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FORMULATION AND EVALUATION OF LULICONAZOLE LOADED INVASOMES GEL

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ABSTRACT

Superficial fungal infection in immunocompromised patients can lead to many disorders and complications. Currently, new topical treatment options are critically needed to treat these fungal infections. Luliconazole is a topical antifungal medicine used for fungal infection treatment. The purpose of this paper was to develop a new topical luliconazole invasomes incorporated gel. Different formulation prepared for Luliconazole loaded in to invasomes by mechanical dispersion technique. The maximum drug content was found in formulation LG-2 (99.12±0.25) and selected as optimized formulation. When the regression coefficient values were compared, it was observed that 'r^2' values of higuchi was maximum i.e. 0.995 hence indicating drug release from formulations was found to follow higuchi release kinetic.

Keywords: Luliconazole, Invasomes, fungal infection, *in-vitro* diffusion study.

INTRODUCTION

Topical treatment of fungal infections has various advantages, including the ability to target the infection site, reduced risk of systemic adverse effects, improved treatment efficacy, and high patient compliance. A range of topical antifungal agents have been employed in the treatment of various dermatological skin infections. The main classes of topical antifungals are polyenes, azoles, and allylamine/benzylamines[1]. These antifungal medications are currently accessible in creams, gels, lotions, and sprays in conventional dosage forms[2]. The effectiveness of topical antifungal treatment is determined on drug penetration through the target tissue. As a result, the effective drug concentration levels in the skin should be obtained. When antifungals are applied topically, the drug components must penetrate through the stratum corneum, the skin's outermost layer, to reach the lower layers, particularly the *viable epidermis*[3].

In this case, the formulation may have a significant impact in medication penetration through the skin. New carrier systems for authorized and investigational medications are being developed as alternate techniques for topical treatment of fungal infections of the skin. Antifungal chemicals can be delivered more effectively into the skin via carriers such as colloidal systems, vesicular carriers, and nanoparticles. For localised or systemic effects, the transdermal route is essential[4]. Many drugs require the stratum corneum, the skin's outer layer, to pass through. Several strategies have been developed to overcome this barrier [5-9], including the use of technologies that alter the continuity of the stratum corneum (SC), such as ultrasound, electroporation, and iontophoresis, as well as the use of vehicles and nanocarriers to increase drug penetration[10-12]. Various types of nanocarriers have recently been developed to increase drug delivery through the dermal and transdermal routes [13-14]. To aid in the penetration of the incorporated agents, lipophilic and hydrophilic medicines can be incorporated into liposomal vesicular systems [15]. Conventional liposomes, on the other hand, are not certified as acceptable methods for transdermal medication delivery because they are unable to penetrate the inner layers of skin, limiting their effects to the top layers [16]. Because of their increased interactions with skin and higher drug penetration, novel elastic vesicles incorporating penetration enhancers outperform standard liposomes [17-18]. Cevc et al. developed the original deformable or elastic vesicles, known as Transfersomes, in the 1990s.These vesicles are composed of phospholipids and edge activators, such as polysorbate or sodium cholate, producing elastic carriers for improved transdermal drug delivery [19–23]. The encouraging results seen with Transfersomes® led to the development of other novel elastic vesicles via alterations in the vesicular composition. In previous examinations, elastic vesicles such as niosome (prepared mostly by non-ionic surfactant and cholesterol) [24] and ethosome (containing high amount of ethanol in their structure) [25] have displayed potential as a drug carrier.

Luliconazole is a new topical antifungal imidazole with broad-spectrum antifungal action that is used to treat superficial mycoses. Although superficial mycoses are not fatal, they can have a significant impact on a patient's quality of life due to the significant discomfort and/or cosmetic deformities they produce. These diseases are found all over the world and impact 20 to 25% of the population. Among the superficial mycoses, dermatophytosis is the most frequent infection. The active molecules related to lanoconazole, a strong antidermatophytic medication, were initially investigated for the compound. Luliconazole is currently available in a 1% cream and a 1% solution for the treatment of superficial mycoses like dermatophytosis, candidiasis, and pityriasis versicolor. Invasomes are a new type of vesicular carrier made of soya lecithin, ethanol, and a little quantity of terpenes (cineole, citral, and d-limonene, essential oils). The purpose of this research is to create and characterise a Luliconazole-loaded invasomes gel as a viable topical delivery.

MATERIALS AND METHODS

Material

Luliconazole was procured from Pharmaceutical Company, Soya phosphatidylcholine Hi Media Pvt. Ltd, and other analytical reagents used throughout the study.

Methods

Formulation Optimization of Luliconazole loaded Invasomes

Luliconazole was loaded in to invasomes by mechanical dispersion technique. Soya Phosphatidylcholine (0.5 to 1% w/v) was added to ethanol and vortexed for 5 minutes [26-27]. Drug (50mg) and terpenes (0.25 to 0.5%) were added under constant vortexing, this mixture was sonicated for 5 minutes. Fine stream of Phosphate buffer saline was added with syringe under constant vortexing. It was vortexed for additional 5 minutes to obtain final invasomal preparation.

Table 1: Formulation optimization of Luliconazole loaded Invasomes

Ingredient (%)	F1	F2	F3	F4	F5	F6
Luliconazole (mg)	50	50	50	50	50	50
Phosphotidylcholine (%)	0.5	1.0	1.5	0.5	1.0	1.5
Terpenes (%)	0.25	0.25	0.25	0.5	0.5	0.5
Ethanol (ml)	5	5	5	5	5	5

Preparation of Gel Base

Carbopol 934 (1-3% w/v -Invasome based gel formulation i.e. LG-1 of 1% w/v, LG 2 of 2% w/v, LG-3 of 3% w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution [28]. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. The same procedure was used to formulate Invasomes containing gel in which previously prepared Invasomes suspension was added. Invasomes preparation corresponding to 0.1% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Evaluation of invasomes

Entrapment efficiency

Entrapment efficiency of luliconazole invasomes formulation was determined using centrifugation method [29]. The entrapment efficiency of acyclovir in invasomes vesicle was determined by ultracentrifugation, 10mL of invasomes formulation were collect in test tube. The amount of drug not entrapped in the invasomes was determined by centrifuging at 3,000 rpm and collect the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was determined at 282 nm using UV spectrophotometer.

$$\%\ Entrapment\ Efficiency = \frac{Therotical\ drug\ content - Practical\ drug\ content}{\text{Therotical drug content}} \times 100$$

Vesicle size

Microscopic analysis was performed to determine the average size of prepared invasomes[30]. Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer. The average diameter was calculated using the flowing formula.

$$Average\ Diameter = \frac{\Sigma n.d}{\Sigma\ \text{n}}$$

Where n = number of vesicles;

d = diameter of the vesicles

Evaluation of invasomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical Invasomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted [31].

Drug content

Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol [32]. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 282 nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load [33]. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer *et al.*,[34]. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80 g of weight was noted. Good spreadibility show lesser time to spread.

$$S = m * l/t$$

Where:

m – Weight tied on upper slide

l – Length of glass slide

t – Time in slide

***In-vitro* drug diffusion study**

The *in-vitro* diffusion study was carried by using franz diffusion cell. Egg membrane was taken as semi permeable membrane for diffusion [35]. The franz diffusion cell had receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14 sq.cms. The egg membrane was mounted between the donor and the receptor compartment. A two cm^2 size patch was taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium was phosphate buffer pH 7.4. The receptor compartment was surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by Teflon coated magnetic bead which was placed in the diffusion cell.

During each sampling interval, samples were withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn were analyzed spectrophotometrically at wavelength of 282 nm.

Results and Discussion

The effect of varying concentration of phosphatidylcholine on average vesicle size was studied. With increase in the phosphatidylcholine concentration the vesicle size increased. The Average vesicle size was found to be in the range of 220.12±0.42 to 274.12±0.34, the minimum vesicle size was found in formulation F-4, 220.12±0.42 nm. From the drug entrapment efficiency results, it is clear that drug entrapment efficiency of Invasomes formulations is reduced with lowering concentration of lecithin. This is because of the fact that low lecithin content provides less drug entrapment efficiency. The ability of formulation to withstand drug molecules in the bilayer membrane of the vesicle was proved by the encapsulation efficiency of Invasomes formulations. The Entrapment Efficiency of formulation F1. F2, F3, F4, F5 and F6 was found to be 63.32±0.23, 65.54±0.35, 68.78±0.45, 78.85±0.41, 71.22±0.32 and 69.98±0.34 percentage, respectively. The maximum percentage entrapment efficiency was found to be in formulation F-4, 78.85±0.41 percentages. Surface potential can play an important role in the behavior of invasomes *in-vivo* and *in-vitro*. In general, charged invasomes were more stable against aggregation and fusion than uncharged invasomes table 2.

The prepared gel at rpm of 10 exhibited a viscosity of 3045±14 to3565±15cps that indicates that the formulation has the desired viscosity required for semisolid formulation for proper packaging. It was found that the viscosity decreases as the rotational speed of viscometer increased suggesting that greater the shearing the lower viscosity favors easy spreadability further confirmed by spreadability and rheological testing. pH of prepared invasomes gel was measured by using digital pH meter. The pH of the gel was found to be in range of 6.75±0.02 to 6.98±0.02 which is good for skin pH. All the formulations of gel were shown pH nearer to skin required i.e. pH of LG1-6.75±0.02, LG2- 6.82±0.03 and LG3-6.98±0.02.Spreadability plays considerable role in patient compliance and ensures uniform application of gel to a larger area of the skin. The spreadability of the formulation LG-2 was calculated as 10.23±0.32cm/sec. The low value of spreadability coefficient of the gel was sufficient suggesting easy spreading and no signs of grittiness. The lower value of spreadability indicates the lesser work required to spread the gel over the skin, which means formulation was easily spreadable by applying small amount of shear. Drug content of Luliconazole incorporated invasomes gel for formulation LG-1, LG-2 and LG-3 was found to be 96.65±0.15, 99.12±0.25 and 98.58 ± 0.32 respectively table 4. The maximum drug content was found in formulation LG-2 (99.12±0.25) and selected as optimized formulation. When the regression coefficient values were compared, it was observed that 'r^2' values of higuchi was maximum i.e. 0.995 hence indicating drug release from formulations was found to follow higuchi release kinetic table 5.

Table 2: Entrapment efficiency and average vesicle size

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F1	63.32±0.23	245.65±0.25
F2	65.54±0.35	265.58±0.36
F3	68.78±0.45	286.65±0.14
F4	78.85±0.41	220.12±0.42
F5	71.22±0.32	242.32±0.22
F6	69.98±0.34	274.12±0.34

(n=3 mean ± SD)

Table 3: Characterization of optimized formulation (F4)of invasomes

Formulation.	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F4	220.12±0.42	78.85±0.41	-37.2

(n=3 mean ± SD)

Table 4: Characterization of gel based formulation of Invasomes

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
LG-1	3565±15	6.75±0.02	96.65±0.15	145.5±0.5	11.25±0.25
LG-2	3325±12	6.82±0.03	99.12±0.25	165.6±0.4	10.23±0.32
LG-3	3045±14	6.98±0.02	98.58±0.32	163.1±0.2	9.85±0.14

(n=3 mean ± SD)

Table 5: In vitro drug release study of prepared optimized gel formulation LG-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	11.85±0.32
2	1	20.32±0.25
3	2	35.54±0.12
4	4	46.65±0.25
5	6	65.54±0.32
6	8	78.85±0.41
7	10	88.85±0.36
8	12	99.12±0.14

(n=3 mean ± SD)

Conclusion

Different formulations of Luliconazole loaded invasomes gel as potential carrier for topical delivery. The maximum drug content was found in formulation LG-2 (99.12±0.25) and selected as optimized formulation. When the regression coefficient values were compared, it was observed that 'r^2' values of higuchi was maximum i.e. 0.995 hence indicating drug release from formulations was found to follow higuchi release kinetic.

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