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Formulation, Development and Evaluation of Invasomes Loaded Gel for Fungal Treatment

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Abstract **Original Research Article**

The transdermal route is an important pathway for localized or systemic effects. The stratum corneum, the outer layer of the skin, is an essential skin permeation barrier for many drugs. To overcome this barrier, several techniques have been developed, including the use of the vehicle and nanocarriers to improve drug penetration. Recently, different types of nanocarriers have been designed to improve the dermal and transdermal delivery of medicines like 'INVASOME'. We made clotrimazole loaded invasome gel which is used in fungal treatment. The procedure used in formulation of invasomal gel is mechanical dispersion method. This preparation is evaluate by many parameters they are appearance, spreadability, solubility, in vitro drug diffusion etc.

Keywords: Invasome Gel, Transdermal, Terpene, Vortex, Clotrimazole, Vesicle.

INTRODUCTION

The trasdermal delivery of drugs is rapidly increasing in the formulation development in enhancing the bioavailability of many drugs. When drugs are administered via transdermal route skin barrier is harmfully affected. In recent years, vesicular systems have been intensively studied as drug carrier systems for the dermal and transdermal administration of drugs. Traditional liposomal formulations, compared to conventional dosage forms, have shown in vitro an enhanced cutaneous drug accumulation allowing a reduction of the dose applied onto the skin. In the last two decades, new classes of lipid vesicles were introduced by different researchers. More recently, researchers investigated the novel vesicular systems called as invasomes. Briefly, invasomes contain not only phospholipids but also ethanol and terpenes, which make the vesicles deformable, and also serve as penetration enhancers [1]. This system has shown to improve skin penetration of hydrophilic and lipophilic drugs. A synergistic effect between terpenes and ethanol on the percutaneous absorption has been significantly observed. In this study, we investigated the ability of invasomes to increase the topical transport of CLOTRIMAZOLE in order to develop a topical formulation with enhanced CLOTRIMAZOLE skin delivery in order to achieve an effective fungal treatment [2]. Ethanol is a good penetration enhancer while terpenes have also shown potential to increase the penetration of many drugs by disrupting the tight lipid packing of the stratum corneum. The aim of the present study was to develop and characterize Clotrimazole-loaded invasomal drug carrier systems. Clotrimazole has been previously identified as a promising candidate for transdermal drug delivery [3].

MATERIAL AND METHOD

Material Used in Formulation

Table No. 1.1 Formulation optimization of Clotrimazole loaded Invasomes

Ingredient (%)	F1	F2	F3	F4	F5	F6
Clotrimazole (mg)	10	10	10	10	10	10
Phosphotidylcholine (%)	0.25	0.5	0.75	0.25	0.5	0.75
Terpenes (%)	0.25	0.25	0.50	0.50	0.75	0.75
Ethanol (ml)	5	5	5	5	5	5

Table No. 1.2 Formulation optimization of gel base

Ingredient (%)	TIG-1	TIG-2	TIG-3
Drug (Invasomes equivalent to 0.1%)	0.1	0.1	0.1
Carbopol 934	1	2	3
Propylene glycol	0.2	0.2	0.2
Water (ml)	100	100	100

Method Used in Formulation

- **Formulation Of Clotrimazole Loaded Invasome**

Clotrimazole (10mg) was loaded in to invasomes by mechanical dispersion technique. Soya Phosphatidylcholine (0.25 to 0.75% w/v) was added to ethanol and vortexed for 5 minutes [11-12]. Drug and terpenes (0.25 to 0.75%) were added under constant vortexing, this mixture was sonicated for 5 minutes. Fine stream of Phosphate buffer saline (upto 10% w/v) was added with syringe under constant vortexing. It was vortexed for additional 5 minutes to obtain final invasomal preparation.

- **Preparation Of Gel Base**

Carbopol 934 (1-3% w/v -Invasome based gel formulation i.e. TIG-1 of 1% w/v, TIG-2 of 2% w/v, TIG-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 [4].

Theobtained 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 PARAMETER

- **Evaluation of Invasomes**
- **Entrapment efficiency**

Entrapment efficiency of Clotrimazole Invasomes formulation was determined using centrifugation method [5]. 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 260 nm using UV spectrophotometer.

- **Vesicle Size**

Microscopic analysis was performed to determine the average size of prepared invasomes [6]. 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 400 X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer.

- **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 [7].

Drug Content

Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol [8]. 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 λmax 260 nm.

- **Extrudability Study**

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load [9]. 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.*, [10]. 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.

- **In-Vitro Drug Diffusion Study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion [8]. The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14 sq. cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell.

- **Stability Studies**

Stability study was carried out for drug loaded invasomal gel at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25- 28 ± 2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any viscosity and % assay.

RESULT AND DISCUSSION

The effect of varying concentration of phosphatidylcholine on average vesicle size was studied. With increase in the phosphatidylcholine concentration the particle size increased the vesicle size. The effect of varying concentration of phosphatidylcholine on vesicle size was determined. With increase in the polymer concentration the vesicle size increased. The Average vesicle size was found to be in the range of 220.12±0.21 to 262.23±0.14, the minimum vesicle size was found in formulation F-3, 220.12±0.21 nm. The Entrapment efficiency of formulation F1, F2, F3, F4, F5 and F6 was found 68.89±0.25, 69.52±0.32, 73.32±0.12, 67.12±0.32, 67.45±0.45 and 69.95±0.25 percentage respectively. 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 prepared gel at least rpm of 10 exhibited a viscosity of 3350±15 to 3570±14cps that indicates that the formulation has the desired viscosity required for semisolid formulation for proper packaging. pH of prepared herbal Gel was measured by using digital pH meter. The pH of the Gel was found to be in range of 6.71±0.02 to 6.81±0.03 which is good for skin pH. 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 TIG-2 was calculated as 11.23±0.15cm/sec. The low value of spreadability coefficient of the Gel was sufficient suggesting easy spreading and no signs of grittiness.

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