DESIGN, DEVELOPMENT AND EVALUATION OF TOPICAL MICROEMULSION

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ABSTRACT

The aim of the present research study was to develop and evaluate the topical microemulsion for antifungal activity by using Terbinafine HCl as antifungal agent. The pseudo ternary phase diagram was method adopted to optimize the amount of oil (Cinnamon oil) (X (1)), Smix (mixture of Span 80 & Isopropyl alcohol as surfactant & co-surfactant respectively) (X (2)) and Water (X (3)) in the microemulsion formulation. The microemulsion which containing drug, cinnamon (67.30 % w/w), 28.84 % w/w Span 80 & Isopropyl alcohol and water (3.84 % w/w) was selected as optimized formulation. With viscosity 18,600 m.Pa.s, refractive index 1.601±0.002 and TEM image shown that micro-droplets were almost spherical in shape. The in-vitro skin permeation and retention studies were also performed using Franz diffusion cell with membrane filter. In-vitro skin permeation was lowest and retention was highest (9.3±3.10; 90.60±3.09 respectively) after 6 hours study. The optimized or selected formulation showed better antifungal activity against Candida albicans than the commercial cream. It was concluded that drug loaded microemulsion could be a promising formulation for effective treatment of topical fungal infection.

Keywords: Topical microemulsion, In-vitro permeation and retention, Terbinafine HCl, Antifungal activity.

INTRODUCTION

Human skin is an important target site for the applications of drugs. Especially in the treatment of local illnesses, a topical drug delivery is an appropriate strategy to restrict the therapeutic effect on the affected area and to reduce systemic incrimination [1].

Colloidal carriers have attracted the main interest because they are promising systems having localized effect. The carriers accumulate in SC or other upper skin layers are not expected to penetrate into viable skin. The common characteristic of all colloidal carriers is the submicron-sized particles which are intended to transport entrapped active molecules to the skin. Microemulsions (MEs) as colloidal carriers are one of the promising systems which have nowadays attracted the main interest due to their localized effect. Due to their special features, MEs offer several advantages for the pharmaceutical use, such as ease of preparation, long-term stability, high solubilization capacity for the hydrophilic and lipophilic drugs, and improved drug delivery [2, 3].

High dose of drug can be incorporated into this system as a consequence of the supersolvent properties of MEs and the dispersed phase can also act as a reservoir, making it possible to maintain an almost constant concentration gradient over the skin for a long time [4].

Terbinafine HCl is chemically (E)-N-(6,6-dimethyl-2-hepten-4-ylnyl)-N-methyl-1-naphthalenemethylhydrochloride, a synthetic allylamine which is one of the most commonly prescribed antifungal drug. Terbinafine HCl is emerging as the good treatment option for virtually all forms of susceptible Candida infections in both immunocompetent and immunocompromised hosts. It acts by blocking the synthesis of ergosterol, an essential component of the fungal cell membrane. Clinical efficacy of topical antifungal therapy depends on the drug ability to penetrate into the SC and the duration of treatment. Hence, ME formulations appeared to be a viable approach for future topical delivery of terbinafine HCl. The solubilisation of terbinafine HCl in MEs would improve its topical availability [5-10].

In this study, water-in-oil (W/O) MEs containing 1% terbinafine HCl have been developed to provide maximal topical (superficial) delivery of terbinafine HCl. Also an attempt was made to study the effect of oil, surfactant/co-surfactant mixing ratios and water on the in vitro permeation of terbinafine HCl using membrane filter. The antifungal activity of optimized ME formulation using Candida albicans as a model fungus has been also evaluated.

MATERIALS AND METHODS

Materials

Terbinafine (purity 99%) was procured as gratis sample from Cipla Pvt. Ltd. (Pune-India). Span (20, 80), Tween (20, 80) purchased from Loba Chemicals Pvt. Ltd., Mumbai; Cinnamon oil, Eucaliptus oil purchased from Sigma Life Science, Mumbai; Peppermint oil purchased from Quest International, Pune; Isopropyl alcohol purchased from Nice Chemical Pvt. Ltd., Cochin; Methanol, Ethanol and propanol as gift sample from DCM Shiram Distillery, Daurala; Peceol, Oleic acid, Capryol 90 purchased from Merck Limited, Mumbai; Whatman filter paper, Membrane filter purchased from Research Labs Fine Chemical, Mumbai. C. albicans (ATCC 10231) gratis sample was procured from Food and Drug Laboratory (Vadodara, India). Double distilled water was used throughout the study. All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

Screening of Excipients

The solubility of drug in various oil (cinnamon), surfactants (span 80), and co-surfactants (propanol, isopropanol, propylene glycol) was determined by dissolving an excess amount of drug in 2ml of each of the selected oils, surfactants, co-surfactants in 5 ml capacity stopper vials or test tube with cap or aluminum foil cap separately. An excess amount of drug was added to each vial and mixed using vortex mixture. The mixture vials were then kept at 37°C ± 5°C in incubator shaker for 72 hours. After 72 hours shaking samples were centrifuged at 5000 rpm (Remi centrifuge) for 15 minutes. The supernatant was taken and filtered through a 0.45µm membrane filter [11-13]. The concentration of the drug was determined in each oil, surfactant and co-surfactant by UV spectrophotometer at their respective λmax (219) in Table 1 and graphically represented in Fig. 1.

Table 1: Solubility of Terbinafine HCl in various oils, surfactants and co-surfactants

<table>
<thead>
<tr>
<th>S. No</th>
<th>Solvent/Excipients</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cinnamon oil</td>
<td>33±5</td>
</tr>
<tr>
<td>2</td>
<td>Eucaliptus oil</td>
<td>19±5</td>
</tr>
<tr>
<td>3</td>
<td>Cardamom oil</td>
<td>52±5</td>
</tr>
<tr>
<td>4</td>
<td>Peppermint oil</td>
<td>95±10</td>
</tr>
<tr>
<td>5</td>
<td>Isopropyl alcohol</td>
<td>90±5</td>
</tr>
<tr>
<td>6</td>
<td>Span 80</td>
<td>42±5</td>
</tr>
<tr>
<td>7</td>
<td>Tween 20</td>
<td>45±5</td>
</tr>
<tr>
<td>8</td>
<td>Peceol</td>
<td>80±10</td>
</tr>
<tr>
<td>9</td>
<td>Propanol</td>
<td>70±5</td>
</tr>
<tr>
<td>10</td>
<td>Span 20</td>
<td>30±2</td>
</tr>
<tr>
<td>11</td>
<td>Tween 80</td>
<td>50±5</td>
</tr>
<tr>
<td>12</td>
<td>Oleic acid</td>
<td>100±5</td>
</tr>
<tr>
<td>13</td>
<td>Capryol 90</td>
<td>90±5</td>
</tr>
</tbody>
</table>
Construction of Pseudo-ternary Phase Diagram

On the basis of solubility studies, Cinnamon was selected as the oil phase. Span 80 and Isopropyl alcohol were selected as surfactant and co-surfactant, respectively. Span 80 was selected also on the basis of their HLB value which is 5.43 and suitable for w/o formulation or microemulsion formulation, according to HBL value description for different formulation (like for w/o preparation surfactant of HLB value range 3-6 recommended best and for o/w preparation surfactant of HLB value range 15-18 recommended best). Double distilled water was used in the formulation so as to prevent the incorporation of surface-active impurities. Surfactant and co-surfactant (S_{coS}) were mixed at different mass ratio. Following ratio were tried – 1:1, 1:2, 1:3, 2:1, 3:1, 4:1, 5:1, 1:1.5, 1.5:1. These ratios were chosen in increasing concentration of surfactant with respect to co-surfactant and increasing concentration of co-surfactant with respect to surfactant for detailed study of the phase diagrams. For each Phase diagram, oil and S_{coS} at a specific ratio were mixed thoroughly to give oil:S_{coS} at different mass ratio from 9:1 to 1:9 in different glass vial or tube with stopper. Following 9 different combination of oil:S_{coS} 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 were made so that maximum ratios were covered for the study of delineate the boundaries of phases precisely formed in the phase diagrams.

Pseudo-ternary phase diagrams of oil, S_{coS} and aqueous phase were developed using the aqueous titration method. Each mixture was mixed thoroughly using vortex mixture until a homogenous dispersion/solution was obtained. Slow titration with aqueous phase was performed for each mass ratio of oil:S_{coS} and visual observation were made for transparent and easily flowable w/o microemulsions. The end point of the titration was the point where the solution became cloudy and/or birefringent. The physical state of the microemulsion was marked on a pseudo-three-component phase diagram with one axis representing the aqueous phase, the second one representing oil and third representing a mixture of surfactant and co-surfactant at a fixed mass ratio [14, 15].

Preparation of Terbinafine HCl-Loaded Microemulsions

Various MEs were selected from the pseudoternary phase diagram with 1:1, 1:2, 1:3, 2:1, 3:1, 4:1, 5:1, 1:1.5, 1.5:1 weight ratio. Terbinafine HCl was added to the mixtures of oil and S/CoS and then an appropriate amount of distilled water was added to the mixture drop by drop and the MEs containing terbinafine HCl were obtained by stirring the mixtures at ambient temperature. All MEs were stored at ambient temperature [16].

Table 2: Compositions of selected microemulsion formulations (% w/w)

<table>
<thead>
<tr>
<th>Final Code</th>
<th>Oil (%wt/wt)</th>
<th>S_{coS} (%wt/wt)</th>
<th>Water (%wt/wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME_{F1}</td>
<td>65.42</td>
<td>28.03</td>
<td>6.54</td>
</tr>
<tr>
<td>ME_{F2}</td>
<td>55.04</td>
<td>36.69</td>
<td>8.25</td>
</tr>
<tr>
<td>ME_{F3}</td>
<td>67.30</td>
<td>28.84</td>
<td>3.84</td>
</tr>
<tr>
<td>ME_{F4}</td>
<td>58.82</td>
<td>39.21</td>
<td>1.96</td>
</tr>
<tr>
<td>ME_{F5}</td>
<td>57.69</td>
<td>38.46</td>
<td>3.84</td>
</tr>
<tr>
<td>ME_{F6}</td>
<td>67.30</td>
<td>28.84</td>
<td>3.84</td>
</tr>
<tr>
<td>ME_{F7}</td>
<td>67.96</td>
<td>29.12</td>
<td>2.91</td>
</tr>
</tbody>
</table>

Characterization of Optimized Microemulsion

Macroscopic and Microscopic Evaluation

Macroscopic and microscopic analysis was carried out (using OLYMPUS Model-CX21I AX7403 microscope and LABLINE optical microscope) in order to observe the homogeneity of microemulsion formulation. Any change in colour and transparency or phase separation occurred during normal condition (37°C) was observed in optimized microemulsion formulation.

Measurement of Droplet Size

The average droplet size of the microemulsions was measured using Zetasizer Nano-ZS (Malvern Instrument, UK). The measurement was performed at fixed refractive index of the respective formulation at 25°C [17, 18].

Determination of pH

The pH values of Microemulsions were determined using digital pH meter (Digital pH meter-III, EI) standardized using pH 4 and 7 buffers before use [19].

Determination of Electrical Conductivity

The electrical conductivity of microemulsions was measured with a conductivity meter (using SIMTRONICS conductivity meter) equipped with inbuilt magnetic stirrer. This was done by using conductivity cell consisting of two platinum plates separated by desired distance and having liquid between the platinum plates acting as a conductor [20].

Viscosity Determination

The viscosity of the formulations was determined as such without dilution using Brookfield viscometer (Brookfield Engineering Laboratories, USA) at room temperature [21-23].

Fig. 1: Graph representing solubility of Terbinafine HCl in various oils, surfactants and co-surfactants

Table 1: Physical characterization of optimized microemulsion formulation

<table>
<thead>
<tr>
<th>Character</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>67.96</td>
</tr>
<tr>
<td>S_{coS}</td>
<td>29.12</td>
</tr>
<tr>
<td>Water</td>
<td>2.91</td>
</tr>
</tbody>
</table>
Transmission Electron Microscopy (TEM) Analysis

Transmission Electron Microscopy (TEM) is the most important technique for the study of microstructures of microemulsions because it directly produces images at high resolution and it can capture any co-existent structure and micro-structural transitions. Morphology and structure of the microemulsion were studied using TEM. In order to perform the TEM observations, a drop of the microemulsion was directly deposited on the holey film grid and observed after drying [25].

In-vitro permeation studies of microemulsion formulation

The evaluation of in vitro drug release was performed on Franz diffusion cells using membranes of mixed cellulose esters type Millipore HA 0.45 μm (membrane surface area of 3.14 cm²).

To simulate usage conditions, experiments were performed by applying 10 mg (‘infinite dose’; n = 3) as the dose. The preparations were evenly distributed on the membranes. Serial sampling was performed after 0.5, 1, 2, 4, and 6 h and fresh receptor liquid was added to receptor compartment to replace the buffer; 5 ml of receptor fluid (PBS) was taken for UV determination of tebinafine HCl concentration at 219 nm [26, 27].

In Vitro anti-fungal activity Studies

In vitro anti-fungal activity studies of optimized ME formulations were carried out using fungus Candida albicans. The antifungal activity of terbinafine HCl from the optimum formulation as well as the reference standard (marketed cream) was determined using Sabouraud dextrose agar as culture medium adopting cup plate method. The mean inhibition zone was calculated for each plate, and this value was taken as an indicator of the antifungal activity.

A single well isolated colony of Candida albicans of at least 1mm diameter was picked from the culture plate and was streaked aseptically to agar slant. The slant was incubated for 24 hrs at 37°C. After incubation, the inhibition zone diameter around each well was measured using a ruler [28, 29].

Stability of Microemulsions

The stability of microemulsion containing terbinafine HCl was investigated via clarity, phase separation observation at 4°C up to 45 days. The centrifuge test was also performed to access the physical stability with centrifuging rate at 5000 rpm for 30 minutes discussed in Chen et al., 2007 [30].

RESULTS

Phase Behavior

Pseudo-ternary phase diagrams were constructed to obtain appropriate components and their concentration ranges for the MEs. The pseudo-ternary phase diagrams with various weight ratios are presented in Fig. 2. The transparent ME region is presented in phase diagrams. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) ME was observed. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. The area of ME isotropic region changed slightly in size with the increasing ratio of S/CoS.

Macroscopic and Microscopic Evaluation

Physical appearance (color, transparency and phase separation) of optimized formulation was studied. Formulation appeared uniform in color and transparency as well as there was no phase separation observed during normal storage (37±2°C) condition under observation for specific period of time.
TEM Study
The positive image of microemulsion (MEF3) was observed TEM as shown in fig. 3. The results of TEM pictures reveal that terbinafine microdroplets were almost spherical in shape.

In-vitro Permeation Release
The relationship of Q (cumulative amount released per surface area of membrane; µg/cm²) versus square root of time, shown in Fig. 3, is derived from the Higuchi model with the assumption that there is a reservoir of the drug always available to diffuse through [31-33], as follows:

\[ Q = \left( C_{n}V + \sum_{i=1}^{n-1} C_{i}S \right) / A \]

\( Q = \) cumulative release of Terbinafine HCl per surface area of membrane
\( V = \) volume of individual Franz cell
\( \sum_{i=1}^{n-1} C_{i} \) = sum of concentration of Terbinafine HCl determined at sampling intervals 1 through \( n - 1 \)
\( S = \) volume of sampling aliquot
\( A = \) surface of sample cell
Total amount of drug (%) delivered from each one of the prepared formulations evaluated after 6 h.

Table 7: Permeation and Retention Data of Microemulsion Formulations:

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Permeation %</th>
<th>Retention %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEF1</td>
<td>7.79±2.64</td>
<td>92.21±2.62</td>
</tr>
<tr>
<td>MEF2</td>
<td>2.92±4.10</td>
<td>97.01±4.10</td>
</tr>
<tr>
<td>MEF3</td>
<td>9.38±3.10</td>
<td>90.62±3.09</td>
</tr>
<tr>
<td>MEF4</td>
<td>13.49±2.30</td>
<td>86.51±2.32</td>
</tr>
<tr>
<td>MEF5</td>
<td>10.21±2.34</td>
<td>89.80±2.34</td>
</tr>
<tr>
<td>MEF6</td>
<td>11.01±3.72</td>
<td>88.88±3.74</td>
</tr>
<tr>
<td>MEF7</td>
<td>15.23±4.63</td>
<td>84.82±4.66</td>
</tr>
</tbody>
</table>

*Mean ± SD, \( n = 3 \)
In-vitro Antifungal Activity

Table 8: In-vitro antifungal activity of 1% TERBINAFINE HCl

<table>
<thead>
<tr>
<th>Formula</th>
<th>Zone of Inhibition (mm)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME P</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ME DL</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33.33</td>
</tr>
</tbody>
</table>

DISCUSSION

Topical formulations for the treatment of skin infections must provide proper concentrations of the drug in the target site for therapeutic activity. In the case of superficial fungal skin infections, in which the main location of the pathogen is the epidermis, the drug must penetrate into the SC in proper concentrations to inhibit the fungus growth [34].

In the present work, different microemulsion formulations were assayed as terbinafine HCl topical delivery system. The main objective was to find a formulation with the capacity to deliver the whole active compound and maintain it within the skin so as to be considered a real benefit either for topical fungal treatment. For the purpose of this work, all the systems remained stable; no significant changes were observed for any of the different prepared microemulsion formulations.

The amount of terbinafine HCl released from the microemulsion formulation studied showed a linear relationship with the square root of time, accordingly to Higuchi model.

The total drug retention in stratum corneum layer of skin from ME 3 was higher than from other formulations so it was considered for evaluation of antifungal activity.

In present study, we used phase titration method to prepare topical microemulsion dosage form for fungal infections. In which we used terbinafine HCl as drug (or antifungal agent), cinnamon as oil (continuous phase), span 80 as surfactant, isopropyl alcohol as co-surfactant and doubled distilled water as dispersed phase.

Droplet size study also proved that the prepared microemulsion droplet size was in desired range of microemulsion formulation (i.e. 10-140 nm).

Other studies like pH determinations, viscosity determinations, and conductivity measurements, TEM, refractive index also done and they also proved formulation was stable and desired for present work and objectives.

In-vitro permeation studies were performed for various microemulsion formulations and on the basis maximum retention or minimum permeation through stratum corneum layer of skin, microemulsion formulation coding ME 3 was found best for topical delivery system and this formulation was furthered selected for antifungal activity study.

It is necessary to point out that these experiments were performed with formulations containing 1% of Terbinafine HCl. It has to be considered as "infinite dose", but it is near the real dose used in clinical practices. The broad differences in the rheological properties shown by the selected compositions obliged us to use this amount so as to allow for an easy application in all cases.

Optimized formulation selected:

The microemulsion formulation ME 3 (containing Terbinafine HCl (1% wt/wt), Cinnamon oil (67.3%) wt/wt), S 80 (28.84% wt/wt) (Span 80: Isopropyl alcohol) and doubled distilled water (3.84% wt/wt) was selected as optimized microemulsion formulation with 90.60 % drug retention (or 9.38 % drug permeation) at 6 hours with droplet size (134 nm) in microemulsion range and also show good antifungal activity on comparison with marketed formulation.

CONCLUSION

In this work, in vitro Terbinafine HCl release was assayed for different topical microemulsion formulation and microemulsion was found perfect for topical delivery dosage form to fungal infections. Using membrane filter, a model that can be related to human skin, our data suggest that high skin (stratum corneum) concentrations could be obtained after topical administration of terbinafine HCl from microemulsions applied at a clinically relevant dose. There are few antecedents of formulations which could provide proper concentrations of the drug in the skin.

ACKNOWLEDGEMENT

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