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Review Article

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Fluconazole Loaded Ethosomes Gel and Liposomes Gel: An Updated Review for the Treatment of Deep Fungal Skin Infection

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Abstract Skin acts as a major target as well as a principle barrier for topical drug delivery. The greatest challenge for dermal delivery is stratum corneum, and in order to improve its permeability, new formulation approaches have been investigated. The main requirement for topical anti-fungal treatment is that the drugs should penetrate into skin layers to ensure effective drug concentrations following topical administration. Types of the formulations as well as the physicochemical characteristics of drug molecules are effective parameters in topical delivery of drugs. Antifungal drugs should reach effective therapeutic levels in the viable epidermis after dermal administration. The drug of choice for topical fungal disease is Fluconazole. Fluconazole has a broad spectrum of activity that includes both dermatophytes and yeasts. The drug is particularly effective in the treatment of mucosal and cutaneous forms of candidiasis. Vesicular system (Liposomes and ethosomes) entrapped in Gel is one of the most promising approaches for topical delivery of active substances and has potential for new opportunities for topical application of Fluconazole in the fungal infections.

Keywords Deep Fungal Skin Infection, Fluconazole, Ethosome Gel, Liposome Gel

Introduction

The incidence of superficial fungal infections of skin, hair and nails has been increased 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 compromise with immune function [1-2]. Dermatophytes are one of the most frequent causes of tinea and onchomycosis. Candidal infections are also among the most widespread superficial cutaneous fungal infections [3]. Even, candida can invade deeper tissues as well as the blood which lead to life threating systemic candidiasis, when the immune system is weakened [4]. 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 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 the viable epidermis. Although topical drugs can provide an immediate reduction in infectivity, are free of systemic adverse effects and are relatively inexpensive. They have some disadvantages such as unable to reach the deep skin layer. In this context, the formulation may play a major role for

penetration of drugs into skin [5]. Development of alternative approaches for topical treatment of fungal infections of skin encompasses new carrier systems for approved and investigational compounds. Delivery of antifungal compounds into the skin can be enhanced with vesicular carriers such as Ethosomes and liposomes. These vesicular carriers are non-invasive delivery carriers that enable drugs to reach the deep skin layer. Liposome is an artificial microscopic single vesicle consisting of an aqueous core enclosed in one or more phospholipid layers used to convey vaccines, drugs, enzymes or other substances to target cells or organs. Ethosomes are ethanolic phospholipids vesicles, which have higher penetration rate through the skin than liposomes. Ethosome vesicles contain phospholipids and alcohol (in relatively in high concentration). The lipid vesicle has higher penetration rate, high efficiency or bio-availability. The physicochemical characteristics of vesicular carriers are such that these transport active substances more efficaciously through the stratum corneum into the deeper layers of the skin.

This review paper comprises of following:-

- Review on drug Fluconazole
- Review on Liposomes as drug delivery carrier
- Review on Ethosomes as drug delivery carrier
- Review on topical gels for incorporating Liposomes and Ethosomes
- Comparison between Liposomes and Ethosomes as topical drug delivery carrier

Review on Drug Fluconazole

Fluconazole is an oral synthetic bis-triazole compound that inhibits the cytochrome P450-dependent 14 alpha-demethylation step in the formation of ergosterol. This leads to alterations in a number of membrane-associated cell functions. Fluconazole has a broad spectrum of activity that includes both dermatophytes and yeasts. The drug is particularly effective in the treatment of mucosal and cutaneous forms of candidiasis. It is currently the drug of choice for controlling oropharyngeal candidiasis in AIDS patients [1-2].

Fluconazole remains one of the most frequent prescribed triazoles because of its excellent bioavailability, tolerability and side-effect profile. More than 80 % of an ingested drug is found in the circulation, and 60 to 70% is excreted in the urine. Only 10% of fluconazole is protein bound. Fluconazole also exhibits excellent tissue penetration. CSF levels are 70% of matched serum levels, and levels reported in saliva, sputum, and other sites are well within therapeutic ranges. The half-life is 27 to 34 h in the presence of normal renal function allowing once-daily dosing. In patients who have a reduced creatinine clearance the normal dose should be reduced by 50%. Fluconazole serum levels are rarely necessary. Currently 50, 100, 150, and 200 mg tablets are available and IV formulation exits in 200 or 400 mg doses [5-16].

Available dosage forms

- Tablets
- Capsule

Numerous dosage forms are used in the topical treatment of superficial fungal infections, including creams, liquids, gels, ointments, lacquers and others. The treatment of athlete's foot and ringworm can easily be accomplished with creams, liquids, gels and ointments.

Side effects

When fluconazole overcomes side effects of other antifungal agents, it also has some side effects in the oral and parenteral dosage forms as pass through the 1st pass metabolism through the liver and excretion through kidneys.

- Headache
- Diarrhea
- Nausea
- Dizziness
- Stomach pain
- Change in the way food tastes.
- Liver and Kidney damage.
- The most common side effects of fluconazole are headache, nausea and pain in the abdomen.

• A few people get diarrhoea, most anti-HIV medications cause problems in the digestive system. Fluconazole could make those problems worse.
• Fluconazole can be hard on the liver.
• Fluconazole can also cause kidney damage.

Due to these side effects of tablet dosage of fluconazole drug, the vesical gel dosage form was formulated [7-8]

Review on Liposomes as Drug Delivery Carrier

Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shell(s) consisting of lipids arranged in a bilayer configuration. The potential use of liposomes as drug carriers was recognized more than 25 years ago and, since that time, liposomes have been used in a broad range of pharmaceutical applications [10].

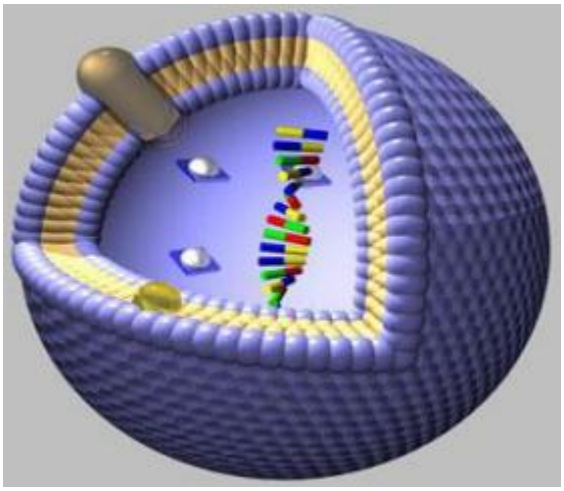

Figure 1: *Structure of a liposome*

They present many advantages since they can be used as carriers for both hydrophilic and lipophilic molecules, as well as drug delivery systems for controlled drug delivery for different therapeutical purposes. An important aspect of liposomes is the protection that they afford as an encapsulating agent against potentially damaging conditions in external environments. Liposomes are also an important system in their own right in medical, cosmetic, and industrial applications. Liposomes can substantially improve drug loading, drug delivery and sustained release, thereby offering clear-cut advantages over traditional dosage forms. Liposomes were first produced in England in 1961 by Alec D. Bangham, who was studying phospholipids and blood clotting. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule was water soluble, while the opposite end is water insoluble. Water-soluble medications added to the water were trapped inside the aggregation of the hydrophobic ends; fat-soluble medications were incorporated into the phospholipid layer [17-21].

Topical drug delivery is an attractive route for local and systemic treatment. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. It also has affinity to keratin of horny layer of skin and can penetrate deeper into skin and hence give better absorption. In the formulation of topical dosage forms, attempts are being made to utilize drug carriers that ensure adequate localization or penetration of drug within or through the skin in order to enhance the local and minimize the systemic effects or to ensure adequate percutaneous absorption. Applied on the skin, liposomes may act as a solubilizing matrix for poorly soluble drugs, penetration enhancer as well as local depot at the same time diminishing the side effects of these drugs. Topical liposome formulations could be more effective and less toxic than conventional formulations. Skin has been considered as a promising route for the administration of drugs because of its accessibility and large surface area [12].

Transdermal drug delivery system, designed to deliver a variety of drugs to the body through diffusion across the skin layers, is appealing for several reasons including avoidance of the variable absorption and metabolic breakdown associated with oral treatments, drug administration can be continuous, and minimal intestinal irritation can be avoided. Liposome is has been used in transdermal drug delivery system because of its much higher diffusivity in

skin compared to most bare drugs. Liposomal formulations are widely used in the pharmaceutical field as drug delivery systems due to their versatility and clinical efficacy and they have been used to administer drugs by several routes such as the oral, parenteral and topical. Among these, topical delivery of drugs carried by liposomes exhibits interesting applications, not only for promoting dermal delivery of drugs which have to act topically, such as local anesthetics, but also for enhancing transdermal delivery of drugs intended for systemic use, thus more effectively exploiting this non-invasive alternative route to oral administration. Due to the aforementioned advantages, in this study liquid-state liposomes were chosen to serve as the drug delivery system [22-23].

Classification of liposomes based on structural parameters

1. Multilamellar Large vesicles > 0.5um
2. Oligolamellar vesicles 0.1-1um
3. Unilamellar vesicles (All size range)

a) Small unilamellar vesicles 20-100nm
b) Medium sized unilamellar vesicles
c) Large unilamellar vesicles >100nm
d) Giant unilamellar vesicles >1um

4. Multivesicular vesicles >1um [24-25]

Advantages

1. Precipitation at the injection site and in the blood circulation can be prevented.
2. Phospholipids are one of the few solubilizers that are well tolerated intravenously.
3. Provide selective passive targeting to tumour tissues.
4. Increase safety and therapeutic index.
5. Increase stability via encapsulation
6. Site avoidance effect [20, 21, 26].

Methods of preparation of liposomes

A) Multilamellar Liposomes (MLV)

1. Lipid hydration method

This is the most widely used method for the preparation of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities. MLV are simple to prepare by this method and a variety of substances can be capsulated in these liposomes. The drawbacks of the method are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous.

2. Solvent Spherule Method

It is a method for the preparation of MLVs of homogeneous size distribution. The process involves dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath.

B) Small Unilamellar Liposomes (SUV)

1. Sanitation Method

Here MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere. The main drawbacks of this method are very low internal volume/encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV along with SUV.

2. French Pressure Cell Method

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are somewhat larger than sonicated SUVs.

The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum).

C) Large Unilamellar Liposomes (LUV)

They have high internal volume/encapsulation efficiency and are nowadays being used for the encapsulation of drugs and macromolecules.

1. Solvent Injection Methods

a) Ether Infusion Method

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65 °C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated by organic solvents or high temperature.

b) Ethanol Injection Method

A lipid solution of ethanol is rapidly injected by a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol.

c) Reverse Phase Evaporation Method

First water in oil emulsion is formed by brief summation of a two phase systems containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. Liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure.

With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication.

These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands. We get a heterogeneous sized dispersion of vesicles by this method. Modified Reverse Phase Evaporation Method was presented and the main advantage of the method is that the liposomes had high encapsulation efficiency (about 80%). The Reverse Phase Evaporation has also been modified to entrap plasmids without damaging DNA strands.

d) Calcium-Induced Fusion Method

This method is used to prepare LUV from acidic phospholipids. The procedure is based on the observation that calcium addition to SUV induces fusion and results in the formation of multilamellar structures in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs. The main advantage of this method is that macromolecules can be encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, although of a heterogeneous size range. The chief disadvantage of this method is that LUVs can only be obtained from acidic phospholipids.

e) Freeze-Thaw Method

SUVs are rapidly frozen and followed by slow thawing. The brief sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is due to the fusion of SUV during the processes of freezing and/ or thawing. This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipid concentration. The encapsulation efficiencies from 20 to 30% were obtained [26-28].

Application of Liposomes

Liposome has its own value in different fields. Liposomes are mostly used for drug delivery to the target gene. Liposomes are also used in mathematics for topology in two dimensional surfaces in three dimensional surface governed only by bi-lipid elasticity. In biophysics liposomes are used for checking of the aggregation behaviour and fractal strength of materials. Liposomes are also used in chemistry for micro compartilization. In pharmaceuticals it is used for studies of drug actions.

Liposomes as drug delivery vehicles in medicine, adjuvants in vaccination, signal enhancers/carriers in medical diagnostics and analytical biochemistry, solubilizers for various ingredients as well as a support matrix for various ingredients and penetration enhancer in cosmetics. Liposomes containing membrane anchored chelators can be used to clean toxic or radioactive metals from solutions. Liposomes increase the efficiency, bioavailability, absorption of certain entrapped dietary and nutritional supplements and are used as topical drug delivery system. But liposomal drug delivery system has certain shortcomings like the need for modification for site specific or organ specific drug delivery, high production cost, leakage and fusion of encapsulated drug/molecule. Moreover unfavorable reactions like oxidation and hydrolysis of phospholipids reduce the half life of the formulation, decreases solubility and stability of drug in the medium [24-25, 27-28].

Review on Ethosomes as Drug Delivery Carrier

Another specially designed vesicle able to allow transdermal delivery is ethosomes, which are non-invasive delivery carriers that allow the drug to reach the deeper skin layers and possibly the systemic circulation. They are a soft, malleable vesicles, which are actively, enhances the delivery of entrapped drugs.

Ethosomes are composed mainly of phospholipids (e.g. phosphatidylcholine, phosphatidylserine, phosphatitic acid), high concentration of ethanol (20-45 %) and water. Toronto (2002) observed that economies might enhance delivery of drug to the deep strata of the skin or to the systemic circulation. Due to ethical high malleability, it may permeate through human skin and into the systemic blood circulation [4, 11].

Mechanism of permeation enhancement using ethosomes

As described previously by Touitou (2002), the exact mode of permeation enhancement using episodes is unclear. However, it is believed that the higher concentration of ethanol in epitomes disrupts the lipid bilayer in the SC making it more permeable and thus giving the epitomes a higher ability to squeeze through the small opening in the SC lipid Substances that reversibly reduce the barrier resistance of the SC, such as ethanol are commonly described as permeation enhancers. Ethanol can interact with the polar head group of the lipid molecules, resulting in reduction of the melting point of the stratum corneum lipid, thereby increasing lipid fluidity and cell membrane permeability. However, due to the interdigitation ethanol causes to the lipid bilayers, it was commonly believed that vesicles could not coexist with high concentrations of ethanol. Currently, ethanol might be used in relatively low concentrations in liposome formulations. Ethanol is a volatile solvent which can be easily evaporated at skin temperature, leaving the supersaturated concentration of the drug in formulation. This may influence the drug flux across the membrane. Also, ethanol may alter the solubility of the SC, facilitating drug delivery [14-15, 17-18].

In current studies, ethanol is found in low concentration in lysosome formulations; 7-10% for transfersomes. However, higher ethanol concentrations are used in proniosome formulations (30-50%). Figure 2 shows how ethosomes may enhance penetration of drugs through lipid bilayers.

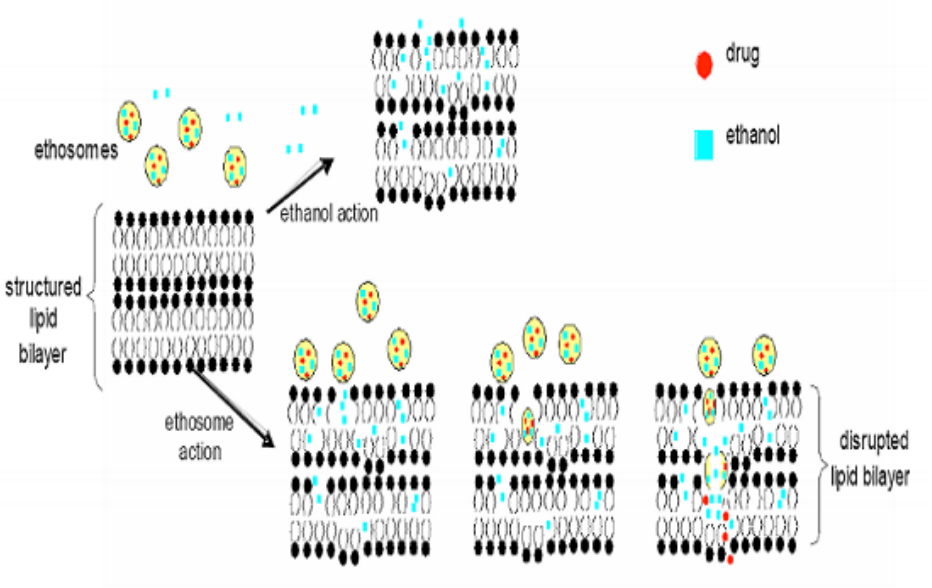

Figure 2: *Diagrams showing the mode of drug penetration through lipid bilayers using ethosomes Composition and preparation of ethosomes [22-24]*

Ethosomes are vesicular carriers composed of hydro-alcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohol(s) is relatively very high.

Ethosomes can be prepared using the conventional thin film hydration technique or by addition of aqueous phase in a controlled manner to the alcoholic solution of phosphatidylcholine.

A. Cold method: In this method, phospholipids, drugs and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Either Propylene glycol or other polyol are added while stirring. The mixture is heated to 30 ºC in a water bath. In a separate vessel the water is heated to 30ºC and added to the mixture followed by stirring for 5 mins. The size of the ethosomal formuation can be decreased using either by sonication or extrusion and the formulation is then finally stored in the refrigerator.

B. Hot method: In this method, phospholipids are dispersed in water by heating in a water bath (40 $^{\circ}$C) until a colloidal suspension is obtained. In a separate vessel, ethanol and propylene glycol are mixed and heated to 40 ºC and once it reaches the desire temperature the organic phase is added to the aqueous one. Depending on the drug hydrophilic/hydrophobic properties, it is either dissolved in water or ethanol solvent. The size of the ethosomal formulation can be decreased using probe sonication or extrusion.

Advantages of ethosomes

In comparison to complicated methods such as iontophoresis and phonophoresis, ethosomes may be more capable of delivering large molecules such as peptides and protein molecules. It offers low risk and high patient compliance when it is administrated as semi-solid formulation (gel or cream). Ethosomes also have applications in pharmaceutical, veterinary, and cosmetic fields [32].

Application of Ethosomes

Ethosomes are mainly used for delivery of drug through transdermal route. Advantages of ethosome such as enhanced permeation of drug molecules to and through the skin, better stability; better solubility of many drugs as compared to conventional vesicles, contrary to deformation liposomes improves skin delivery of drugs.

There are some common drugs administered by transdermal route like NSAIDS (Diclofenac), Acyclovir, Antibiotic, Cannabidol, Zidovudine, Ketoconazole. Ethosomal system is non-invasive, passive and available for immediate

commercialization. Ethosomes provide a large platform for drug delivery, its composition is safe and the components are approved for pharmaceutical and cosmetic use. Ethosomes are also used for pilosabeaceous targeting. Pilosabeacous is an epidermal invagination found on the most surfaces of human body and mostly composed of hair follicles and sebaceous glands. It has low risk profile [30-32].

Review on Topical Gels for Incorporating Liposomes and Ethosomes

Transdermal drug delivery, the practical application of these formulations onto the skin is less. However, these can be incorporated into the gels than can apply onto the skin. It has been found that liposomes and ethosomes incorporate into the gels are stable.

Hydrogels are clinically acceptable systems that offer many advantages, such as suitable rheological properties, good tissue compatibility and convenience in handling and ease of application. Carbopol gels are approved for pharmaceutical use in several different administration routes. Cutaneous use of these gels is advantageous as they possess good rheological properties resulting in long residue times at the site of administration and they provide higher and sustained skin concentrations of drugs compared to conventional gels and creams. Moreover, carbopol gels are anionic hydrogels with good buffering capacity, which may contribute to the maintenance of the desired pH [3, 33-34].

Comparison between Liposomes and Ethosomes as Topical Drug Delivery Carrier

Ethosomes are similar in their structure to liposomes only difference in composition. Liposomes composed of phosphatidylcholine and cholesterol whereas ethosomes are made of high concentration of ethanol. The value of ethosomes lies in their capability of increasing the transdermal permeation of the entrapped material compared to liposomes or drug solutions of mixture containing ethanol and water. Ethosome has its ability to permeate intact through the human skin due to its high deformability. The physicochemical characteristic of ethosome allows this vesicular carrier to transport active substances more efficaciously through the stratum corneum into the deeper layers of the skin than conventional liposomes.

Addition of ethanol in ethosomes can provide high flexibility of the vesicles to squeeze through the skin pores that are much smaller in diameter than the vesicles.

Thus, ethosomes are much more efficient in delivery of the drug in terms of quantity and depth when compared to conventional liposomes that tend to accumulate at the upper layer of the skin due to the stratum corneum barrier. In other research articles, it has been observed that due to the presence of ethanol, ethosomes provide good storage stability of the vesicles [13, 35-38].

Conclusion

Fungal infections of the skin are one of the often faced with dermatological diseases in worldwide. Topical therapy is an attractive choice for the treatment of the cutaneous infections due to its advantageous such as targeting of drugs to the site of infection and reduction of the risk of systemic side effects. Currently, antifungal drugs are generally used as conventional cream and gel preparations in topical treatment. The efficiency of that treatment depends on the penetration of drugs through the target layers of the skin at the effective concentrations. However, stratum corneum, the outermost layer of the skin, is an effective barrier for penetration of drugs into deeper layers of the skin. The physico-chemical characteristics of drug molecules and the types of the formulations are effective factors in topical drug delivery. Therefore, a number of formulation strategies such as use of liposomes and ethosomes entrapped in gels have been investigated for delivering antifungal compound Fluconazole through targeted site of the skin.

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