Development and evaluation of microemulsion based gel (MBGs) containing econazole nitrate for nail fungal infection

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ABSTRACT

System containing microemulsion-based gels (MBGs) composed of propylene glycol, Oleic acid, Tween 80 and H2O, loaded with drug (econazole nitrate), have been prepared and characterized by rheological measurements, particle size, zeta potential, spreadability, gel strength, mucoadhesive force, FTIR and scanning electron microscope (SEM). The viscosity of the systems was found to be in the range (24995 to 58488 cps) for the plain gels, whereas for the microemulsion-based gels it was up to (20696 to 34133 cps). The maximum gel strength and mucoadhesion was found to be up to (129 seconds) and (18215 dynes/cm²) respectively.

Keywords: fungal nail infection, econazole nitrate, microemulsion-based gels

INTRODUCTION

Onychomycosis is a fungal infection of the nail plate affecting 2 to 18% of the American population accounting for up to 50% of all nail disease [1-3]. The conventional formulations for the local delivery of drugs to a nail fungal infection are the tablet, creams and varnish. As conventional drug delivery systems do not remain on the nail surface for prolonged periods, they are unable to deliver the antifungal agents to the site of infection in effective concentrations and in fully active forms. Before beginning treatment with oral antifungals, a blood test is necessary to monitor the function of your liver. In rare instances, these medications can cause liver disease. Other side effects include headache, taste disturbance, stomach upset, dizziness, and skin rash [4]. As compared to conventional formulations microemulsions are wider better as they have enhanced drug solubility, good thermodynamic stability, ease of manufacturing and enhancement effect on transdermal delivery [5-6]. This system is suitable for delivery of both water insoluble drugs and water soluble drugs. Water insoluble drugs may be delivered through oil-in-water (o/w) microemulsions [7-9], while water soluble drug may be delivered through water-in-oil (w/o) microemulsions. Recently researchers have focused on microemulsions for transdermal delivery of various drugs of anti-inflammatory [10-16], anaesthetics [17-18], antifungals [19-20], steroids [21-22], etc. Based on this special network structure, the MBGs have received particular attention especially as drug delivery systems. One important consequence is that the stability of the MBGs is much better compared to that of conventional hydro-gels. One reason for this is that the MBGs are prepared from W/O microemulsions which are thermodynamically stable systems, and the organic solvent as external phase which could offer superior resistance to microbial contamination compared to aqueous phase [23]. Moreover, due to the increasing of viscosity of the system by incorporating gelatin into W/O microemulsions, the MBGs are suitable to be used as a kind of sustained release drug delivery systems [24]. Other properties that make the MBGs attractive as drug delivery vehicles include their electrical conductivity to be applied in iontophoretic drug delivery systems [25-26]. Objective of the present study is to prolong the delivery of the active drug on nail fungal infection using a suitable carrier such as microemulsion based gels which can effectively deliver the drug for an extended duration of time hence not only reduce the systemic side effects but also improve the therapeutic efficacy, patient compliance.

MATERIALS AND METHODS

Construction of pseudo-ternary phase diagrams

In order to find out the concentration range of components for the existing range of microemulsions, pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature (25°C). Oleic acid was selected as the oil phase. Tween 80 and Polylethylene glycol 200 were selected as surfactant and co-surfactant, respectively. Distilled water was used as an aqueous phase. Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratios of tween 80 to Polylethylene glycol 200, respectively. For each phase diagram at a specific surfactant/co-surfactant weight ratio, the ratios of oil to the mixture of surfactant and co-surfactant were varied as 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5:5.5, 5:5, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1, 9.5:0.5. The mixtures of oil, surfactant and co-surfactant at certain weight ratios were diluted with water drop wise, under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions, crude emulsions or gels. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous type microemulsions (Table 1 and Fig.1).

Preparation of microemulsions based gels

After the microemulsions regions in the phase diagrams were identified, the microemulsion formulations were selected at different component ratios as described in Table1. In order to prepare the drug loaded microemulsions, a stock solution containing econazole nitrate was prepared with the mixture of oleic acid and Polylethylene glycol. The clear oily phase containing econazole nitrate was obtained by diluting the weighed amount of stock solution with Oleic acid and Polylethylene glycol. Tween 80 was taken and solubilized in the distilled water. Then water was added to the clear oily phase drop by drop. The W/O microemulsions (econazole nitrate) were obtained at ambient temperature using magnetic stirrer. Appropriate amount of xanthan gum was dispersed slowly in 10 ml of the econazole nitrate microemulsion with the help of overhead stirrer. The suitable gelling agent was selected on the basis of compatibility with microemulsion structure, feel and ease of spreadability.

Measurement of droplet size and zeta potential

The average droplet size and zeta potential of the microemulsions were measured using a Zetasizer Nano ZS (Malvern Instruments, UK). The measurement was done at 25°C (Table 2).

Determination of pH

The pH of the F4 and F5 gel containing econazole nitrate were determined using calibrated pH meter standardized using pH 7.0 standard buffers before use.
Preparation of Sabauroud’s media

Antifungal Activity

Detachment stress (dyne/cm²) measurement. Detachment stress (dyne/cm²) until the gel and the mucosal tissue were detached. Mucoadhesive force, the rate of 5 mL/min. The weight of the water in the glass vial (B) kept increasing opened to make the water drop into the glass vial (B) with a constant flow contact was given. Then, the switch (C) of the infusion apparatus was two minutes time of test membrane. Then the height of second vial was so adjusted that the MBG was added onto the mucosa of first vial. Before applying the gel, instantly fixed with mucosal side out onto each glass vial (E) using rubber setup is presented in Figure. 4. The mucoadhesive force of the formulations has been derived from a previously published method [30,31]. The experimental technique used for determining the bioadhesive force has spreadability of the gel was determined using the following technique [27-29]: 0.5 g gel was placed within a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 1000 g was allowed to rest on the upper glass plate for 5 minutes (figure 2). The increase in the diameter due to spreading of the gels was noted. The calculation of spreadability is as follows; S = ML/T Where M = Weight tide to the Upper Slide (g) L = Length moved on the glass slide (cm) T = Time taken

Measurement of Gel Strength

A sample of 50 g of gel was placed in a 100 ml graduated cylinder [30]. The apparatus for measuring gel strength (weigh or apparatus as shown in figure 3, weighing 10 g) was allowed to penetrate in econazole nitrate MBG. The gels strength, which means the viscosity of the gels, was determined by the time (seconds); the apparatus took to sink 5 cm down through the prepared gel (Fig.3).

Determination of mucoadhesive Force

The experimental technique used for determining the bioadhesive force has been derived from a previously published method [30,31]. The experimental setup is presented in Figure. 4. The mucoadhesive force of the formulations was determined as follows; a section of mucosa was cut from the chicken and instantly fixed with mucosal side out onto each glass vial (E) using rubber band. The vial with chicken mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan (A). Econazole MBG was added onto the mucosa of first vial. Before applying the gel, 150µL of phosphate buffer pH 7.4 was evenly spread on the surface of the test membrane. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then, the switch (C) of the infusion apparatus was opened to make the water drop into the glass vial (B) with a constant flow rate of 5 mL/min. The weight of the water in the glass vial (B) kept increasing until the gel and the mucosal tissue were detached. Mucoadhesive force, the detachment stress (dyne/cm²), was determined from the minimal weights that detached the gel. The chicken mucosa pieces were changed for each measurement. Detachment stress (dyne/cm²) = mg/A

Where, m = weight of the water added to the balance (g) g = acceleration due to gravity taken as 980 cm/s² A = area of tissue exposed

Antifungal Activity

Preparation of Sabauroud’s media

Table 1: Composition of the microemulsion formulations

<table>
<thead>
<tr>
<th>Composition</th>
<th>S:CoS-1 ratio</th>
<th>S:CoS-2 ratio</th>
<th>S:CoS-3 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Econazole nitrate (mg)</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Oleic acid (ml)</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Tween 80 (ml)</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Polyethylene glycol (ml)</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Water</td>
<td>Upto100 ml</td>
<td>Upto 100 ml</td>
<td>Upto 100 ml</td>
</tr>
</tbody>
</table>

Rheological studies

Brookefield programmable DVII+ Model pro II type viscometer was used for rheological studies. The prepared microemulsion formulations (100 ml) were placed in a beaker and were allowed to equilibrate for 5 minutes before measuring the dial reading using spindle No. 63 for F4 and F5 formulations. The viscosity of plain gel and MBGs were determined at different angular velocities and averages of two reading were used to calculate the viscosity.

Spreadability

The spreadability of the gel was determined using the following technique. A = area of tissue exposed

Where, A = area of tissue exposed

Measurement of gel strength, which means the viscosity of the gels, was determined by the following technique:

\[
T = \frac{M}{L^2}
\]

Where

- \( T \) = Time taken
- \( M \) = Weight tide to the Upper Slide (g)
- \( L \) = Length moved on the glass slide (cm)

Determination of mucoadhesive Force

The viscosity of plain gel and MBGs were determined at different angular velocities and averages of two reading were used to calculate the viscosity.

\[
\text{Spreadability} = \frac{S}{ML/T}
\]

Where, S = ML/T

The above solution was mixed and well boiled for one minute. Chloramphenicol was added to prevent the growth of any bacteria [32]. It was autoclaved for 15 minutes, at pressure of 15 lb, at 118° to 120°C temperature. The plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl 10^4 CFU) and spread evenly on the plate. After 20 min, the wells were filled with drops of compound at different concentrations. The control plates with standard antibiotics were also prepared. All the plates were incubated at 27°C ± 1°C for 12 hrs and the diameter of inhibition zone were noted Antifungal activity of econazole nitrate -MBGs was evaluated against Aspergillus niger (ATCC 16404) by using a zone of inhibition by Petri plate method. The mean zone of inhibition was recorded for the formulations.

Diffusion studies

The diffusion medium used was phosphate buffer pH 7.4. Assembly of diffusion cell for in – vitro diffusion studies the diffusion cell was designed as per the dimension given. Diffusion cell with an effective diffusion area of 3.14 cm² was used for in vitro permeation studies. The diffusion cells were placed on the magnetic stirrers. The donor compartment consisting of 1 g of microemulsion based gel containing econazole nitrate. The receptor compartment was filled with fluid. Then the chicken membrane [33] was mounted on the cell carefully so as to avoid the entrapment of air bubble under the chicken membrane. Intimate contact of chicken membrane was ensured with receptor fluid by placing it tightly with clamp. The speed of the stirring was kept constant throughout the experiment. With the help of 1ml pipette 1ml of sample was withdrawn at a time intervals of 30 minutes from sampling port of receptor compartment and same volume was replaced with receptor fluid solution in order to maintain sink condition. The samples were appropriately diluted and the absorbance was measured at 232 nm using UV- VIS spectrophotometer.

Drug content

For determination of drug content about one ml of each microemulsion formulation was weighed in a 10 ml volumetric flask and dissolved in methanol. It was diluted appropriately and analyzed spectrophotometrically at 232 nm.

FTIR spectra

The FT-IR spectra were recorded in KBr on Beckman Coulter DU 800 spectrophotometer.

SEM Analysis

The surface characteristics of microemulsion were performed on the formulation selected on the basis of particle size the formulation using scanning electron microscopy (Phenom World EMS 550X).

RESULTS AND DISCUSSION

Measurement of droplet size and zeta potential
The droplet size and zeta potential for the formulations are represented in Figure 5. The result shows that the droplet diameter decreases with increasing ratio of oil: surfactant/co-surfactant. These results are in accordance with the report that the addition of surfactant to microemulsion system causes the interfacial film to condense and to be stable, while the co surfactant causes the film to expand. Average droplet size of optimized formulations ranged from 30 to 130 nm.

**Table 2: Composition of the microemulsion based gel (MBGs) of Econazole nitrate**

<table>
<thead>
<tr>
<th></th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthan Gum (g)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.75</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Distilled water (ml)</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
</tr>
</tbody>
</table>

**Table 3: Characteristics of optimized MBGs**

<table>
<thead>
<tr>
<th>Optimized MBG</th>
<th>pH</th>
<th>Spreadability g.cm/sec</th>
<th>Gel strength sec</th>
<th>Mucoadhesive force dynes/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4</td>
<td>6.9</td>
<td>98.68</td>
<td>42.02</td>
<td>15136.66</td>
</tr>
<tr>
<td>F5</td>
<td>7.0</td>
<td>117.96</td>
<td>129.77</td>
<td>18215.15</td>
</tr>
</tbody>
</table>

**Table 4. Zone of inhibition (Marketed cream and MBGs)**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of Inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marketed cream</td>
<td>1.8</td>
</tr>
<tr>
<td>Econazole nitrate MBG</td>
<td>2.2</td>
</tr>
</tbody>
</table>

**Determination of pH**

Physicochemical properties such as pH and clarity were performed and the results are recorded in Table 3.

**Rheological studies**

The viscosity of polymer plain gels and MBGs of various formulations was determined at various shear rates. As the shear rate is increased, the viscosity of plain gels and MBGs decreased (Figure 6 and Figure 7). In general increase in ratio of polymer concentration to drug caused an increase in viscosity.

**Spreadability of MBG**

The values of spreadability indicate that the gel is easily spreadable by small amount of shear. The spreadability (98.68 to 117.96 g.cm/seconds) of formulations F4 to F5 was found to be more as compared to other optimized formulations. This indicates spreadability of in situ system containing gel having higher ratio of polymer was good as when compared with lower ratio.

**Gel Strength of MBGs**

Gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out of the targeted site. The formulations F4 to F5 (42.02 to 129.77 sec) exhibited good gel strength among all optimized F code formulation which may be due to increase in concentration of viscosity enhancer (XG).

**mucoadhesive Force of MBGs**

Mucoadhesive drug delivery system to mucosal membrane leads to an increase in the drug concentration at the absorption site and therefore im-

Figure 6. Showing the viscosity of plain gels of optimized formulation

Figure 7. Showing the viscosity of econazole nitrate MBGs of optimized formulation

Figure 8: Showing the drug release of F4 and F5 formulations (MBG)

Figure 9. SEM micrograph of the xanthan gum plain gel

Figure 10. SEM micrograph of the econazole nitrate MBGs

Figure 11. FTIR Spectra

proved bioavailability of systemic delivery drugs. In addition, adhesive system has been used to target local disorders at the mucosal surface to reduce the overall dosage required and minimize side effects that may be caused by systemic administration of drug. The mucoadhesive force is an important physicochemical parameter for topical application. The formulation F4 to F5 (122.40 to 127.55 dynes/cm²) showed maximum mucoadhesive force than all optimized formulations.

Diffusion studies of MBGs
The formulations F4 and F5 released 72.1% and 73.2 % of drug respectively at 7th hour. The optimized formulations F4 and F5 containing higher concentration of xanthan gum (1.0 -1.5g). The in vitro drug release of the formulations F4 to F5 was 72.1% to 73.2 %, which leads to extended drug release the MBGs (Fig 8).
Figure 12. Zone of inhibition

SEM Analysis of MBGs

SEM is a powerful method for visualizing the structure of microemulsions. Recently, we were able to show that this special preparation technique can be successfully used for the detection of plain gel and w/o microemulsions based gel. (Figure.9,10)

FTIR spectra:
The characteristic bands of pure econazole nitrate (3434.17, 2918.76, 1401.39, 1074.12, 822.37, 665.73 and 562.23 cm⁻¹). The FTIR spectra of econazole nitrate and xanthan gum showed band at (3432.03, 2918.76, 1401.39, 1068.53, 825.17, 665.73 and 570.62 cm⁻¹). These findings indicate there was no interaction occurs between econazole nitrate and xanthan gum combination. Therefore, econazole nitrate and xanthan gum can be used as excipients in the formulation of MBGs (Figure .11).

Antifungal activity of MBGs

It was observed from the results that the zone of inhibition for marketed cream was 1.8 cm, whereas it was 2.2 cm for the econazole nitrate MBGs. It is clearly understood that the formulations showed better antifungal activity against Aspergillus niger in comparison to brand used in the study (Figure 12 and table 4).

CONCLUSION:
The purpose of this study was to construct formulation and in vitro evaluation of microemulsion based nail antifungal gel of Econazole nitrate. The Econazole nitrate-MBGs could be successfully formulated for the treatment of nail fungal infection. A microemulsion-based system was chosen due to its good solubilizing capacity and permeation capabilities. Oleic acid and Propylene Glycol were screened as the oil phase. The prepared formulations of good solubilizing capacity and permeation capabilities. Oleic acid and Pro-

REFERENCES

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