ABSTRACT

Objective: Terbinafine is a broad spectrum antifungal drug. The aim of present study was to develop topical nano emulgel of terbinafine using carbopol 934 as a gelling agent. The objective behind the formulation was to avoid dosing frequency and to increase the stability and bioavailability by avoiding the first pass metabolism.

Methods: The formulations were prepared by using oleic acid, carbopol 934, span 20, propylene glycol in different ratios and analyzed by pseudo tertiary phase diagram. All the five prepared nano emulsion formulations have shown satisfactory physiochemical properties. The stability and particle size is been determined by zeta potential.

Results: The highest drug release 82.38 %was found in formulations of batch F4, which follows non-fickian mechanism. The studies showed that changing the concentration of oil, surfactant, co surfactant and double distilled water as aqueous phase has an impact on the behavior and thermodynamic stability of the nanoemulsion.

Conclusion: Study concludes that of Terbinafine can be delivered effectively by nano emulgel formulations.

Keywords: Antifungal drug, nano emulsion, nano emulgel, Terbinafine, topical drug delivery, tertiary phase.

INTRODUCTION

Nanoemulgel has emerged as one of the most interesting topical delivery system as it has dual release control system i.e. hydrogel and nanoemulsion. Nanoemulgel having nanosize (10 to 100μm) rapidly penetrates and deliver active substance deeper and quicker. Gelling agent promotes better stability of nanoemulsion by reducing the surface and interfacial tension and also enhancing viscosity of the aqueous phase for drug administration topically[1,2]. Drug delivered through nanoemulgel has better adhesion on the surface on the surface of the skin and high solubilizing capacity which leads to larger concentration gradient towards the skin, hence influences better skin penetration. Nanoemulsions are thermodynamically stable, transparent, or translucent dispersion of two immiscible liquids, such as oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having the droplet size of less than 100 nm. It also retard dosing frequency of drug[3,4].

Terbinafine [(2E)-6, 6-dimethylhept-2-en-4-yn-1yl][(methyl)(naphthalene-1-ylmethyl)amine is a broad spectrum antifungal drug active against dermatophytes. Dermatophytes cause infections of the skin, hair and nails, obtaining nutrients from keratinized material. Some of these skin infections are known as ringworm or tinea. Terbinafine has first pass effect due to this shows poor oral bioavailability. It inhibits ergosterol synthesis by inhibiting squalene epoxidase, an enzyme that is a part of fungal cell membrane synthesis pathway. Because terbinafine prevent conversion of squalene to lanosterol, ergosterol cannot be synthesized, and caused fungal cell lysis[5].

The objective of present study was to develop a most effective topical preparation to avoid first pass metabolism of drug, with enhanced pharmacological action on local area, enhanced penetration of drug with the help of penetration enhancer, improved and better drug release profile of the drug by preparing a suitable nanoemulgel for the treatment of fungal infection[6].

MATERIALS AND METHODS

Terbinafine was obtained from Yarrow chem. product Uttarakhand, India, Oleic acid, Span 20, propylene glycol, Carbopol 934 were obtained from Molychem.
pvt. Ltd. All other ingredients, chemicals and solvents used were of analytical grade.

**Fourier Transform Infrared (FTIR) spectral analysis**

IR analysis was done on IR spectrometer with KBr disc. In IR the spectrum was recorded in the wavelength region of 4000 to 400cm⁻¹. 10mg of drug was mixed with KBr and triturated then it was placed in holder and pressed to form a pellet. It was placed under IR beam and a spectrum was obtained on computer. The IR spectrum of drug exhibit maxima only at the same wavelength as that of similar preparation of the corresponding reference standard, thus IR spectrum of substance being examined should be concordant with the reference spectrum of the drug.

**Solubility Study**

Solubility of Terbinafine was determined in various oils such as oleic acid, isopropyl myristate, clove oil, castor oil and olive oil by shake flask method. An excess amount of drug was taken in 10 ml of the oil in vials, and mixed using vortex mixer. The vials were then kept at 25 ± 1°C in an isothermal shaker. The samples were then centrifuged at 3,500rpm for 15min. The supernatant was filtered through whatman (no. 41) filter paper. The filtrate was suitably diluted. The amount of drug dissolved in the oil was determined using UV spectrophotometer at their respective wavelength.

**Partition coefficient**

It is a ratio of unionized drug distribution between organic and aqueous phase at equilibrium. It was determined in n-octanol: water system, by taking 25ml of both n-octanol and water in separating funnel. Shake this mixture for 30 minutes and keep it for 24 hour. Then 10 mg drug mixed with saturated solution of n-octanol:water in separating funnel. The separating funnel was shaken for 24 hours. The two phases were separated and the amount of the drug in aqueous phase was analyzed by UV at 282.7 nm after appropriate dilution.

**Preparation of standard stock solution**

-100mg of drug dissolve in 10ml of methanol in 100ml volumetric flask and volume was adjusted with methanol up to the mark to obtained 1000µg/ml (solution A). The solution was filtered through whatman filter paper No. 41

**Determination of λ_max**

A10ml solution was pipette out from solution A in 100ml volumetric flask and diluted with methanol up to the mark to obtained 100µg/ml. The solution was filtered through Whatman filter paper No. 41(solution B). From these aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml were pipette out in to a 10ml volumetric flask and diluted to methanol up to the mark and get the concentration 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10
μg/ml respectively. Absorbance of this solution was measured at 282 nm using UV spectroscopy against blank (methanol).

**Table 1: Screening and selection of oil, surfactant and co-surfactant**

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oils</strong></td>
<td></td>
</tr>
<tr>
<td>Olive oil</td>
<td>32.92</td>
</tr>
<tr>
<td>Castor oil</td>
<td>19.12</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>49.22</td>
</tr>
<tr>
<td>Isopropyl myristate</td>
<td>43.16</td>
</tr>
<tr>
<td>Clove oil</td>
<td>39.36</td>
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<tr>
<td><strong>Surfactants</strong></td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>98.42</td>
</tr>
<tr>
<td>Span 20</td>
<td>106.31</td>
</tr>
<tr>
<td>Polyethylene glycol 4000</td>
<td>72.18</td>
</tr>
<tr>
<td><strong>Co- Surfactants</strong></td>
<td></td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>86.04</td>
</tr>
<tr>
<td>Glycerine</td>
<td>63.82</td>
</tr>
</tbody>
</table>

**Preparation of phosphate buffer pH 7.4 (PBS)**

Dissolve 2.3gm of disodium hydrogen phosphate, 0.19gm of potassium dihydrogen phosphate and 8gm of sodium chloride in sufficient water to produce 1000ml.

**Figure 5: Calibration curve of terbinafine in 7.4 pH Phosphate buffer**

**Calibration curve of terbinafine in Phosphate buffer pH 7.4 solution**

A10mg of drug dissolve in 20ml of methanol and 8ml of phosphate buffer in a 100ml volumetric flask and volume was adjusted up to the mark to obtained 1000μg/ml. The solution was filtered through Whatman filter paper No. 41(1000 ml). From this solution an aliquot of 1ml was withdrawn and diluted to 10ml with PBS pH7.4 to get concentration of 100 μg/ml (solution B), filtered out all solution by whatman filter no. 41. From these aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml were pipette out in to a 10ml volumetric flask and diluted to PBS pH 7.4 up to the mark and get the concentration 2 μg/ml, 4 μg/ml, 6 μg/ml, 8 μg/ml, 10 μg/ml respectively. Absorbance of this solution was measured at 282.7nm using UV spectroscopy against blank 2:8. (Methanol: PBS pH7.4)

**Construction of pseudo ternary phase diagram**

The phase diagram was developed using water titration method to determine the appropriate components and their concentration ranges. Oleic acid was used as the oil phase, span 20 and propylene glycol was selected as surfactant and cosurfactant, respectively. Distilled water was used as an aqueous phase. Surfactants and cosurfactant \( S_{max} \) were mixed in different weight ratios (1:1, 1:2 and 2:1) to determine the optimum ratio which can result in maximum nanoemulsion area. For each phase diagram, oil and specific \( S_{max} \) were mixed well in different ratios from 1:9 to 9:1 in different vials. The ratio of oil to surfactant varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The mixtures were titrated with the aqueous phase, and visual observations were made for transparent and easily flow able oil-in-water (o/w) nanoemulsion. The physical state of the true nanoemulsion was marked on a pseudoternary phase diagrams with one axis representing the aqueous phase, and the other representing a mixture of surfactant and cosurfactant at fixed weight ratios (\( S_{max} \) ratios).

**Formulation of Terbinafine loaded nanoemulsion**

The experimental design based on a three component system: Oil phase (oleic acid), \( S_{max} \) (span20: propylene glycol) and aqueous phase (water). The total conc. of the three phases summed is 100%. Based on the results of pseudo ternary diagram appropriate range of the component was selected. The o/w NE was prepared by water titration method. The formulations were further sonicated (Sonica ultrasonic, 2000 MH,) for 5 minutes and stored at room temperature until their use in subsequent studies.

**CHARACTERIZATION OF NANOEMULSION**

**Physical characterization**

The prepared nanoemulsion formulations were inspected visually for their color, appearance, consistency, phase separation and homogeneity.

**Table 2: Particle size analysis of drug loaded nanoemulsion formulations**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Polydispersity Index</th>
<th>Particles size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.728</td>
<td>521</td>
</tr>
<tr>
<td>F2</td>
<td>0.709</td>
<td>95.96</td>
</tr>
<tr>
<td>F3</td>
<td>0.652</td>
<td>536</td>
</tr>
<tr>
<td>F4</td>
<td>0.400</td>
<td>144</td>
</tr>
<tr>
<td>F5</td>
<td>0.462</td>
<td>215.8</td>
</tr>
</tbody>
</table>

**Droplet size and size distribution**

The globules size distribution, polydispersity index and droplet size of the resultant nanoemulsion was determined by dynamic light scattering with zeta sizer, 1ml of the optimized nanoemulsion formulation was diluted with water to 10ml in a test tube, and gently mixed by glass rod and then analyzes the fluctuations in light scattering due to Brownian motion of the particles. Light scattering was monitored at 25°C at a 90° angle. Globule diameter and distribution was obtained.

**Table 3: Viscosity of Nanoemulsion Formulation**

<table>
<thead>
<tr>
<th>Spindle speed (rpm)</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>0.3</td>
<td>960</td>
<td>982</td>
<td>861</td>
<td>946</td>
<td>883</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>829</td>
<td>830</td>
<td>720</td>
<td>871</td>
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<td></td>
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<tr>
<td>1.5</td>
<td>740</td>
<td>724</td>
<td>648</td>
<td>730</td>
<td>647</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>629</td>
<td>604</td>
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<td>6</td>
<td>552</td>
<td>526</td>
<td>424</td>
<td>552</td>
<td>458</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>385</td>
<td>437</td>
<td>335</td>
<td>382</td>
<td>317</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>240</td>
<td>352</td>
<td>227</td>
<td>218</td>
<td>241</td>
<td></td>
</tr>
</tbody>
</table>
Zeta-potential analysis
Zeta potential is a technique which is used to measure the surface charge properties and further the long term physical stability of nanoemulsion. The potential is measure of the electric potential at the slip plane between the bound layer of diluents molecules surrounding the particle and the bulk solution. A higher level of zeta potential results in greater electro-static repulsion between the particles, minimizing aggregation/flocculation\(^\text{10}\).

Measurement of pH
1ml of nanoemulsion was dissolved in 10 ml of distilled water. At first pH meter reading was calibrated using known pH solution (pH4 and pH7) and the electrode was then dipped in to NE formulation and constant reading was noted\(^\text{11}\).

Measurement of viscosity
The viscosity of true nanoemulsion was determined without any dilution using Brookfield viscometer. The sample (30mL) was taken in a beaker and allowed to equilibrate for 5min before measuring the reading using a spindle at 2, 2.5, and 5, 6, 10, 12, 20, 30rpm. At each speed, the corresponding reading on the viscometer was noted\(^\text{12}\).

Table 4: Viscosity of Nanoemulsion Gel

<table>
<thead>
<tr>
<th>Spindle speed (rpm)</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>0.3</td>
<td></td>
<td>9600</td>
<td>12000</td>
<td>6000</td>
<td>8400</td>
<td>6500</td>
</tr>
<tr>
<td>0.6</td>
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<td>4000</td>
<td>8000</td>
<td>4000</td>
<td>6200</td>
<td>2200</td>
</tr>
<tr>
<td>1.5</td>
<td></td>
<td>2000</td>
<td>7600</td>
<td>2600</td>
<td>2300</td>
<td>1650</td>
</tr>
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<td>3</td>
<td></td>
<td>1700</td>
<td>3700</td>
<td>1820</td>
<td>1200</td>
<td>940</td>
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<td>1300</td>
<td>730</td>
<td>820</td>
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<tr>
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<td>1158</td>
<td>1600</td>
<td>780</td>
<td>620</td>
<td>760</td>
</tr>
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<td>30</td>
<td></td>
<td>720</td>
<td>900</td>
<td>480</td>
<td>320</td>
<td>550</td>
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</tbody>
</table>

Centrifugation
This technique of centrifugation helps to determine the phase separation of nanoemulsion. 10ml NE was placed in centrifugation tube and put in apparatus at 3000rpm for 30mint and examined for any phase separation.

Table 5: Spreadability and percentage drug content of nanoemulsion gel

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Spreadability</th>
<th>% Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1*</td>
<td>5.14</td>
<td>88.9</td>
</tr>
<tr>
<td>F2*</td>
<td>5.46</td>
<td>90.3</td>
</tr>
<tr>
<td>F3*</td>
<td>6.15</td>
<td>81.9</td>
</tr>
<tr>
<td>F4*</td>
<td>6.47</td>
<td>92.7</td>
</tr>
<tr>
<td>F5*</td>
<td>6.31</td>
<td>86.37</td>
</tr>
</tbody>
</table>

Dye test
It is used to check the nature of the nanoemulsion (o/w or w/o). Water soluble dye is added in o/w NE. The NE takes up the color uniformity. Conversely, if the emulsion is w/o type and the dye being soluble in water, the emulsion takes up the colour only in dispersed phase and emulsion is not uniformly colored\(^\text{13}\).

Formulation of nanoemulsion gel
1% carbopol 934 was selected as a gelling agent. Carbopol 934 solution (1% carbopol 934 added in warm water with continuous stirring) added drop wise into the nanoemulsion with continuous stirring until the nanoemulsion convert into nanoemulgel\(^\text{14}\).

CHARACTERIZATION OF NANOEMULGEL

pH determination
One gram of nanoemulgel was dissolved in 10 ml of distilled water and the pH meter was prior standardized with standard buffers of pH 4 and pH 7\(^\text{14}\).

Viscosity
The viscosity of formulations is determined using Brookfield DV-III at temperature 25°C. 50grams of the sample is tested using a 50 ml capacity vessel using spindle 5 at different speed\(^\text{12}\).
Spreadability
An excess of emulgel (about 1g) under study was placed on this ground slide. The emulgel preparation was then sandwiched between this slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 100 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the emulgel between the two slides. Measured quantity of weight (35g) was placed in the pan attached to the pulley with the help of hook. Time in seconds taken by two slides to slip off from emulgel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula.

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

Where S is spreadability, m is weight placed on upper slide, l is length of upper slide, and t is the time taken.

**Figure 8:** Graphical representation of Zeta Average

Drug content determination
Quantity of Terbinafine in nanoemulsion gel was determined by UV-Spectrophotometer. 1.0 g of formulation was accurately weighed, dissolved in 100 ml of methanol: phosphate buffer (2:8). It was filtered and diluted if required. Absorbance was determined using UV spectrophotometer at 282.7nm. A Brookfield Viscometer was used to measure the viscosity of nanoemulsion and nanoemulgel by different spindle speeds. Viscosity reveals the rheological properties of all formulation. Spreadability of NEG was determined by spreadability apparatus. Spreadability is measured on the basis of ‘slip’ and ‘Drag’ characteristics of nanoemulsion gel. Spreadability is an important property of topical formulation from patient compliance point of view.

RESULTS AND DISCUSSION

The pre-formulation studies were performed as per given procedures. The partition coefficient (log P) was determined by shake flask method. The log P value of drug sample was obtained 5.51. 10mg Terbinafine was dissolve in 10ml of ethanol than 1ml of this solution was taken and diluted up to 10ml with ethanol. This dilution were scanned for determined absorption maxima in range 200-300nm. The observed absorbance maxima were found to be 282.7 nm. The calibration curve of Terbinafine in 7.4 pH PBS was determined in conc. range of 2-10µg/ml. The compatibility of nanoemulsion containing all excipients oleic acid as oily phase, span20 as a surfactant and propylene glycol as a co-surfactants and drug (Terbinafine), by FTIR. It was found that there was no chemical reaction between drug and excipients because in the characteristics peaks of terbinafine, there no any changes was observed when compared to the IR spectra of pure drug.

In the all formulation the particle size range were observed from 95.96 to 536 (nm) and the polydispersity index was found to be 0.400 to 0.709. The particle size study explain that the effect of different ratio of surfactant, cosurfactant, oil and water. F4 has 144 nm zeta average due to 1:2 proportion of surfactant and cosurfactant and less amount of oil phase. Higher size average was found to be 536 nm for formulation F1. From the all 4 formulations, best formulations graphs and figure are given below. First graph is F2, its size was found to be 95.96 nm and polydispersity index was found to be 0.709. Second graph is F4, its size was found to be 144 nm and polydispersity index was found to be 0.400. Third graph is F5, its size was found to be 215.8 nm and polydispersity index was found to be 0.462. Zeta Potential of all formulation was found to be -4.32 to -32.6. The higher zeta potential of any formulation shows more stability because due to the high zeta potential of particles are not allow getting aggregate because of electrical repulsive force between particles. The pH value for NE formulation was recorded 5.73 to 6.82. The pH of the NE was found to be within the range of pH of skin and would not cause any irritation to the skin. A Brookfield Viscometer was used to measure the viscosity of nanoemulsion and nanoemulgel by different spindle speeds. Viscosity reveals the rheological properties of all formulation.

In-vitro drug release kinetics
To study the release kinetics of in-vitro drug release, data was applied to kinetic models such as zero order, first order, Higuchi and Korsmeyer-Pappas. In short, the result obtained from in-vitro release studies were plotted in four kinetic models of data treatment as follows:

- Cumulative % drug release Vs. Time (zero order rate kinetics)
- Log cumulative % drug release Vs. Time (First order rate kinetics)
- Cumulative % drug release Vs. Time √(T) (Higuchi’s classical diffusion equation)
- Log cumulative % drug release Vs. log Time (Korsmeyer Pappas equation).
Drug content is the drug concentration in gellified nanoemulsion, which was measured by UV spectrophotometer. The range of percentage drug content of nanoemulsion gel was 75.3% to 92.7%. The range of percentage drug content of formulations was found to be satisfactory. The in-vitro % cumulative drug release studies of NEG were found to be 66.90% to 82.69%. All the formulation shows different release...
rate because of different ratio of surfactant and co-
surfactant. F4* NE shows best drug release 82.69% in
6hrs and F2* shows lowest drug release 66.90% in
6hrs. For the determination of drug release data of all
NEG formulation were fitted into zero order kinetics,
first order kinetics, Koresymer Peppas release kinetics.
Higuchi release kinetics, Bakar losandale release
kinetics to know the drug release pattern from the NEG
formulation.

CONCLUSION
The principle object of the present experimental work
was to make a most effective topical preparation for
avoid the first pass metabolism of terbinafine in the
treatment of antifungal infections with maximum drug
release and reduce GIT side effects. There was a
spontaneous formation of clear nanoemulsion, presumably
due to orientation of surfactant and cosurfactant at the interface, which is a direct
consequence of high thermodynamic stability at the
attained interface of the system. In this study,
nanoemulsion and NEG were prepared and evaluated.
The results showed that nanoemulsion components had significant effect on the response. The nanoemulsion
formulation containing % surf: co surf 48.91, %oil 5.43
and %water 45.65 was best for forming NEG. For all
studies the nanoemulsion gel F4* has best release and most effective formulation. Study concludes that nano
emulgel is a promising area for continued research with the aim of achieving controlled release with enhanced
bioavailability and for drug targeting to affected sites.

AUTHOR’S CONTRIBUTION
The manuscript was carried out, written, and approved in collaboration with all authors.

CONFLICT OF INTEREST
No conflict of interest associated with this work.

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