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DEVELOPMENT AND CHARACTERIZATION OF NOVEL CARRIER FOR DRUG DELIVERY OF ANTIFUNGAL DRUG

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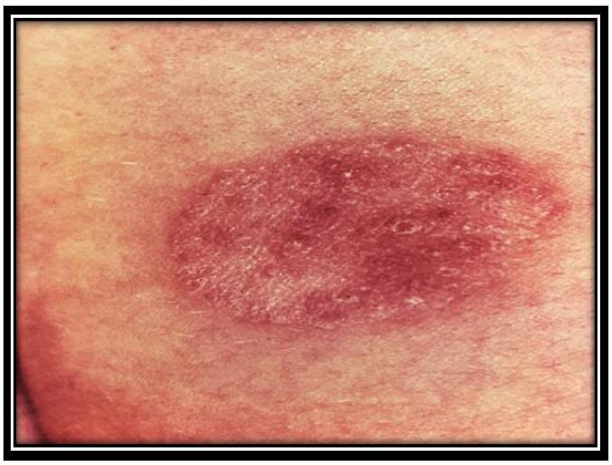
ABSTRACT
Fungal infections are the most common diseases found in tropical countries like India, but these infections are neglected and hence spread to other parts of the body. The most commonly used antifungal agents are Miconazole, these molecules being imidazole derivatives with high lipophilicity. It act against most pathogenic fungi and some Gram- positive bacteria. Usually they are well tolerated and their low toxicity allows them to be safely used for treating several cutaneous or systemic infections. However, anaphylactic reactions and cardio respiratory toxicity of Miconazole, which have been fatal in several cases, have led to premature cessation of therapy in some cases. Similarly, the bioactivity of Miconazole is found to be lower than the Ketoconzole. Encouraging results have been obtained on the treatment of topical fungal infections with liposomal or novel vesicular formulations of these antifungal drugs, but due to their size and rigid lipid bilayer they were not able to penetrate efficiently across the skin layer. However, ethosomes with high alcohol content are capable of enhancing penetration into deep tissues and systemic circulation. After nearly ten years of extensive research, ethosomes have proved to be good delivery carriers in transdermal field and their penetration enhancement capability has been widely accepted. The Transfersome preparations containing Miconazole are conceptually sophisticated; they are characterized by simplicity in their preparation, good stability, safety and efficacy- a combination that can highly expand their application in the treatment of fungal infections.

KEYWORDS: Fungal infection, Miconazole, Transfersome, Drug delivery system, Antifungal drugs.

Fungal infections
Having entered the thousand years with extraordinary expectations, conveying some terrific achievements of the past, humankind deals with difficult issues and difficulties revolved around developing parasitic, bacterial and viral diseases. Amazing accomplishments in drug, clinical sciences and nanotechnology have driven the analysts across the globe put unremitting endeavors to check exceptional ascent in these infections.[1] We currently have a much more clear comprehension of how medications are assimilated into, appropriated inside and cleared from the body. Most regular dose structures discharge the medication at first at a quicker rate, along these lines prompting a speedy ascent in the blood level of the medication and afterward falls dramatically until a further portion is administered.[2] It has been seen that individuals will in general disregard the contagious contaminations enormously and lion's share doesn't think about it as a sickness and like to go for flighty treatments.[3]

Abnormally, parasitic contaminations on one piece of the body can cause rashes on different pieces of the body that are not tainted. For instance, a contagious contamination on the foot may cause an irritated, rough ill-advised on the fingers. These ejections (dermatophytids or id responses) are hypersensitive responses to the parasite. They don't come about because of contacting the tainted territory.[4,5]



Human skin

One of the significant regions of interest is in the field of treatment. The interest in this has prompted work on the improvements of novel particles, conveyance frameworks just as plans which can handle these parasitic contaminations to a more noteworthy degree than previously. The vast majority of the parasitic diseases are show up over the skin, the treatment routine of these contaminations consistently includes outer application details, for example, creams, salves, moisturizers etc.[6]

The skin has three layers—the epidermis, dermis, and fat layer (likewise called the subcutaneous layer). Each layer performs explicit undertakings. Given its part as the defensive shell for the inside organs and design, the skin supports consistent contact and thusly, it is the most harmed human organ.[7]

Epidermis

The epidermis is the moderately slight, intense, external layer of the skin. The vast majority of the cells in the epidermis are keratinocytes. They start from cells in the most profound layer of the epidermis called the basal layer. New keratinocytes gradually move up toward the outside of the epidermis. When the keratinocytes arrive at the skin surface, they are bit by bit shed and are supplanted by more youthful cells pushed up from beneath.[8]

Dermis

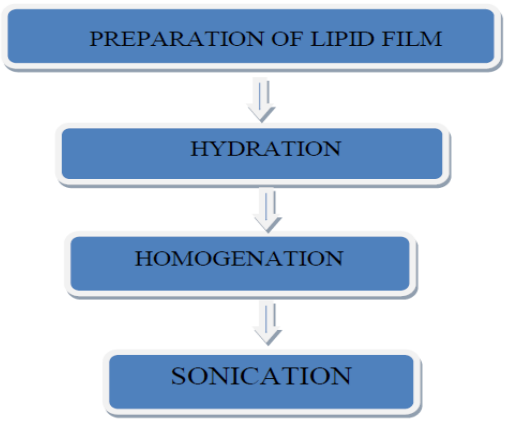
The dermis, the skin's next layer, is a thick layer of sinewy and versatile tissue (made generally of collagen, elastin, and fibrillin) that gives the skin its adaptability and strength. The dermis contains sensitive spots, sweat organs and oil organs, hair follicles, and veins. The sensitive spots sense torment, contact, pressing factor, and temperature. A few zones of the skin contain more sensitive spots than others. For instance, the fingertips and toes contain numerous nerves and are incredibly delicate to contact.[9]

Fat layer

Beneath the dermis lies a layer of fat that protects the body from warmth and cold, gives defensive cushioning, and fills in as an energy stockpiling region. The fat is contained in living cells, called fat cells, held together by sinewy tissue. The fat layer differs in thickness, from a small amount of an inch on the eyelids to a few creeps on the midsection and bottom in some people.[10]

Trasferosome

The term transfersome and the hidden idea were presented in 1991 by Gregor Cevc. In broadest sense, a transfersome is an exceptionally versatile and stressresponsive, complex total. Its favored structure is a ultradeformable vesicle having a watery center encircled by the intricate lipid bilayer. Interdependency of neighborhood organization and state of the bilayer makes the vesicle both automatic and selfoptimising. This empowers the Transfersome to cross different vehicle hindrances effectively, and afterward go about as a Drug transporter for non-intrusive focused on medication conveyance and supported arrival of remedial specialists Delivery through the transdermal course is a fascinating choice with regards to this regard on the grounds that a transdermal course is helpful and safe.[11]



Flow diagram for preparation of transfersome

Materials used

Phospholipids	Ozone Chemicals Mumbai.
Distilled Alcohol	Changshu Yangyuan Chemical China.
Phosphate Buffer	Ranchem Laboratory Chemicals Pvt. Ltd
Chennai Trioton X-100	Ozone Chemicals, MUMBAI.
Propylene Glycol	Ozone Chemicals, Mumbai.
Soya Phosphatidyl Choline	Vesicles Forming Component
Tween 80	For Providing Flexibility
Methanol & Chloroform	As A Vehicle
Saline Phosphate Buffer (Ph 6.5)	As A Hydrating Medium

Size and Shape analysis

The mean size was calculated by microscopy. In order to observe individual vesicles, a slice of Transfersome was diluted correctly with purified water and a drop of diluted suspension was analyzed under a microscope (magnification 15 x 45 X) using a matched eyepiece micrometer with a stage micrometer. The 150 vesicle diameters were calculated arbitrarily. Using formula101, the average diameter was determined.[12,13,14,15]

Average diameter (d_{ave}) $\frac{\sum nd}{\sum n}$

n = number of vesicles

d = diameter of the vesicles

Drug entrapment studies (% Entrapment efficiency studies)

Ultracentrifugation was used to assess the entrapment potential of drugs (MICO) into transfersome vesicles. 1 ml of 1 percent triton X-100 solution was combined with 10 ml of Transfersome formulation. For 2 periods of 5 minutes, each sample was vortexed, with 2 minutes of rest between the cycles. In various centrifugal tubes, 1.5ml of each vortexed sample and fresh untreated TRANSFERSOME formulations were brought in. Ses samples were centrifuged for 3 hours at 20,000 rpm. The supernatant coating was isolated, suitably diluted with water, and drug concentration in both vortexed and unvortexed samples was estimated at 260.2 and 222.4nm respectively.[16,17,18,19]

The effectiveness of the trap was determined as follows:

$$\text{Entrapment Efficiency} = \frac{t-c}{t} \text{ X } 100$$

'T' is total amount of drug detected from supernatant layer of vortexed sample.

'C' is the amount of drug unentrapped and detected from supernatant layer of unvortexed sample.

Stability studies

A stability analysis was performed at two different temperatures, i.e. cooling temperature (4 ± 3 °C) and at room temperature (30 ± 3 °C) for 8 weeks, for Transfersome formulations. The formulations submitted for the stability analysis were placed in a borosilicate jar in order to prevent some kind of contact between the preparation of the Transfersome and the container glass that could influence the observations. For any physical modifications such as colour and shape, the Transfersome formulations were analysed. Changes in vesicular size and shape, trapping ability and drug quality were other studies conducted.[20,21,22]

Vesicular Shape and Size during stability studies

Under the microscope, samples of Transfersome formulations were oriented for an interval of two weeks to consider any alteration in shape and scale as previously stated under the characterization heading.

Preparation of transfersomes containing miconazole

Three different independent variables were used which include: Phospholipids, Methanol, Tween 80, Drug;. The independent variables were screened using a multilevel factorial design and three different formulations of Miconazole Transfersomes were obtained, as represented in Table below. All formulations were prepared using the thin lipid film hydration technique and then evaluated for entrapment efficiency, particle size. Transfersome carriers are a device consisting primarily of phospholipids, comparatively high amounts of methanol and water comprising soft vesicles. After optimizing process and formulation variables, Transfersome were prepeared.

At 2 percent concentration, the transfersome vesicles contained Miconazole. The transfersome phospholipid concentration was set at 2.5 percent. The concentration of Methanol ranged from 25-45 percent. The formulation contained Tween80 15 percent concentration. The compositions of the various transferosomal formulations prepared in this analysis are listed in the table below.[22,23,24]

Composition of different transfersome formulations MICO.

Formulation Code	%Phospholipids	%Methanol	%TWEEN 80	%Drug
FEt1	2.5	25	15	2.5
FEt2	2.5	35	15	2.5
FEt3	2.5	45	15	2.5

The collected transfersomal suspensions were mildly brownish in color and creamy in appearance. Different characteristics of Transfersomes and the influence of different methanol concentrations have been further assessed and findings have been recorded. Miconazole optimized transfersomal vesicles were formulated with a basic sonication process.[25]

Characterization

Since the physical characterization is supposed to clarify the physical integrity of the shape of the dosage, the data were pooled at one venue. The effects of prepared transfersomal formulations and their similarities have been addressed under the same heading.[26,27]

Size and Shape analysis

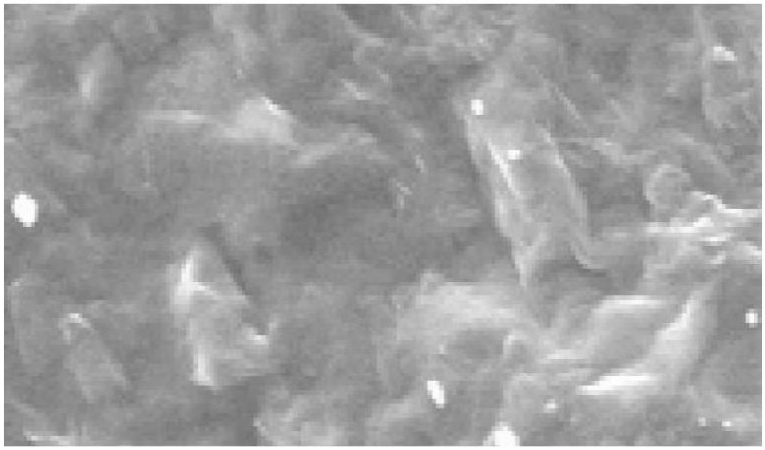
The miconazole transfersomal plan fet1 with 25% methanol and 2.5% phospholipids was discovered to be inside the size scope of 0-18.120 µm and the normal width was discovered to be 4.575 µm[28,29]

Size distribution of miconazole transfersomes of formulation FET1

S. no	Size range	Average size (d)	No. of vesicles (n)	% vesicles	(n) x (d)
1.	0.0-3.5	1.6	45	31.0	72
2.	3.5-6.5	4.7	55	35.0	258.85
3.	6.5-9.5	8.0	25	15.0	200
4.	9.5-12.5	11.0	05	3.0	55

5.	12.5-15.5	9.5	02	1	19

Average diameter (d_{ave}) = $\frac{\sum nd}{\sum n} = \frac{604}{132}$ = 4.575 µm



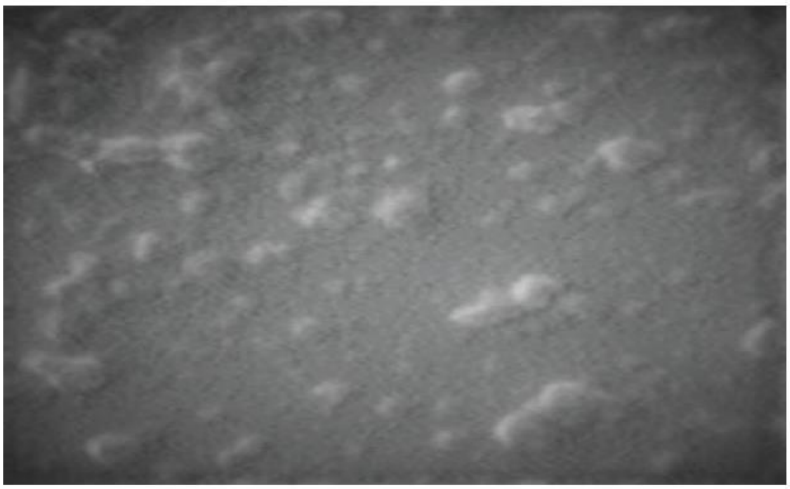
Photographic view of miconazole transfersomes of formulation fet1

The transfersomal formula FET2 of Miconazole had a vesicle size range of 0-16 µm with 35 percent methanol concentration and 2.5 percent phospholipids. The average vesicle diameter was estimated to be 4.240 µm.[30,31]

Size distribution of miconazole transfersomes of formulation FET2

S. no.	Size range	Average size (d)	No.of vesicles (n)*	% vesicles	(n) x (d)
1.	0.0-3.5	1.6	60	35	96
2.	3.5-6.5	4.7	70	50	329
3.	6.5-9.5	8.0	20	12	160
4.	9.5-12.5	11.0	06	2	66
5.	12.5-15.5	9.5	02	1	19

Average diameter (dave) = $\frac{\sum nd}{\sum n} = \frac{670}{158}$ = 4.240 µm



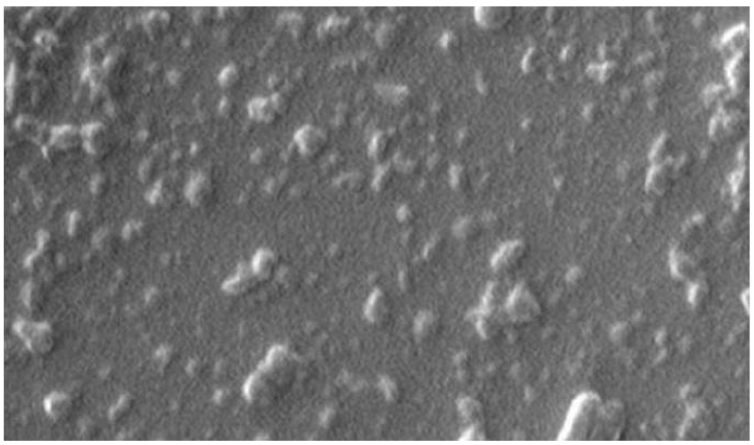
Photographic view of miconazole transfersomes of formulation FET2

With 45 percent methanol concentration and 2.5 percent phospholipids, the Miconazole transferosomal formula FET3 had a vesicle size range of 0-16 µm. The mean vesicle diameter was estimated to be 3.216 µmm.[32,33]

Table 5.5. Size distribution of Miconazole transfersomes of formulation FET3.

S.no	Size range	Average size (d)	No.of vesicles (n)*	% vesicles	(n) x (d)
1.	0.0-3.5	1.6	90	35	144
2.	3.5-6.5	4.7	45	50	211.5
3.	6.5-9.5	8.0	07	12	56
4.	9.5-12.5	11.0	05	2	55
5.	12.5-15.5	9.5	01	1	9.5

Average diameter (dave) = $\frac{\sum nd}{\sum n} = \frac{476}{148}$ = 3.216 µm



Photographic view of miconazole transfersomes of formulation FET3

The effect of methanol concentration on vesicle size was demonstrated by the findings obtained by vesicular size analysis. The effect of the concentration of methanol on micnazole-containing transfersomes is observed to be similar. As the methanol concentration rose, the size of transfersomes vesicles decreased[34,35] with the largest vesicle size of 4,575 µm, with 25 percent methanol and the smallest 3,216 µm, with 45 percent methanol for transfersomes based on miconazole. As per the results, there was a decrease in transfersomes average vesicular size with a rise in methanol concentration from 25% to 45%. But methanol concentration above 50 percent, along with loss of lamilarity, can increase vesicular diameter. The number of research groups observed this kind of phenomena and examined it in their scientific literature.[36,37,38]

Entrapment efficiency

Size and efficacy of trapping are often known as criteria for optimizing vesicular formulations. After the existence of vesicle bilayers in the transfersomes system has been verified, the capacity of vesicles to capture drugs has been studied using the process of ultracentrifugation. To evaluate the trapment quality, ethosomal vesicles containing drugs and un-trapped or free drugs were segregated using the ultracentrifugation process. The findings obtained are given in the table.[39,40]

Table: Entrapment efficiency.

S. no.	Name of formulation	Entrapment efficiency %
1.	FET1	49.06 ± 0.5
2.	FET2	55.51±0.5
3.	FET3	45.51±0.5

In vitro skin permeation studies

In-vitro skin saturation study directed on FET3 was demonstrating less noteworthy reports contrasted with FET1 and FET2. It required over 1 hour to start the arrival of Miconazole from FET3. Following 6 hours of dispersion, the rate drug discharge from FET3 was under 15%. Following 12 hours of dispersion study, over 73% of medication was discovered to be unreleased from the FET3 detailing. At the point when rate drug discharge was determined after 24th, 48th and 60th long periods of dispersion, it was 34.06±0.05%, 43.00 ±0.05% and 47.63±0.05% individually for MET3. At the point when dissemination study was finished following 72 hours just minimal over half Miconazole was delivered and almost half was discovered to be unreleased from MET3 containing 45% methanol focus.

Table: Percentage drug release.

Time	Dilution factor	% drug released	%drug unreleased
1 hour	10	100± 0.10	0.00 ± 0.10
06 hour	10	87± 0.10	13± 0.10
12 hour	10	68.9± 0.10	31.1± 0.10
24 hour	10	59.8± 0.10	40.2± 0.10
48 hour	10	49.6± 0.10	50.4± 0.10
60 hour	10	45.5± 0.10	54.5± 0.10
70 hour	10	42.9± 0.10	57.1± 0.10

Stability studies

All administrative bodies acknowledge just ongoing information for any medication or drug to evaluate the timeframe of realistic usability and quickened strength studies may just fill in as an instrument for plan screening and security issues identified with delivery or capacity at room temperature. The quickened soundness examines were done as per the ICH rules. The capacity of vesicles to hold the medication was evaluated by keeping the transfersome suspension at various temperature. Streamlined transfersome details were chosen for solidness investigations of vesicles.

Misfortune in rate drug content was not over 5% incase of Miconazole based transfersome formulations. The most noteworthy Miconazole misfortune was seen at room temperature following two months when contrasted with refrigeration temperature. Like the discoveries of Miconazole definitions, FET2 with 35% methanol fixation indicated least rate misfortune in medication content out of other two plans FET1 and FET3. The rate misfortune in medication content found in FET2 was just 2%, 3.5% under refrigeration and room temperature. There was no critical misfortune seen in any of the plans containing Miconazole.The drug content in various definitions showing the steadiness of medication Miconazole even following two months.

CONCLUSION

The point of our examination was to assess the capability of novel vesicular transporter, transfersome containing antifungal specialists (Miconazole). The definitions were exposed to physicochemical, in vitro, ex vivo pervasion reads and tried for antimicrobial potential. The size, shape and surface morphology of definitions was contemplated utilizing Zeta sizer, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) strategies. The photomicrographs of plans Fet1, Fet2, Fet3 uncovered almost circular shape, smooth surface, discrete, separate with no accumulation and nanometer size range. Ensnarement effectiveness considered as a huge boundary for improving vesicular details was discovered to be most elevated at 35% methanol fixation. Further expansion in methanol fixation diminished the size and ensnarement efficiency. Studies demonstrated great medication maintenance in created vesicles in presence of ethanol. Steadiness considers were done on completely created definitions for a time of about two months. Results demonstrate that transfersome with 35% methanol focus were the most steady. The misfortune in rate drug content just as drop in entanglement productivity were insignificant for transfersome with 35% ethanol fixation. Methanol at ideal focus was found to apply a balancing out impact on the created definitions. The definitions which were kept at refrigeration and 30 + 2 °C were discovered to be steady. Study demonstrated the meaning of methanol focus on medication discharge from transfersomes. Transfersome with 35% methanol had prevalent medication discharge, which showed that 35% is the ideal fixation for creating ethosomal vesicles for antifungal specialists (***Miconazole***).

They can be put away at ordinary room temperature and have amazing solidness, wellbeing and viability profile. Further examination might be needed to investigate the extent of transfersomes innovation in medication conveyance.

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Conflict of interest

The authors declare no conflict of interest.

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