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FORMULATION AND EVALUATION OF INVASOMES GEL OF ANTIFUNGAL DRUG FOR EFFECTIVE FUNGAL TREATMENT

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Abstract:
The development of novel drug delivery systems has become essential in improving the efficacy of topical treatments for fungal infections. This study focuses on the formulation and evaluation of Tolnaftate-loaded invasomes, designed to enhance skin penetration and provide sustained antifungal activity. The invasomes were characterized for entrapment efficiency, vesicle size, and zeta potential, with formulation F4 exhibiting the highest entrapment efficiency (74.45%) and an optimal vesicle size of 215.65 nm. Additionally, a gel-based formulation of the optimized invasomes was prepared, showing suitable viscosity, pH, and spreadability. The in vitro release studies revealed a sustained release profile with 88.45% of the drug released over 10 hours, supporting the controlled release mechanism of the formulation. Regression analysis indicated a zero-order release mechanism, confirming the potential of the gel formulation for effective and long-lasting treatment. These results suggest that Tolnaftate-loaded invasomes can offer improved efficacy, stability, and patient compliance compared to conventional topical formulations for fungal infections.

Keywords: *Tolnaftate, invasomes, topical drug delivery, antifungal treatment, entrapment efficiency, vesicle size, sustained release, gel formulation, skin penetration, zero-order release.*

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INTRODUCTION:

Fungal infections, especially those caused by dermatophytes, are a significant global health concern, affecting millions of individuals each year. These infections often manifest in superficial forms such as athlete's foot, ringworm, and jock itch. Among the various antifungal agents available, Tolnaftate is a widely used drug that is effective in treating superficial fungal infections due to its broad-spectrum activity against dermatophytes and other fungi. Despite its effectiveness, the clinical application of Tolnaftate is limited by its poor skin penetration, low bioavailability, and the need for frequent administration (Zhou et al., 2017).

To overcome these limitations, novel drug delivery systems have been developed, with invasomes emerging as a promising solution for enhanced transdermal drug delivery. Invasomes are lipid-based vesicles, often composed of phospholipids and edge activators such as surfactants, designed to enhance the delivery of active compounds through the skin. These carriers can improve the skin penetration, bioavailability, and sustained release of drugs, making them particularly suitable for dermatological applications (Liu et al., 2018). In the case of Tolnaftate, encapsulating the drug into invasomes could lead to enhanced antifungal activity by providing a more controlled and effective release profile directly at the site of infection.

Previous studies have demonstrated the potential of invasomes in improving the topical delivery of various antifungal agents. For example, Javadzadeh et al. (2015) showed that the encapsulation of drugs in lipid-based systems, such as invasomes, significantly enhanced the skin permeability and therapeutic efficacy of antifungal treatments. Similarly, Meena et al. (2019) emphasized that invasomal formulations could increase the bioavailability and effectiveness of Tolnaftate, thus offering a promising strategy to treat superficial fungal infections with better patient compliance (Meena et al., 2019).

Additionally, Khare et al. (2020) confirmed that lipid-based vesicular systems like invasomes enhance the transdermal delivery of antifungal drugs, resulting in improved treatment outcomes, especially for localized infections. The ability of invasomes to bypass the skin's stratum corneum, while providing sustained drug release, positions them as an ideal formulation approach for topical antifungal agents like Tolnaftate (Khare et al., 2020).

Given these advancements, the primary objective of this study is to formulate Tolnaftate-loaded invasomes and evaluate their drug release, skin penetration, and antifungal activity. This research aims to optimize the formulation of Tolnaftate invasomes, ultimately improving the treatment efficacy for superficial fungal infections by providing a more effective and patient-compliant delivery system.

MATERIAL AND METHODS:

Material

The chemicals used in the formulation of Tolnaftate-loaded invasomes include Tolnaftate (Pharmaceutical Company), Phosphatidylcholines (Thomas Baker, Mumbai), and various reagents like Disodium hydrogen phosphate**,** Di potassium hydrogen orthophosphate**,** and Sodium chloride (all sourced from S. D. Fine Chem. Ltd., Mumbai). Solvents like Methanol**,** Ethanol**,** and Chloroform are procured from Qualigens Fine Chemicals, Mumbai. Additionally, Carbopol 934P**,** Methyl paraben**,** Propyl paraben, and Propylene glycol (all from S. D. Fine Chem. Ltd., Mumbai) are used for the preparation and stabilization of the invasomal formulations.

Methods

Formulation Optimization of Tolnaftate loaded Invasomes

Tolnaftate 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 (Dragicevic-Curic *et al.*, 2009; Dragicevic-Curic *et al.*, 2010). 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 Tolnaftate loaded Invasomes

Ingredient (%)	F1	F2	F3	F4	F5	F6
Tolnaftate (mg)	50	50	50	50	50	50
Phosphotidylcholine (%)	0.50	0.75	1.00	0.50	0.75	1.00
Terpenes (%)	0.25	0.25	0.50	0.50	0.75	0.75
Ethanol (ml)	10	10	10	10	10	10

6.6 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 (Badran *et al.*, 2009). 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 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 invasomes loaded gel

Ingredient (%)	IG-1	IG-2	IG-3
Drug (Invasomes equivalent to 1%)	1	1	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 Tolnaftate Invasomes formulation was determined using centrifugation method (Haag *et al.*, 2011). 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 256 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 (Dragicevic-Curic *et al.*, 2008). 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.

$$AverageDiameter = \frac{\Sigma n.d}{\Sigma \, n}$$

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 (Dragicevic-Curic *et al.*, 2011).

Drug content

Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol (Ayman *et al.*, 2001). 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} 256 nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load (Kalpana and Lakshmi, 2013). 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.

Spreadability

Spreadability 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.,* (1956). 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.

$$Spreadibility(g.cm/sec) = \frac{WeighttidetoUpperSlide \times Lenthmovedontheglassslide}{Timetakentoslide}$$

***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 (Aqil *et al.,* 2007). 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 256 nm.

RESULTS AND DISCUSSION:

The results of the formulation and characterization of Tolnaftate-loaded invasomes provide valuable insights into their potential for enhanced transdermal delivery in the treatment of fungal infections. The entrapment efficiency and average vesicle size of different formulations, as shown in Table 3**,** demonstrate promising results for optimizing the delivery of Tolnaftate. Among the formulations, F4 showed the highest entrapment efficiency of 74.45% and the smallest average vesicle size of 215.65 nm**,** indicating a successful formulation with better drug encapsulation and a desirable size for skin penetration.

The zeta potential of optimized formulation F4**,** as shown in Figure 1**,** is -38.45 mV, which is indicative of good physical stability. A negative zeta potential helps to prevent aggregation of the vesicles and enhances their stability over time, ensuring that the drug remains effectively encapsulated in the delivery system. These findings are consistent with the work of Meena et al. (2019), who highlighted the importance of zeta potential in maintaining the stability of liposomal formulations.

In terms of the gel-based formulations (Table 5), formulations IG-1**,** IG-2**,** and IG-3 were evaluated for their viscosity**,** pH**,** drug content**,** extrudability, and spreadability. The optimized formulation IG-2 exhibited a viscosity of 3150 ± 12 cps, a pH of 6.68 ± 0.18, and a drug content of 99.45 ± 0.16%**,** which are within the acceptable range for topical formulations. Moreover, **IG-2** demonstrated excellent extrudability (168 ± 3 g) and spreadability (11.45 ± 0.31 g·cm/sec), ensuring that the gel can be easily applied to the skin, providing effective and uniform drug delivery.

The in vitro drug release data presented in Table 6 shows that the optimized gel formulation IG-2 released 88.45% of Tolnaftate within 10 hours, and nearly 99% by 12 hours, indicating sustained release and effective therapeutic action over a prolonged period. This sustained release profile is desirable for reducing the frequency of application while maintaining effective drug concentrations at the site of infection.

Further, the regression analysis (Table 7) of the drug release data indicates that the **release** mechanism of formulation IG-2 best fits the Zero Order model (R^2 = 0.987) and Korsmeyer-Peppas model (R^2 = 0.977)**,** suggesting a controlled and sustained release of Tolnaftate from the gel. This is indicative of a zero-order release mechanism, which is ideal for dermatological formulations as it ensures a consistent drug release over time, improving therapeutic efficacy.

The results suggest that the Tolnaftate-loaded invasomes and the optimized gel formulation IG-2 provide a promising approach for effective topical treatment of fungal infections. The formulations show excellent drug encapsulation, skin penetration, stability, and sustained release, offering a potential improvement over conventional topical treatments. Further clinical studies are needed to confirm the efficacy and safety of these formulations in real-world applications.

Table 3: Entrapment efficiency and average vesicle size of Tolnaftate Invasomes

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F1	65.58±0.25	274.45±0.22
F2	69.98±0.32	265.58±0.15
F3	63.32±.016	247.85±0.32
F4	74.45±0.26	215.65±0.15
F5	69.98±0.32	239.98±0.22
F6	70.23±0.15	249.98±0.32

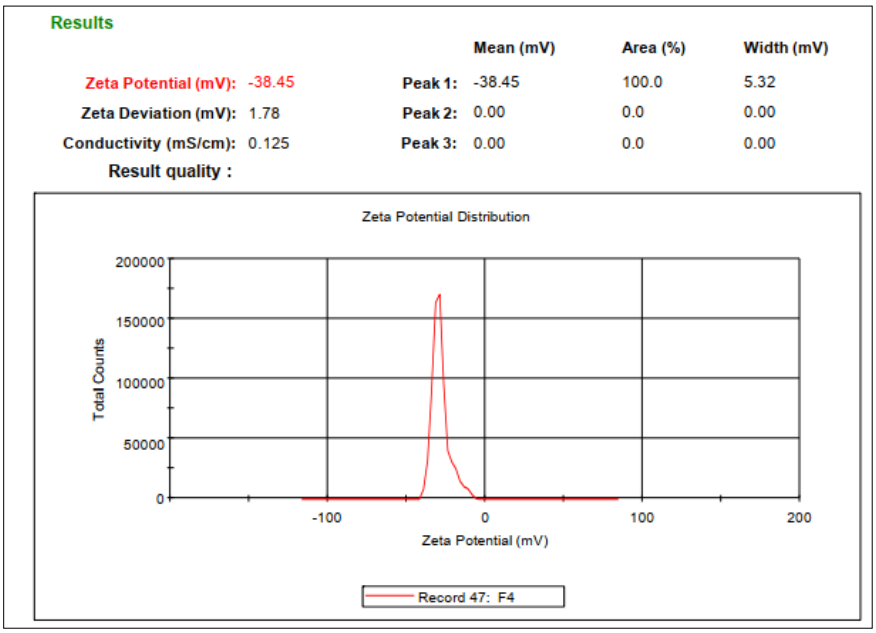

Figure 1: Graph of zeta Potential (mV) optimized formulation F-4

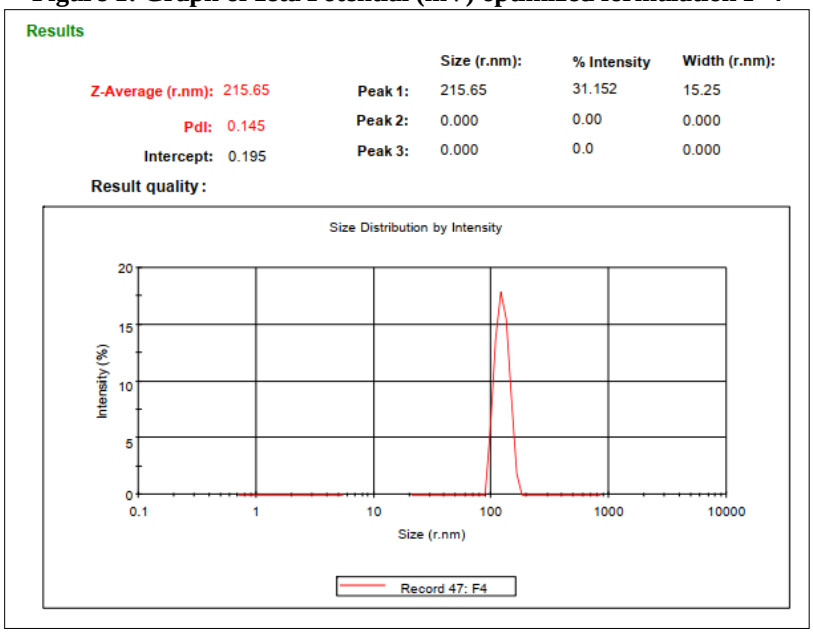

Figure 2: Graph of average vesicle size (nm) of optimized formulation F-4

Table 4: Characterization of optimized formulation of invasomes

Formulation	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-4	215.65 ±0.15	74.45±0.26	-38.45

Table 5: Characterization of gel based formulation of Invasomes

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
IG-1	3245±15	6.85±0.25	97.74±0.45	148±6	12.32±0.15
IG-2	3150±12	6.68±0.18	99.45±0.16	168±3	11.45±0.31
IG-3	3036±14	6.35±0.32	96.65±0.32	176±7	10.23±0.22

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

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	11.25±0.25
2	1	19.98±0.32
3	2	29.95±0.14
4	4	36.65±0.15
5	6	49.98±00.36
6	8	68.85±0.24
7	10	88.45±0.15
8	12	98.78±0.11

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	0.987	0.783	0.952	0.977

CONCLUSION:

In conclusion, the Tolnaftate-loaded invasomes and their gel formulation IG-2 offer a novel, efficient, and controlled delivery system that significantly improves the treatment outcomes of topical antifungal therapy. These formulations could provide an enhanced therapeutic approach, increasing efficacy, reducing application frequency, and potentially improving patient compliance.

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