FORMULATION DEVELOPMENT AND EVALUATION OF SUSTAINED RELEASE ANTIFUNGAL EMULGEL OF VORICONAZOLE

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ABSTRACT

Voriconazole is an imidazole derivative and used for the treatment of local and systemic fungal infection. Commercially Voriconazole topical gel preparation are not available in the market, thus this formulation is made for better patient compliance and to reduce the dose of drug and to avoid the side effects like liver damage and kidney damage. The sustained release emulgel was formulated by changing the polymer ratio. FT-IR study confirmed the purity of drug and revealed no interaction between the drug and excipients. Emulgel formulations were characterized for drug content, pH determination, viscosity measurement, in vitro diffusion, antifungal activity and skin irritation. The objective of this project was to develop Emulgel for sustain release of voriconazole. In present work we prepare emulsion and then incorporated in carbopol gel. The result of studied revealed that the optimized batch shows 94.27% release in 24 hours and stable for around there. Hence it can be concluded that emulsion based system is more effective and safe system for sustained delivery of antifungal agent(s). Efficient delivery of drug to skin application was found to be highly beneficial in localizing the drug to desired site in the skin and reduced side effects associated with conventional treatment.

KEYWORDS: Emulsion, Gel, Emulgel, Voriconazole, Topical drug delivery.

INTRODUCTION

Voriconazole is the newest agent in the armamentarium against fungal infections. It has a spectrum of activity comparable to that of itraconazole. Voriconazole was approved by the
Food and Drug Administration in May 2002 for the treatment of invasive aspergillosis and refractory infections of *Scedosporium apiospermum* and *Fusarium* spp. Studies have also shown it to be a promising agent for empiric treatment in febrile neutropenia. Voriconazole (VFEND, Pfizer Ireland Pharmaceuticals. Ringaskiddy, Ireland) is a triazole antifungal agent that inhibits fungal ergosterol biosynthesis. It is structurally related to fluconazole, with the major difference being the substitution of a fluoropyrimidine grouping in place of a triazole moiety. Voriconazole is indicated for the treatment of invasive aspergillosis. It is also indicated for the treatment of fungal infections caused by *S.apiospermum* or *Fusarium* spp. are refractory to other antifungal agents. Voriconazole works principally, by inhibition of fungal cytochrome P-450 mediated 14alpha-halanosterol demethylation, an essential step in fungal ergosterol biosynthesis. Voriconazole is designated chemically as (2R 3S)-2-(2,4-difluorophenyl)-3-(5fluoro-4-pyrimidinyl)- 1-(1H-1,2,4-triazol-1-yl)-2 butanol and a molecular weight of 349.31. Compared to fluconazole, voriconazole has an enhanced antifungal spectrum that includes filamentous fungi. Voriconazole was designed to enhance the potency and spectrum of activity of fluconazole used against a broad spectrum of significant clinical isolates like Aspergillus, Candida, Scedosporium and Fusarium.

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. There are wide spectrum of preparations for both cosmetic and dermatological use for their diseased skin. These formulations range in physicochemical nature from solid through semisolid to liquid. Drug substances are seldom administered alone, but rather as part of a formulation, in combination with one or more non medicated agents that serve varied and specialized pharmaceutical function. Drugs are administered topically for their action at the site of application or for systemic effects. Drug absorption through the skin is enhanced if the drug substance is in solution, if it has a favorable lipid/water partition coefficient and if it is a nonelectrolyte. For the most part, pharmaceutical preparations applied to the skin are intended to serve some local action and as such are formulated to provide prolonged local contact with minimal systemic drug absorption. Drug applied to the skin for their local action include antiseptics, antifungal agent, skin emollients and protectant. The main advantage of topical delivery system is to bypass first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption like pH changes, presence of
enzymes, gastric emptying time are other advantages of topical preparations.\cite{3-4} The topical drug delivery system is generally used where the other system of drug administration fails or it is mainly used in fungal infection. Human skin is uniquely engineered organ that permits terrestrial life by regulating heat and water loss from the body whilst preventing the ingress of noxious chemicals or microorganisms. It is also the largest organ of the human body, providing around 10\% of the body mass of an average person, and it covers an average area of 1.7m. Such a large and easily accessible organ apparently offers ideal and multiple sites to administer therapeutic agents for both local and systemic actions. Human skin is a highly efficient self-repairing barrier designed to keep the insides in and the outside out.\cite{5}

Gels are a relatively newer class of dosage form created by entrapment of large amounts of aqueous or hydroalcoholic liquid in a network of colloidal solid particles, which may consist of inorganic substances, such as aluminum salts or organic polymers of natural or synthetic origin.\cite{6} They have a higher aqueous component that permits greater dissolution of drugs, and also permit easy migration of the drug through a vehicle that is essentially a liquid, compared with the ointment or cream base.\cite{7} These are superior in terms of use and patient acceptability.\cite{8} In spite of many advantages of gels a major limitation is in the delivery of hydrophobic drugs. So to overcome this limitation, emulgels are prepared and used so that even a hydrophobic therapeutic moiety can enjoy the unique properties of gels.\cite{9-11} In fact, the presence of a gelling agent in the water phase converts a classical emulsion into an emulgel.\cite{12} Both oil-in water and water-in-oil emulsions are used as vehicles to deliver various drugs to the skin. Emulgels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, nonstaining, long shelflife, bio-friendly, transparent & pleasing appearance.\cite{13-14} Use of topical agents requires an appreciation of the factors that influence percutaneous absorption. Molecules can penetrate the skin by three routes: through intact stratum corneum, through sweat ducts, or through sebaceous follicle. The surface of the stratum corneum presents more than 99\% of the total skin surface available for percutaneous drug absorption.\cite{15} Passage through this outer most layer is the rate limiting step for percutaneous absorption. The major steps involved in percutaneous absorption include the establishment of a concentration gradient, which provides the driving force for drug movement across the skin, release of drug from the vehicle (partition coefficient), and drug diffusion across the layers of the skin (diffusion coefficient). Preferable characteristics of topical drugs include low molecular mass (600 Da), adequate solubility in oil and water, and a high partition coefficient. Except for
very small particles, water soluble ions and polar molecules do not penetrate intact stratum corneum. Topical formulation can be used to manipulate the barrier function of the skin, for example, topical antibiotics and antibacterials help a damaged barrier toward off infection, sun-screening agents and the horny layer protect the viable tissues from Ultraviolet radiation and emollient preparations restore pliability to a desiccated horny layer.[16] During development of semi-solid preparations for cutaneous application whose formulation contains an antimicrobial preservative, the need for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in efficacy of antimicrobial preservation. Sterile semi-solid preparations for cutaneous application are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of microorganisms.[17] The efficacy of an antimicrobial preservative may be enhanced or diminished by the active constituent of the preparation or by the formulation in which it is incorporated or by the container and closure used. Preparation for topical use should have microbiological quality and it is checked with test for sterility. Total viable aerobic count should not be more than 102 micro-organisms (aerobic bacteria plus fungi) per gram. It should not have more than 101 enterobacteria, certain other gram-negative bacteria per gram and completely devoid of Pseudomonas aeruginosa and Staphylococcus aureus.[18-19] Many widely used topical agents like ointment, cream, lotion have many disadvantages. They are very sticky causing uneasiness to the patient when applied. Moreover they also have lesser spreading coefficient and need to apply with rubbing. And they exhibit the problem of stability also. A gel is colloid that is typically 99% wt liquid, which is immobilized by surface tension between it and a macromolecular network of fibers built from a small amount of a gelating substance present. Inspite of many advantages of gels a major limitation is in the delivery of hydrophobic drugs. So to overcome this limitation an emulsion based approach is being used so that even a hydrophobic therapeutic moiety can be successfully incorporated and delivered through gels.[20]

EXPERIMENTAL

MATERIALS AND METHODS

Voriconazole was received as a gift sample from Ranbaxy laboratories Ltd., Indore (India). Carbopol 934; Light liquid paraffin; Tween 20; Span 20; Propylene glycol; Methyl paraben; Propyl paraben; Ethanol were purchased from loba chemie, Mumbai. Distilled water was used for all experiments. All chemicals were of pharmaceutical grade and used without
further modification.

**Preparation of Emulgel**

The two different phases were prepared. The oil phase of the emulsion was prepared by dissolving Span 20 in light liquid paraffin while the aqueous phase was prepared by dissolving Tween 20 in purified water. The drug (Voriconazole) was dissolved in ethanol. Both the oily and aqueous phases were separately heated to 70° to 80°C; then the oily phase was added to the aqueous phase with continuous stirring until cooled to room temperature. The gel formulations were prepared by dispersing Carbopol 934 in purified water with constant stirring at a moderate speed in then the pH were adjusted to 6 to 6.5 using Tri Ethanol Amine (TEA). Methyl and Propyl paraben were dissolved in aqueous phase of propylene glycol Glutaraldehyde was added during the mixing of gel and emulsion phase in 1:1 ratio to obtain the emulgel.

Optimization of emulsified gel Experimental design: Eight voriconazole emulgel formulations (Table 1) were prepared according to a $2^3$ factorial design employing the qualitative factors and levels show in table 1 and table 2.

**Table 1: Factor level for the $2^3$ factorial designs.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>[A] Gelling agent type</td>
<td>$+7.5%$ - $5%$</td>
</tr>
<tr>
<td>[B] Liquid paraffin concentration</td>
<td>$+5%$ - $2.5%$</td>
</tr>
<tr>
<td>[C] Emulsifying agent concentration</td>
<td>$+2.5%$ - $1.5%$</td>
</tr>
</tbody>
</table>

**Table 2: Composition of Voriconazole Emulgel formulation.**

<table>
<thead>
<tr>
<th>Formulation Batches</th>
<th>Compositions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>F1</td>
<td>+</td>
</tr>
<tr>
<td>F2</td>
<td>+</td>
</tr>
<tr>
<td>F3</td>
<td>+</td>
</tr>
<tr>
<td>F4</td>
<td>-</td>
</tr>
<tr>
<td>F5</td>
<td>+</td>
</tr>
<tr>
<td>F6</td>
<td>-</td>
</tr>
<tr>
<td>F7</td>
<td>-</td>
</tr>
<tr>
<td>F8</td>
<td>-</td>
</tr>
</tbody>
</table>

A-Gelling Agent type, B-liquid paraffin concentration, C-emulsifying agent concentration, Factor at low level [-], factor at high level [+].
Characterization of Emulgel

**Physical appearance:** The prepared voriconazole emulgel formulations were inspected visually for their color, homogeneity, consistency.

**pH:** The pH values of 1% aqueous solutions of the prepared emulgel were measured by a pH meter.

**Spreadability:** One of the criteria for Emulgel is to meet the ideal quality is that it should possess good spreadability. It is the term expressed to denote the extent of area to which gel readily spread on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value.

Spreadability is expressed in terms of 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 (Fig. 1).

\[ S = \frac{M \times L}{T} \]

Where \( M \) = wt. tied to upper slide \( L \) = length of glass slides.
\( T \) = time taken to separate the slides

**Extrudability study:** In conducting the test, a closed collapsible tube containing above 20 grams of gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and gel was extrudes until the pressure was dissipated (Fig. 2).

**Viscosity Study:** The viscosity of different emulgel formulation was determined using a brook field viscometer (Brookfield DV-E viscometer). The recorded viscosities are in figure 3.

**Drug Content Determination:** Drug concentration in emulgel was measured by spectrophotometer. Voriconazole content in emulgel was measured by dissolving known quantity of emulgel in solvent (ethanol) by Sonication. Absorbance was measured after suitable dilution at 256 nm in UV/VIS spectrophotometer.

**In Vitro Release Study:** Franz diffusion cell was used for the drug release studies. Emulgel
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(200 mg) was applied onto the surface of cellophane membrane evenly. The cellophane membrane was clamped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared PBS solution to solubilize the drug. The receptor chamber was stirred by magnetic stirrer. The samples (1.0 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV visible spectrophotometer at 256 nm after appropriate dilutions. Cumulative corrections were made to obtain the total amount of drug release at each time interval. The cumulative amount of drug released across the cellophane membrane was determined as a function of time.

**Microbiological assay:** Disc plate technique was used. It is a technique used for evaluation of fungistatic activity of a compound. It is mainly applied for semisolid formulations. Previously prepared Sabouraud’s agar dried plates were used. Three grams of the emulgel are placed in a disc cut in the plate. Freshly prepared culture loops are streaked across the agar at a right angle from the ditch to the edge of the plate. After incubation for 48 hours at 25°C, the fungal growth was observed and the percentage inhibition was measured as follows: % inhibition = L2 / L1 × 100 Where L1 = total length of the streaked culture, and L2 = length of inhibition.

**Accelerated stability studies of emulgel:** Stability studies were performed according to ICH guidelines. The formulations were stored in hot air oven at 37 ± 2° and 45 ± 2° for a period of 3 months. The samples were analyzed for its appearance and drug content every two weeks by UV-Visible spectrophotometer at 256 nm. Stability study was also carried out by measuring the change in pH of gel at regular interval of time.

**RESULTS AND DISCUSSION**

**Physical appearance:** The prepared voriconazole emulgel formulations were white to slightly yellowish, viscous creamy preparation with a smooth and homogeneous appearance.

**pH:** The pH values of all formulations were found in the range 6.50 to 6.80. Hence all the formulations are satisfactorily complying with pH values needed for topical application and pH of skin.

**Spreadability:** The observations for spreadability of all formulations are shown in [fig 1](#). The spreadability of the formulation depends on its viscosity. The greater the viscosity the longer will be the time taken for spreading on the skin. F5 formulation showed moderates
spreadability of 28.13±1.0246 and was most easily spread on skin as compared to other formulations.

**Extrudability**: The observations for extrudability of all formulations are shown in **fig 2**. F5 formulation shows highest extrudability when compared with other formulations.

![Fig 1: Spreadability of the various emulgel formulations (Mean ± S.D.)](image1)

**Extrudability**

![Fig. 2: Extrudability of the various emulgel formulations (Mean ± S.D.)](image2)

**Viscosity studies**: The measurement of viscosity of the prepared Emulgel was done with a Brookfield cone and plate type Viscometer (CAP 2000+) was used to determine viscosity (cp) of the formulations. The viscosity was measured at 10 rpm after 60 seconds. At each speed, the corresponding dial reading was noted. The viscosity of the emulgel was obtained (Fig.3)
Drug content determination: 1 g of the prepared emulgel was mixed with 100 ml of suitable solvent (ethanol). Aliquots of different concentration were prepared by suitable dilution after Sonication and filtering the stock solution and absorbance was measured. Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve. The drug content of all emulgel formulation is given below (Fig.4).

In vitro Drug Release
The in vitro release profiles of voriconazole from its various Emulgel formulations are represented in Figure 5. It was observed that all the formulation had become liquefied and diluted at the end of the experiments, indicating water diffusion through the membrane. In general, it can be observed from figures that the better release of the drug from all Emulgel formulation. The release of the drugs from its Emulgel formulation can be ranked in the following descending order: F5 > F4> F7 > F6> F2 > F8 > F3 > F1, Where the amounts of
the drug release of the drug released after 24 hours were 94.27%, 92.31%, 91.73%, 91.67%, 90.47%, %, 89.66% and 88.51% respectively. Thus the higher drug release was observed with formulation F5. Add 0.1% of glutaraldehyde to give the retard the release of drug from emulgel formulation. It’s proved that the presence of liquid paraffin led to retardation of voriconazole release from its emulgel formulation. Thus the 3 studied factors can be arranged according to their effect on the drug release from the emulgel formulation as follows: the emulsifying agent concentration > liquid paraffin concentration > the gelling agent type.

![Graph showing release profiles of voriconazole from its emulgel formulations at 24 hours (Mean ± S.D.).](image)

**Fig. 5: Release profiles of voriconazole from its emulgel formulations at 24 hours (Mean ± S.D.)**

**Microbiological assay:** The use of control plates showed that the plain Emulgel bases were microbiologically inert toward the tested *Candida albicans* strains. The antifungal activity of voriconazole in its optimized formulation is shown table 3. Percentage inhibition was taken as a measure of the drug antifungal activity. The percentage inhibition of optimized formulation F5 was found to be 40.7%. This percentage inhibition was compared with the marketed preparation which was found to be 40.5%.

**Table 3: Percentage inhibition of Voriconazole in its different Emulgel formulations.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Zone of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>40.7%</td>
</tr>
<tr>
<td>Marketed Preparation</td>
<td>40.5%</td>
</tr>
</tbody>
</table>

**Accelerated stability studies of emulsion based gel:** The accelerated stability studies were performed according to ICH guidelines for 3 months and the results were found to be stable in varying temperature as shown in Table 5 and 6.
Table 5: Accelerated stability study of optimized emulsion based gel formulation F5 at 25°C.

<table>
<thead>
<tr>
<th>Storage Temp. °C</th>
<th>Period of Studies in month</th>
<th>1 month</th>
<th>2 month</th>
<th>3 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 ± 2</td>
<td>Appearance</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Drug content</td>
<td>97.86 ± 0.05</td>
<td>97.29 ± 0.11</td>
<td>97.19 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6.78 ± 0.07</td>
<td>6.79 ± 0.019</td>
<td>6.80 ± 0.027</td>
</tr>
</tbody>
</table>

Table 6: Accelerated stability study of optimized emulsion based gel formulation F5 at 45°C.

<table>
<thead>
<tr>
<th>Storage Temp. °C</th>
<th>Period of Studies in months</th>
<th>1 month</th>
<th>2 months</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 ± 2</td>
<td>Appearance</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Drug content</td>
<td>96.96 ± 0.01</td>
<td>96.89 ± 0.03</td>
<td>96.43 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6.78 ± 0.09</td>
<td>6.79 ± 0.013</td>
<td>6.80 ± 0.014</td>
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</table>

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