTIR studies confirmed the drug-excipient compatibility.

[Fig 1: FTIR of Pur Drug]
[Fig 2: FTIR of Drug with Lecithin and Span 60]
4.2 Melting Point
The melting point was found to be at 142°C by capillary method.

4.3 Surface Morphology of Transferosomes
The formulated bifonazole transferosomes were found to be almost spherical in shape and vesicle size was found to be in the range of 166-291um.

4.4 Particle Size Distribution and Zeta Potential Determination
Zeta potential of bifonazole loaded transferosomes of formulation showed very low stability.
4.5 Drug Content
The drug content of all 12 formulations was determined. Among 12 formulations, span 60 F2 formulations showed highest drug content followed by F1.

4.6 Entrapment Efficiency
The entrapment efficiency for all 12 formulations was determined. Among all 12 formulations, span 60 F2 of 4:1 formulation showed highest entrapment efficiency followed by F1.
4.7 Viscosity
Viscosity of 12 formulations was determined by DV-E Brookfield Viscometer and was in the range of 34800-36200cp

4.8 Ph Determination
pH of all 12 formulations was measured using pH meter and was in the range of 6.3-6.6.

4.9 Spreadability

![Spreadability chart]

Spreadability of bifonazole loaded transferosomal gel was measured and was found to be in the range of 43.03-45.21 g.cm/sec.

4.10 In-Vitro Diffusion Studies
A diffusion study of 12 formulations was carried out using Franz diffusion cell through dialysis membrane from Himedia Dialysis membrane. The diffusion study was done for 6 hours. Among 12 formulations, F2 formulation of 4:1 ratio showed highest diffusion followed by F1.

As per the above evaluation of transferosomal formulations, Span 60 showed better results compared to other surfactants and among 4 formulations of Span 60 F2 followed by F1 showed highest drug content, entrapment efficiency and highest diffusion of drug over the span of 6 hours but the problem arises for stability of formulation. Based on drug content, entrapment efficiency and in vitro diffusion of drug, span 60 (F2) formulations showed highest results and hence it was selected for further studies which include increasing the time of sonication to reduce vesicle size and enhancing the stability of formulation by including cholesterol in the F2 formulation in 2:1 and 4:1 ratio of phospholipid to cholesterol [14].
5. Formulation of Transfersomal Gel of F2 By Incorporating Cholesterol In Formulation

Transfersomes was prepared by reverse phase evaporation method. Soya lecithin, cholesterol, span 60 was dissolved in a mixture of chloroform and methanol (2:1) and drug was added to above mixture. It was kept for 24 hours at room temperature for evaporation of solvent until thin film is formed. After evaporation of solvent thin film was hydrated with phosphate buffer 6.8pH at temperature of 55 °C. Then it was sonicated at frequency of 20 HZ for 5min by using probe sonicator and unentrapped drug was removed by centrifugation at 4000 rpm for 45min at 4 °C and incorporated in 1%w/v carbopol gel and stored at 4 °C.[14, 15]

Table 2: Formulation chart of bifonazole loaded transfersomal gel prepared after modification in formulation

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>F2 (1)</th>
<th>F2 (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Drug (mg)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2.</td>
<td>Lecithin:cholesterol</td>
<td>2:1</td>
<td>4:1</td>
</tr>
<tr>
<td>3.</td>
<td>Lecithin: span 60</td>
<td>4:1</td>
<td>4:1</td>
</tr>
<tr>
<td>4.</td>
<td>Chloroform:Methanol</td>
<td>2:1</td>
<td>2:1</td>
</tr>
<tr>
<td>5.</td>
<td>Carbopol 934 %W/V</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6.</td>
<td>Sodium Benzoate% w/v</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

5.1 Characterization of Transfersomal Gel

5.1.1 Surface Morphology: The formulated bifonazole transfersomes were found to be almost spherical in shape and vesicle size was found to be in the range of 120nm-550nm.

![Fig 13: SEM image of F2 (2:1) formulation](image1)

5.1.2 Particle Size Distribution and Zeta Potential

Zeta potential of bifonazole loaded transfersomes of formulation F (2:1) and F2 (4:1) was found to be -35.4MV and -25.7MV respectively.

![Fig 14: Zeta potential of F2 (2:1) Formulation](image2)

![Fig 15: particle size distribution of F2 (2:1) formulation](image3)
5.1.3 Drug Content And Entrapment Efficiency: Drug content and entrapment efficiency of F2 (2:1 and 4:1) was determined and samples were analyzed.

5.1.4 In-Vitro Diffusion: Diffusion studies of F2 (2:1 and 4:1) were done for 6 hours and samples were analyzed by UV spectrophotometer at 255nm.
F2 (2:1) formulation was selected as optimized formulation based on the zeta potential value, drug content, entrapment efficiency and in-vitro diffusion studies.

6. Release Kinetic Profile For Transderosomal Gel of Optimized Formulation F2 (2:1):
The drug release kinetics studies were estimated to determine the type of release mechanism followed. Release kinetic study of optimized formulation was studied for different kinetic equations (zero order, first order, Higuchi, and Korsemeyer-peppas equation) [16].

![Higuchi plot](image)

**Figure 22:** Higuchi plot of F2 (2:1)

![Peppas plot](image)

**Figure 23:** Peppas plot of F2 (2:1)

$R^2$ values for the optimized formulation were found to be highest for the Higuchi model. This indicated that the drug release from all the formulations followed diffusion controlled release mechanism.

7. Stability Studies of Optimized Formulation
Optimized formulation F2(2:1) was stored at refrigerated temperature(4 °C), Room temperature and for short term accelerated stability studies at 40°C±5° and 75±5% RH as per International Conference on Harmonization (ICH) states Guidelines. Samples were analyzed for homogeneity, pH, drug content, entrapment efficiency and in-vitro diffusion studies after 30, 60 and 90 days. Stability studies showed that Transerosomal gel is more stable at 4 °C when compare to other temperatures. There was a change in color for the samples kept at room temperature and accelerated temperature and leakage of drug was minimum due to gel formulation because it is viscous in nature and also decreases the fusion of vesicles which otherwise will be responsible for drug leakage [17, 18].

<table>
<thead>
<tr>
<th>s.no.</th>
<th>Duration</th>
<th>% Drug content</th>
<th>% Entrapment efficiency</th>
<th>pH</th>
<th>Visual appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>30 days</td>
<td>90.37 83.14 80.7</td>
<td>79.32 77.5 78.57</td>
<td>6.5 6.3 6.5</td>
<td>N.A  N.A  N.A</td>
</tr>
<tr>
<td>2.</td>
<td>60 days</td>
<td>90.05 78.12 75.54</td>
<td>79.01 77.21 78.21</td>
<td>6.5 6.3 6.5</td>
<td>N.A  N.A  N.A</td>
</tr>
<tr>
<td>3.</td>
<td>90 days</td>
<td>90.05 70.2 66.51</td>
<td>78.9 71.9 68.47</td>
<td>6.5 6.3 6.5</td>
<td>N.A  C.C  C.C</td>
</tr>
</tbody>
</table>

4±2°C-Refigerated temperature, R.T-Room Temperature, A.T-Accelerated temperature and N.A-No aggregates. C.C-change in color
8. Conclusion
Transdermal drug delivery by vesicular system provides various benefits. Bifonazole loaded transferosomal gel was prepared by reverse phase evaporation method and characterization of transferosomal gel was done by determination of vesicle size, zeta potential, drug content, entrapment efficiency and in-vitro diffusion studies. 12 formulations were prepared by taking soya lecithin, surfactant and drug; but it was noticed that formulations based on zeta potential value. Hence to increase the stability and as well as to decrease the vesicle size, further two formulation were prepared by taking F2 formulation because it showed highest drug content, entrapment efficiency and in-vitro diffusion among all12 formulation. Two formulations were named as F2 (2:1) and F2 (4:1) which had cholesterol in their formulation in a ratio of 2:1 and 4:1 with respect to phospholipid. F2 (2:1) was selected as optimized formulation after its evaluation and was kept at three different temperatures for stability studies.

9. References
4. Cevc G, Schätzlein A, Richardsen H, Vierl U. "Overcoming semi-permeable barriers, such as the skin, with ultra-deformable mixed lipid vesicles, Transfersomes, liposomes or mixed lipid micelles. Langmuir, 2003; 19(26):10753-10763