DESIGN AND DEVELOPMENT OF TRANSFEROSOME OF FLUCONAZOLE FOR TOPICAL DRUG DELIVERY SYSTEM

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ABSTRACT
Transferosome are ultra deformable carriers, well known for their potential in topical drug delivery have been chosen to avoid the side effect and drug interaction of Fluconazole during oral administration and increase skin penetration power of Fluconazole. In present work for the formulation of Transferosome of fluconazole using Factorial Design approach was undertaken. Phospholipid (PL 90 G) and surfactant ratio and different surfactant (Span-80, tween-80 and tween-20) were taken at three different levels and Transferosome were prepared using thin film Hydration Technique. The Prepared formulation were characterized by Vesicle size (nm), % Entrapment efficiency, Transmission electron microscopy (TEM) (Optimized batch), in-vitro drug diffusion, Gels Containing Transferosome (Optimized Batch) were prepared in Carbopol 934 with different concentration 1%, 1.5%, 2%, 2.5% and 3% and characterized for pH, viscosity, Drug content, Spreadability, permeation and in vitro drug diffusion study and stability study. Result of regression analysis revealed that vesicle size and % Entrapment efficiency were dependant on the phospholipid and surfactant ratio and different surfactant. Formulation (TF2) showed maximum drug Entrapment 69.34% and minimum vesicle size 187 nm. TEM study of (TF2) Formulation showed that vesicles have a uniform spherical shape with nano size. Rheological studies of all Transferosomal gels gave clear idea 2% carbopol required. Transferosomal suspension and gel were found to highest % release (92.96%) and increase the skin penetration than control gel. Transferosomal suspension and gel were found to be stable in freezing temperature for 30 days. It is evident from this study that transfersomes are a promising prolonged delivery system for Fluconazole and have reasonably good stability characteristics. This research suggests that Fluconazole loaded transfersomes can be potentially used as a Topical drug delivery system.

KEYWORDS: Transferosome; Fluconazole; Factorial Design; Gels; Topical.

INTRODUCTION
Topical drug delivery is an attractive route for local and systemic treatment. The delivery of drugs onto the skin is recognized as an effective means of therapy for local dermatologic diseases. Topical drug delivery systems the major limitation of is the permeability of the skin, it is permeable to small molecules, lipophilic drugs and highly impermeable to macromolecules and hydrophilic drugs. The main barrier and rate-limiting step for diffusion of drugs across the skin is provided by the outermost layer of the skin, the stratum corneum (SC)\(^[[1]\). Recent approaches in
modulating vesicle compositions have been investigated to develop systems that are capable of carrying drugs and macromolecules to deeper tissues. These approaches have resulted in the design of two novel vesicular carriers, ethosomes and ultraflexible lipid-based elastic vesicles, transfersomes. Transfersomes are ultradeformable vesicles possessing an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimizing. Transfersomes have recently been introduced, which are capable of transdermal delivery of low as well as high molecular weight drugs. Transfersomes are specially optimized, ultraflexible lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact and then act as a drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents. Each transfersome consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties, due to the incorporation of "edge activators" into the vesicular membrane. Surfactants such as sodium cholate, sodium deoxycholate, Span 80, and Tween 80, have been used as edge activators. Due to their deformability, transfersomes are good candidates for the non-invasive delivery of small, medium, and large sized drugs. Materials commonly used for the preparation of transfersomes are phospholipids (soya phosphatidyl choline, egg phosphatidyl choline), surfactants (Tween 80, sodium cholate) for providing flexibility, alcohol (ethanol, methanol) as a solvent, dye (Rhodamine-123, Nile-red) for confocal scanning laser microscopy (CSLM) and buffering agent (saline phosphate buffer pH 7.4), as a hydrating medium. Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss.

Fluconazole, a synthetic antifungal agent, is a triazole derivative. It is used in the treatment of oropharyngeal, esophageal, or vulvovaginal candidiasis as well as other serious systemic candidal infections. It is also effective against superficial fungal infections and dermatophytoses. Fluconazole is available commercially as tablets and injections only in spite of its well known adverse effects including nausea, vomiting, bloating and abdominal discomfort. Oral fluconazole cannot be taken in conjunction with a number of medications. In order to bypass these disadvantages, the Transferosomal gel formulations have been proposed as topical application. The system was developed and evaluated for its physicochemical characteristics, such as particle size, entrapment efficiency, stability, and In vitro skin permeation. The compositions of lipid in the transfersomes (soya Phosphatidyl choline) was evaluated.

**MATERIALS AND METHODS**

**Materials**
Fluconazole (Cadilla pharmaceutical Ltd. Ahmedabad) and soya Phosphatidyl choline Phospholipon 90G) was a generous gift Lipoid Germany. Span-80, Tween 80 (TW80), Tween-20, Carbopol® 934 NF (poly acrylic acid polymer) and methanol were purchased from Shital pharmachem, Ahmedabad. All other chemicals used were of HPLC or analytical grade.

**PREPARATION OF FLUCONAZOLE LOADED TRANSFERSOMES**

Soya-phosphatidylcholine was taken in a round bottom flask. Span 80 or Tween 80 or Tween-20 was put in the same round bottom flask. Methanol was then added to the same flask. The drug was also loaded in the same RBM. These were then dissolved by shaking. Thin film was then formed by keeping it in the rotatory vaccum evaporator at 60°C. This thin film was then hydrated by phosphate buffer saline to get the Transferosome.\(^8\)

**Effect of variables**

To study the effect of variables on Transferosome performance and characteristics, different batches were prepared using \(^3^2\) factorial design approach. Ratio of PL 90G and Surfactant and effect of different surfactant were selected as two independent variables. Vesicle sizes, entrapment efficiency (EE) were selected as dependent variables. Amount of methanol (4ml) and fluconazole (100 mg) were kept constant. Values of all variables and batch codes are shown in Table 1.

**Experimental design with coded levels of variables and actual values**

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>(X_1) (Phospholipid: surfactant ratio)</th>
<th>(X_2) (Effect of various surfactant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF1</td>
<td>(-1) 75 :25</td>
<td>(-1) span-80</td>
</tr>
<tr>
<td>TF2</td>
<td>(-1) 75 :25</td>
<td>(0) Tween-20</td>
</tr>
<tr>
<td>TF3</td>
<td>(-1) 75 :25</td>
<td>(1) Tween-80</td>
</tr>
<tr>
<td>TF4</td>
<td>(0) 80:20</td>
<td>(-1) span-80</td>
</tr>
<tr>
<td>TF5</td>
<td>(0) 80:20</td>
<td>(0) Tween-20</td>
</tr>
<tr>
<td>TF6</td>
<td>(0) 80:20</td>
<td>(1) Tween-80</td>
</tr>
<tr>
<td>TF7</td>
<td>(1) 85:15</td>
<td>(-1) span-80</td>
</tr>
<tr>
<td>TF8</td>
<td>(1) 85:15</td>
<td>(0) Tween-20</td>
</tr>
<tr>
<td>TF9</td>
<td>(1) 85:15</td>
<td>(1) Tween-80</td>
</tr>
</tbody>
</table>
Size distribution

Mean vesicle size and size distribution profile of Trasferosome was determined by using Malvern particle size analyzer model SM 2000, which follows Mie's theory of light scattering. Diluted liposome suspension was added to the sample dispersion unit containing stirrer and stirred at 2000 rpm in order to reduce the interparticle aggregation, and laser obscuration range was maintained between 10-20%. The average particle size was measured after performing the experiment in triplicate.\[13-15\]

Entrapment efficiency

Fluconazole associated with Transferosome was separated from unentrapped drug using centrifugation method. Transferosome were centrifuged at 20000 rpm for 1 h at controlled temperature of 4°C. Supernatant containing unentrapped fluconazole was withdrawn and measured UV spectrophotometrically at 260 nm against phosphate buffer saline (pH 7.4). The amount of fluconazole entrapped in liposome was determined as follow

\[
EE(\%) = \left(\frac{C_d - C_f}{C_d}\right) \times 100 \quad (1)
\]

Where \(C_d\) is concentration detected of total fluconazole and \(C_f\) is concentration of free fluconazole. The entrapment efficiency was obtained by repeating the experiment in triplicate and the values were expressed as mean standard deviation.\[16,17\]

Transmission Electron Microscopy (TEM)

TEM (H-7500, Japan) was used to visualize the transfersomal vesicles. The sample were negatively stained magnification at an accelerating voltage of 100 kV with a 1% aqueous solution of phototungustic acid (PTA). After drying, the sample was viewed under the microscope at 10–100 k.\[19\]

In-vitro Drug Diffusion Study

Cellulose membrane (0.45μm, obtained from sigma chemicals) was used for this study. A sample of 1g of the preparation was spread on a cellulose membrane previously soaked overnight in the release medium. The loaded membrane was firmly stretched over the edge of a glass tube of 2 cm diameter; the membrane was tied up with a rubber to prevent leakage. Tubes were then immersed in the dissolution vessel which contained 50 ml of the release medium, phosphate buffer pH 7.4, and maintained at 37°C ± 0.5°C. The shafts were rotated at 50 rpm and aliquots each of 3 ml were withdrawn from the release medium at specified time intervals. Withdrawn samples were replaced by equal volumes of fresh release medium. The samples were assayed spectrophotometrically at λmax 260 nm and the concentration of the drug was determined from the previously constructed calibration curve. Each data point represented the average of three
determinations. *In vitro* release studies were recorded for a four hour period. Previous solubility tests were made so as to ensure sink conditions for drug dissolution in the donor medium.²⁰⁻²⁵

**Skin permeation and drug deposition studies**

Skin permeation studies with fluconazole containing Transferosome formulations were carried out using Cellophane membrane, employing modified Franz-diffusion cell. The results obtained were compared with that of non-Transferosomal formulations of fluconazole. The cellophane membrane was prepared by mounting on the receptor chamber with cross-sectional area of 3.91 cm exposed to the receptor compartment. The receptor compartment was filled with (100 ml) phosphate buffer pH 7.4. It was jacketed to maintain the temperature 37 ± 0.5 C and was kept stirring at 50 rpm. Prior to application of formulations, the membrane was allowed to equilibrate at this condition for 1 h. Transferosomal or non-Transferosomal fluconazole formulation (amount equivalent to 5 mg of drug) was applied uniformly on the dorsal side of membrane. Aliquots of 2 ml were withdrawn periodically and replaced with same amount of saline solution to maintain the receptor phase volume at a constant level. The samples were quantified spectrophotometrically at 260 nm after suitable dilution and filtration.²⁶⁻²⁷

**Preparation of liposomal gel (Optimized Batch TF2)**

On the basis of factorial design approach, Transferosome batch was selected for further formulation studies of Transferosomal gel. Gel was prepared using carbopol® 934 NF (1, 1.5, 2, 2.5 and 3%). The appropriate quantity of carbopol 934 powder was dispersed into distilled water under constant stirring with a glass rod, taking care to avoid the formation of indispersible lumps and allowed to hydrate for 24 h at room temperature for swelling. Topical transferosome gel formulations were prepared by incorporation of transferosome’s containing fluconazole (separated from the unentrapped drug) were mixed into the carbopol gel with a mechanical stirrer (25 rpm, 2 m). The dispersion was neutralized using triethanolamine (0.5% w/w). Control gels were made under the same conditions.²⁴

**Table 2. Preparation of Formulation Transferosomal gel**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation Code</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferosomal suspension (ml)</td>
<td>Equivalent to 0.5% drug</td>
<td>Equivalent to 0.5% drug</td>
<td>Equivalent to 0.5% drug</td>
<td>Equivalent to 0.5% drug</td>
<td>Equivalent to 0.5% drug</td>
<td>Equivalent to 0.5% drug</td>
</tr>
<tr>
<td>Carboxol 934 (%)</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Water (ml)</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>
Drug content

The gel sample (100 mg) was withdrawn and drug (fluconazole) content was determined using UV spectrophotometer at 260 nm. In case of transferosomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analyzed by using UV spectrophotometer at 260 nm.[22]

Stability studies

Stability study by keeping the transferosomal gel at two different temperature conditions, i.e., 4-8 °C (Refrigerator; RF), 25±2 °C (Room temperature; RT), for a period of 30 days. Samples were withdrawn periodically and analyzed for the drug content and drug release for Transferosomal gel.[23]

RESULTS AND DISCUSSION

| Table 3. Formulation and evaluation of batches in $3^2$ full factorial design |
|----------------|----------------|----------------|----------------|----------------|
| Batch Code   | $X_1$ (Phospholipid: surfactant ratio) | $X_2$ (Effect of various surfactant) | Vesicle size (nm) | % Entrapment Efficiency |
| TF1          | -1             | -1             | 580             | 54.22           |
| TF2          | -1             | 0              | 187             | 69.34           |
| TF3          | -1             | 1              | 220             | 63.22           |
| TF4          | 0              | -1             | 1092            | 47              |
| TF5          | 0              | 0              | 282             | 55.50           |
| TF6          | 0              | 1              | 392             | 53.98           |
| TF7          | 1              | -1             | 694             | 51.20           |
| TF8          | 1              | 0              | 280             | 60.25           |
| TF9          | 1              | 1              | 295             | 60              |
Ratio of phospholipid:surfactant and effect of different surfactant were found to be critical in preparation and stabilization of transferosomes and hence selected as variables in the $3^2$ factorial designs.

In a preformulation study the ratio of phospholipid:surfactant and different surfactant were determined to obtain stable transferosomes devoid of aggregation, fusion and sedimentation. The 80:20 ratio of phospholipid:surfactant(span-80) was found to be optimum to prevent aggregation of transferosomes. Transferosomes were prepared using film hydration technique and method was found to be well suited for the production of transferosomes without aggregation. Responses of different batches were obtained by using factorial design (Table  ). Obtained data were subjected to multiple regression analysis using “design expert 9" software and obtained data were fitted in Eq. (2).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_11X_1X_1 + b_22X_2X_2 + b_12X_1X_2$$

**Effect of variables on particle size**

The most important parameter, which needs to monitor during transferosomes preparation its best performance, is the vesicle size and size distribution of transferosome. From the number of reports, it was observed that the size and size distribution of the transferosome determines their in vivo or ex-vivo performance. There are some reports, which showed the effect of transferosome size on the drug release as well as drug deposition in the skin. It was observed that the relative ratio of phospholipid:surfactant was found to play important role in vesicle size. Size of vesicles found to be in the range of 187-1092nm. To understand the effect of surfactant and lipid concentration on vesicle size, coefficient observed for liposome size fitted in Eq

$$Y=391.44 - 243.166X_1 + 295.833X_1^2$$

The reduced model was tested in proportion to determine whether $X_1$ and $X_1^2$ the coefficient contribute significance information to the prediction of Vesicle size. The results of regression analysis reveal that on decrease the values for $X_1$ and $X_1^2$ means decrease surfactant concentration and increase phospholipid concentration that time, increase in Vesicle size observed, because coefficient $b_1$ and $b_11$ bears a negative, so result find that increase the surfactant concentration decrease the size of vesicle and decrease the concentration of phospholipid decrease the vesicle size, but in surfactant vesicle size decrease at concentration from 5 to 20% above 20% vesicle size increase because above 20% formation of micelle structure instead of vesicles.
Figure 1. Effect ratio of phospholipid : surfactant and different surfactant on vesicle size

Effect of variables on entrapment efficiency

Determination of EE is an important parameter in case of transferosomes as it may affect the drug release and skin deposition. EE is expressed as the fraction of drug incorporated into transferosomes relative to total amount of drug used. In the present study, the observed EE for all-batches were in the range of 47-69.34%. To understand the effect of surfactantant lipid concentration on EE fitted in Eq.:

\[ Y = 56.44 + 3.96X_1 - 2.55X_2 - 0.05X_1X_2 - 6.926X_1^2 + 70878X_2^2 \]

Some coefficients were found to be \( P > 0.05 \), so there was need to generate reduced model from the full model.

Reduced model from the full model:

\[ Y = 56.44 + 3.96X_1 - 6.926X_1^2 + 70878X_2^2 \]

The results of statistical analysis are shown in Table 8. The reduced model was tested in proportion to determine whether \( X_1, X_1^2 \) and \( X_2^2 \) the coefficient contribute significance information to the prediction of % Entrapment efficiency. The results of regression analysis reveal that on increase the values for \( X_1, X_1^2 \) and \( X_2^2 \) increase surfactant concentration and decrease phospholipid concentration that time increase in % entrapment efficiency observed, because coefficient \( b_1, b_{11} \) and \( b_{22} \) bears a positive. So, but in surfactant concentration entrapment efficiency increase at concentration from 5 to 20% above 20% gets decrease because above 20% formation of micelle structure instead of vesicles. These mixed micelles were reported to have a lower drug carrying capacity and poor skin penetration due to their structure features.
Figure 2. Effect ratio of phospholipid : surfactant and different surfactant on Entrapment Efficiency

Vesicle shape (For optimized batch)

when prepared Transfersome examined by Transmission electron microscope (TEM) appeared as a unilamellar vesicles with a predominant spherical shape shown in figure 3.

Drug content uniformity and pH measurement

There was no significant difference observed in the % drug at various locations, indicating that the method used to disperse the liposomal dispersion in the gel base is satisfactory. The pH of the developed formulations was in accordance with that of human skin pH rendering them more acceptable. The developed formulations had pH near to that of skin. So, we can conclude that prepared transferisomal gel was suitable for topical application. EE%, selected for the further study of gel formulation.
In vitro drug diffusion study:

**Figure 4. In Vitro release of batches (F1 to F5) and comparative study**

In terms of Drug content and percentage cumulative drug release, batch F3 shows the highest drug content (99.01%) and 92.96% drug release in 12 hours, so we consider it as an optimized batch. Batch F3 was selected for stability study and analysis of drug release mechanism.

**Skin permeation and drug deposition studies**

Results obtained from in-vitro drug permeation studies conducted with different formulations of fluconazole are shown in Table. Significant augmentation in the skin permeation of fluconazole has been observed (Fig.) with transferosomal formulations vis-à-vis plain carbopol gel. Higher values of flux obtained with transferosomal suspension 0.22 mg/cm²/h, and transferosomal gel 0.21 mg/cm²/h, than that obtained with control gel 0.17 mg/cm²/h, for the permeation enhancing effect of vesiculation on the drug. Results of this study clearly depict that the amount of drug retained in the skin was considerably higher in case of transferosomal preparations, than with non-transferosomal. This gave an understanding that transferosomes could not only enhance the penetration of drug molecules but also helped localize the drug in the skin. Improved skin permeation of fluconazole coupled with its enhanced retention in the skin with transferosomal formulation can be ascribed to the lipo-solublized state of fluconazole molecules. The latter was achieved in the presence of aqueous and non-aqueous phase of bilayered systems, a state most ideally suited for drug penetration. The Transferosome phospholipids (also one of the natural constituent of skin lipids) helped generating and retaining the required physico-chemical state of the skin for enhanced permeation of the fluconazole.
phospholipid-rich domains of vesicles might have helped to produce the depot effect for drug molecules. The latter has been reflected as higher amount of drug retention within the skin layers in case of Transferosomal formulations. Thus, the transferosomal fluconazole formulation, with desired characteristics for topical administration, could be successfully prepared. The formulated fluconazole transferosomes have shown an appreciably enhanced skin permeation as well as retention of drug molecules in the skin.

<table>
<thead>
<tr>
<th>Table 4. Permeation study of fluconazole formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconzole Formulations</td>
</tr>
<tr>
<td>Transfersomal suspension</td>
</tr>
<tr>
<td>Transfersomal gel</td>
</tr>
<tr>
<td>Control gel</td>
</tr>
</tbody>
</table>

Figure 5. Comparative study of Fluconazole formulation by permeation study

RELEASE KINETIC STUDY

<table>
<thead>
<tr>
<th>Table 5. Release kinetic data of batch F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Regression Values (R2)</td>
</tr>
<tr>
<td>Slope</td>
</tr>
<tr>
<td>Intercept</td>
</tr>
</tbody>
</table>

Transfersomal formulation was fitted for the Higuchi kinetic equation as the formulation coefficient values over Zero order and first order kinetics. This indicates the drug permeation mechanism by diffusion, slow and sustained permeation of the drug from membrane.

STABILITY STUDY

Stability of 2% w/w carbopol transferosomal gel containing 0.5% fluconazole was carried out for 30 months at 4-8 °C and room temperature. Responses obtained for different parameters of
liposomal gel during stability period are as shown in Table. transfersome were found to be reasonably stable in terms of aggregation, fusion and/or vesicle disruption tendencies, over the studied storage period. From results it can be concluded that at room temperature and freeze temperature there was slightly but insignificantly decrease in %drug release and in drug content. Result suggests that keeping the transfersomal product in refrigeration conditions minimizes stability problems of transfersomes.

**Table 6. Stability studies of Transfersomal formulation**

<table>
<thead>
<tr>
<th>Time period for Sampling</th>
<th>Drug content(%) (For F3 batch)</th>
<th>In vitro drug release for (F3 batch)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-8 °C</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Initial</td>
<td>99.0</td>
<td>99.0</td>
</tr>
<tr>
<td>After 1 month</td>
<td>97.56</td>
<td>96.03</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Preparation of Transfersome using Factorial design was found to be well suited to obtain stable transfersomal formulation. Variables such as phospholipid:surfactant ratio and various surfactant have a profound effect on the vesicle size and entrapment efficiency. Increase in surfactant concentration and decrease phospholipid concentration with increase entrapment efficiency and decrease vesicle size but up to 20% concentration of surfactant above this start the formation of micelle structure instead of vesicles so decrease in Entrapment efficiency and increase particle size. Transfersome determined by TEM show the spherical shape and nano size range. Rheological studies of transfersomal gel prepared with 1%, 1.5%, 2%, 2.5% and 3% carbopol gave a clear idea of concentration of carbopol required 2% carbopol. Transfersomal formulation was fitted for the Higuchi kinetic equation as the formulation coefficient values over Zero order and first order kinetics. This indicates the drug permeation mechanism by diffusion, slow and sustained permeation of the drug from membrane. Transfersomal dispersion and gel were found to increase skin penetration than control gel. Transfersome have more stability at freezing temp. Hence from result obtained it can be concluded that transfersomal gel containing fluconazole has application in topical delivery.
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