CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.6498218

Available online at: http://www.iajps.com *Research Article*

FORMULATION, DEVELOPMENT AND EVALUATION OF ANTIFUNGAL ECONAZOLE LOADED INVASOMES GEL FOR TOPICAL FUNGAL TREATMENT

Durgesh Kumar Patel*, Dr. R.B. Goswami
Sagar Institute of Research Technology & Science–Pharmacy Ayodhya Bypass Road, Bhopal

Article Received: March 2022 **Accepted:** March 2022 **Published:** April 2022

Abstract
Topical drug administration is a localised drug delivery system that can be used to administer drugs to any part of the body via ophthalmic, rectal, vaginal, and cutaneous channels. The principal route of topical medication delivery is through the skin, which is one of the most easily accessible organs on the human body for topical administration. Fungus can cause a variety of skin illnesses. Antifungal medications are fungicides that are used to treat mycoses such athlete's foot, ringworm, and candidasis. Antifungal kills the fungal organism without harming the host by leveraging distinctions between mammalian and fungal cells. Hence we attempted to develop Econazole-loaded invasomes hydrogel. Invasomes were prepared by conventional thin layer evaporation technique using Phosphatidylcholine, terpene (Limonene) and ethanol. In present study was to develop and characterize Econazole - loaded invasomal drug carrier systems. Different Formulations (F1 to F6) of invasomes were prepared and evaluated for average vesicle size, zeta potential and entrapment efficiency. Drug content of econazole incorporated invasomes gel for formulation IG-1, IG-2 and IG-3 was found to be *98.85±0.15, 99.45±0.10 and 98.74±0.23 respectively. The maximum drug content was found in formulation IG-2 (99.45±0.10), select as optimized formulation. When the regression coefficient values of were compared, it was observed that 'r^2' values of Higuchi was maximum i.e. 0.985 hence indicating drug release from formulations was found to follow Higuchi kinetics.*
Key words: *Econazole, Invasomes, Fungal Treatment, formulation, Evaluation*

Corresponding author:
Durgesh Kumar Patel,
Sagar Institute of Research Technology & Science–Pharmacy,
Ayodhya Bypass Road, Bhopal
durgeshsidhi@gmail.com

QR code

Please cite this article in press Durgesh Kumar Patel et al, ***Formulation, Development And Evaluation Of Antifungal Econazole Loaded Invasomes Gel For Topical Fungal Treatment.,*** *Indo Am. J. P. Sci, 2022; 09(04)*

INTRODUCTION:

The incidence of superficial fungal infections of skin, hair and nails has been increased in worldwide. It has been estimated that about 40 million people have suffered from fungal infections in developing and under developed nations. The progression of fungal infections can be rapid and serious due to compromising with immune function [1]. *Dermatophytes* are one of the most frequent causes of *tinea* and o*nchomycosis*. Candidal infections are also among the most widespread superficial cutaneous fungal infections even, *candida* can invade deeper tissues as well as the blood which leads to lifethreating systemic candidiasis, when the immune system is weakened.

Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different type of topical effective antifungal compounds has been used in the treatment of a variety of dermatological skin infections. The main classes of topical antifungals are polyenes, azoles, and allylamine/benzylamines. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels, lotions and sprays [2]. The efficiency of the topical antifungal treatment depends on the penetration of drugs through the target tissue. Hence, the effective drug concentration levels should be achieved in the skin. In topical administration of anti-fungals, the drug substances should pass the *stratum corneum*, which is the outermost layer of the skin, to reach lower layers of the skin, particularly into *viable epidermis* [3].

The transdermal route is an important pathway for localized or systemic effects [4]. 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 [5-9], including the use of methods that change the stratum corneum (SC) continuity, such as ultrasound, electroporation, and iontophoresis, and the use of the vehicle and nanocarriers to improve drug penetration [10-12]. Invasomes are novel and flexible vesicles containing a mixture of soy phosphatidylcholine (PC), terpenes, lyso PC, and ethanol with improved skin penetration in comparison with liposomes. Furthermore, invasomes have the same structural constituents as liposomes but contain terpene in their structure. Terpenes are hydrocarbon compounds and are known to be the primary constituents of essential oils from many plants. Addition of terpenes creates deformable vesicles, which can increase the fluidity of the lipid bilayers of the skin [13-16]. The ability to permeate through skin layers enhances the activity of invasomes, which exert their effects by fluidizing the bilayer structure of SC lipids and disturbing lipids and intracellular protein interactions [17]. Invasomes are novel vesicular systems that exhibit improved transdermal penetration compared to conventional liposomes. These vesicles contain phospholipids, ethanol, and terpene in their structures; these components confer suitable transdermal penetration properties to the soft vesicles. The main advantages of these nanovesicles lie in their ability to increase the permeability of the drug into the skin and decrease absorption into the systemic circulation, thus, limiting the activity of various drugs within the skin layer.

MATERIAL AND METHODS:

Material

Itraconazole was obtained as a gift sample from pharmaceutical company. Phosphatidylcholine, terpene, Carbopol 934 was purchased from Sigma-Aldrich Chem, Germany. High purity 99.9% Ethanol were obtained from SD Fine chemicals, Mumbai, India. All other chemical and materials were of analytical grade. Triple distilled water was generated in house.

methods:

Formulation Optimization of Econazole loaded Invasomes

Econazole 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 [18-19]. Drug and terpenes (0.5 to 1.5%) 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.

Table 1: Formulation optimization of Econazole loaded Invasomes

Ingredient (%)	F1	F2	F3	F4	F5	F6
Econazole (mg)	50	50	50	50	50	50
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

Preparation of Gel Base

Carbopol 934 (1-3% w/v -Invasome based gel formulation i.e. IG-1 of 1% w/v, IG-2 of 2% w/v, IG-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 [20]. 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.

Table 2: Formulation optimization of gel Base

Ingredient (%)	IG-1	IG-2	IG-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

Evaluation of Invasomes

Entrapment efficiency

Entrapment efficiency of Econazole Invasomes formulation was determined using centrifugation method [21]. 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 224 nm using UV spectrophotometer.

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

Vesicle Size

Microscopic analysis was performed to determine the average size of prepared invasomes [22]. 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. The average diameter was calculated using the flowing formula.

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

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 [23].

Drug content

Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol [24]. 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} 224 nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load [25]. More the quantity of gel extruded shows better extrudability. It was determined by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadability

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.*,[26]. 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 [27]. 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 cm^2 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.

During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of 224 nm.

RESULTS 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 210.01±0.15 274.45±0.65, the minimum vesicle size was found in formulation F-5, 210.01±0.15 nm. The Entrapment efficiency of formulation F1, F2, F3, F4, F5 and F6 was found 67.78±0.48, 68.85±0.41, 67.74±0.35, 68.98±0.25, 75.45±0.14 and 68.85±0.23 percentage respectively.
From the drug entrapment efficiency results, it is clear that drug entrapment efficiency of Invasomes formulations is reduced with lowering concentration of lacithin. 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 proves the encapsulation efficiency of Invasomes formulations. The maximum percentage entrapment efficiency was found to be in formulation F-5, 75.45±0.14 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.

The prepared gel at least rpm of 10 exhibited a viscosity of 3045±14 to 3565±15 cps 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 favours easy spreadability further confirmed by spreadability and rheological testing.

pH of prepared herbal Gel was measured by using digital pH meter. The pH of the Gel was found to be in range of 6.72±0.06 to 6.85±0.05 which is good for skin pH. All the formulation of Gel was shown pH nearer to skin required i.e. pH of IG1-6.85±0.05, IG2-6.72±0.06 and IG3-6.75±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 IG-2 was calculated as 10.65±0.14 cm/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 econazole incorporated invasomes gel for formulation IG-1, IG-2 and IG-3 was found to be 98.85±0.15**,** 99.45±0.10 and 98.74±0.23 respectively. The maximum drug content was found in formulation IG-2 (99.45±0.10), select as optimized formulation.

When the regression coefficient values of were compared, it was observed that 'r^2' values of Higuchi was maximum i.e. 0.985 hence indicating drug release from formulations was found to follow Higuchi kinetics.

Table 3: Entrapment efficiency and average vesicle size

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F1	67.78±0.48	245.65±0.32
F2	68.85±0.41	222.12±0.14
F3	67.74±0.35	225.65±0.15
F4	68.98±0.25	235.45±0.32
F5	75.45±0.14	210.01±0.15
F6	68.85±0.23	274.45±0.65

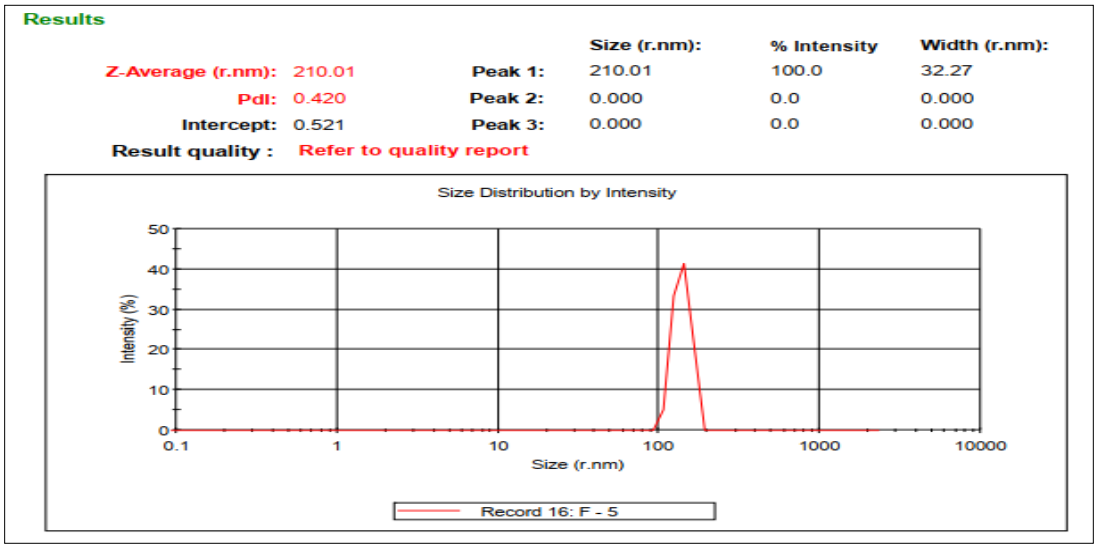

Figure 1: Graph of average vesicle size (nm) of optimized formulation F-5

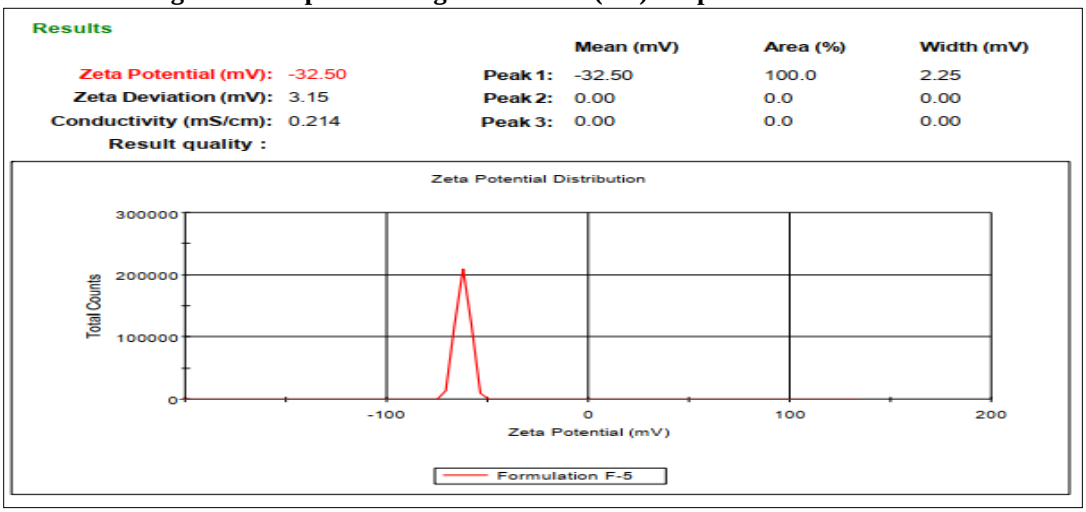

Figure 2: Graph of zeta Potential (mV) optimized formulation F-5

Table 4: Characterization of optimized formulation of invasomes

Formulation .	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-5	210.01±0.15	75.45±0.14	-32.50

Table 5: Characterization of gel-based formulation of Invasomes

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
IG-1	3645±15	6.85±0.05	98.85±0.15	175±4	11.65±0.23
IG-2	3545±10	6.72±0.06	99.45±0.10	185±6	10.65±0.14
IG-3	3465±12	6.75±0.02	98.74±0.23	192±8	9.45±0.26

Table 6: *In vitro* drug release study of prepared optimized gel formulation IG-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	23.36±0.32
2	1	39.98±0.25
3	2	41.15±0.12
4	4	59.95±0.18
5	6	68.85±0.41
6	8	79.98±0.32
7	10	88.45±0.14
8	12	99.45±0.25

Table 7: Regression analysis data of optimized gel formulation IG-2

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
IG-2	0.966	0.761	0.985	0.972

CONCLUSION:

Econazole loaded invasomes formulation was successfully prepared by conventional thin layer evaporation technique using phospholipon 90H, terpene (limonene), ethanol and invasomes hydrogel-based formulations were prepared with hydrophilic polymer carbopol 934p. It can serve as a useful vehicle for the delivery of Econazole through the affected part of the skin for extended period of time. This study also revealed that invasomes hydrogel (IG-2) resides at targeted site for a relatively longer period of time with a first order model of drug release kinetics profile. It signifies the improved patient compliance.

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