ARTICLE INFO

**Article History:**
Received on 11th July, 2018
Peer Reviewed on 26th July 2018
Revised on 13th August, 2018
Published on 27th August, 2018

**Keywords:**
Econazole nitrate, Liposomes, Topical gel, Carbopol 934

ABSTRACT
This project aimed to developing liposomes of econazole nitrate (EN) and formulating them as a suitable dermatological gel for improved therapeutic efficacy, better dispersity, and good storage stability. Liposomes were prepared by Dry film and evaluated for the mean diameter, surface charge, and entrapment efficiency. Optimized liposomes with vesicle size and entrapment efficiency of 358.4 nm and 95.08 $\pm$ 0.17 %, respectively, have very low leaking capacity. There were formulated as Carbopol 934 NF gels with and compared with conventional and marketed cream. The pharmacotechnical evaluation of gels demonstrated HCPG with a flux rate 0.476 $\pm$ 0.003 mg/cm²/h as the best formulation that was able to exhibit controlled release of Econazole nitrate for 12 hr and percent drug diffused from liposomes was higher than conventional and marketed cream. Stability profile of the prepared system assessed for 30 days revealed very low aggregation and insignificant growth in vesicular size. The results collectively suggest that because of the controlled drug release, better antifungal activity, and good storage stability, EN liposomes loaded gel has tremendous potential to serve as a topical delivery system.

Corresponding Author: Harshil M Patel, Ph.D Research Scholar, Dept. of Pharmacy, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan-333001
INTRODUCTION:

Fungal Infection

Fungal infections (mycoses) are widespread in the population; they are generally associated with skin or mucous membranes. In the countries with cold climate these infections are mainly benign and become more of a nuisance than a threat. Fungi are eukaryotic cells and therefore represent a more complex and evolved organism, which is predominantly parasitic in nature among the thousands of species around. Approximately 50 among them are pathogenic in humans. Clinically they are classified in to four main types on the bases of their morphological and their characteristics.

1. Yeasts (e.g. Cryptococcus neoformans)
2. Yeast-like fungi that produce a structure resembling a mycelium (e.g. Candida albicans)
3. Filamentous fungi with a true mycelium (e.g. Aspergillus fumigatus)
4. ‘Dimorphic’ fungi that, depending on nutritional constraints, may grow as either yeasts or filamentous fungi (e.g. Histoplasma capsulatum).

The current therapeutic agent to treat fungal infections are broadly classified in to two groups

A. The naturally occurring antifungal antibiotics such as the polyenes and echinocandins, and
B. Synthetic drugs includingazole and fluorinated pyrimidines.

Superficial fungal infections can be classified into two broad category i.e., dermatomycoses and candidiasis. Dermatomycoses include infections of the skin, hair and nails (onychomycosis). Whereas in case of candidiasis (superficial), the yeast-like organism may infect the mucous membranes of the mouth, vagina (thrush) or the skin.

Fungal infections are among the most widespread diseases known to man. They target the parts of the body as diverse in form and function as the skin, the nail, the buccal cavity, the eye and the vagina. Fungi staticazole drugs, i.e. imidazole and triazole – containing compounds, have been the mainstay of antifungal therapy for many years.

Econazole nitrate is an antifungal agent which belongs to imidazole derivative/ ring structure. The spectrum of activity includes dermatophytes, candida, yeasts, dimorphic fungi and gram positive bacteria.

Introduction to topical formulation

The topical drug delivery system is generally used in pain management, contraception, and urinary incontinence. Topical formulation would be based on the facility to reach the target into the skin (biopharmaceutics) and the demonstration of the local therapeutic effect (pharmacology of the drug to the surface of the skin or within the skin).

Topical delivery includes two basic types of product:

- External topicals that are spread, sprayed, or otherwise dispersed on to cutaneous tissues to cover the affected area.
- Internal topicals that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity.

1.2.1. Advantages of topical dosage forms

- They can avoid gastrointestinal drug absorption difficulties caused by gastrointestinal pH and enzymatic activity and drug interaction with food and drinks.
- They can substitute for oral administration of medication when that route is unsuitable.
- To avoid the first pass effect, that is, the initial pass of drug substance through the systemic and portal circulation following gastrointestinal absorption, possibly avoiding the deactivation by digestive and liver enzyme.
- They are non-invasive and have patient compliance.
- They are less greasy and can be easily removed from the skin.
- They are cost effective. They reduce doses as compare to oral dosage forms.
- Their effect is localized with minimum side effects.

Disadvantages of topical dosage forms

- Drugs with reasonable partition coefficient and possessing solubility both in oil and water are most ideal, as drug must diffuse through lipophilic stratum corneum and hydrophilic viable epidermis to reach the systemic circulation.
Only drugs, which are effectively absorbed by the percutaneous routes as such or by using penetration promoters, can be considered.

The route is not suitable for drugs that irritate or sensitize the skin.

The route is restricted by the surface area of delivery system and the dose that needs to be administered in the chronic state of disease.

Topical drug delivery systems are relatively expensive compared to conventional dosage forms. They may contain a large amount of drug, of which only a small percentage may be used during the application period.

**Topical gels**

The U.S.P. defines gels as a semisolid system consisting of dispersion made up of either small inorganic particle or large organic molecule enclosing and interpenetrated by liquid. Most topical gels are prepared with organic polymers, such as carbomers, which impart an aesthetically pleasing, clear sparkling appearance to the product, and are easily washed off the skin with water. Gels are two-component semisolids system rich in liquid. Their one characteristic feature is the presence of a continuous structure providing solid-like properties. In a typical polar gel, a natural or synthetic polymer builds a three-dimensional matrix throughout a hydrophilic liquid. Typical polymers used include the natural gums such as tragacanth, carrageenin, pectin, agar and alginic acid; semi synthetic materials such as methylcellulose, hydroxyl ethyl cellulose, hydroxyl propyl methylcellulose, and carboxy methylcellulose; and a synthetic polymer carbopol. We may also use certain clays such as bentonite, veegum, and laponite. Provided that the drug does not bind to the polymer or clay, such gels release medicaments well; the pores allow relatively free diffusion of molecules, which are not too large.

**Skin**

Most of topical preparations are meant to be applied to the skin. So basic knowledge of skin and its physiology, function and biochemistry is very important for designing topical preparations. The skin is the most extensive and readily accessible organs of the human body. The skin of average adult covers over 20,000 cm² of surface area and receives overall of all blood circulation through the body. It is a multi-layered organ.

**Physiology of the skin**

The skin has several layers. The overlaying outer layer is called epidermis; the layer below epidermis is called dermis. They dermis contain a network of blood vessels, hair follicle, sweat gland & sebaceous gland. Beneath the dermis are subcutaneous fatty tissues. Bulbs of hair project in to these fatty tissues.

![Figure 1 Structure of skin](image_url)
Routes of penetration

There are two main pathways by which drugs can cross the skin and reach the systemic circulation.

Transcellular Pathway

The direct route is known as the transcellular pathway. By this route, drugs cross the skin by directly passing through both the terpene lipids membranes and the cytoplasm of the dead keratinocytes that constitute the stratum corneum. Although this is the path of shortest distance, the drugs encounter significant resistance to permeation. This is because the drugs must cross the lipophilic membrane of each cell, then the hydrophilic cellular contents containing keratin, and then the terpene lipids bilayer of the cell one more time.

Intercellular Pathway

The second more common pathway through the skin is via the intercellular or paracellular route. Drugs crossing the skin by this route must pass through the small spaces between the cells of the skin, making the route more tortuous. Although the thickness of the stratum corneum is only about 20 µm, the actual diffusional path of most molecules crossing the skin is on the order of 400 µm. The 20-fold increase in the actual path of permeating molecules greatly reduces the rate of drug penetration.

Introduction of liposomes

Liposomes are microscopic vesicles composed of one or more lipid bilayers arranged in concentric fashion enclosing an equal number of aqueous compartments. Various amphipathic lipid molecules have been used to form the liposomes and the method of preparation can be used to control their size and morphology. Drug molecules can either be encapsulated in the aqueous space or intercalated into the lipid bilayer; the exact location of a drug in the liposome will depend upon its physicochemical characteristics and the composition of the lipids. Liposomes, i.e., phospholipid vesicles, are widely applied for the topical treatments of diseases in dermatology. Many drugs encapsulated into liposomes show enhanced skin penetration. Because of their ability to provide a sustained and controlled release of the incorporated material, liposomes also have a potential for being applied vaginally. The major disadvantage of using liposomes topically and vaginally lies in the liquid nature of the preparation. To achieve the viscosity desirable for application, liposomes should be incorporated into a suitable vehicle. It has been well established that liposomes are fairly compatible with viscosity increasing agents (methylcellulose) and polyacrylic acid (Carbopol). Several techniques have been explored to increase the drug penetration rate across skin including
iontophoresis and penetration enhancement, particularly for the delivery of peptides and proteins. Here focus on a third alternative method, the encapsulation of drugs in lipid vesicles prepared from phospholipids (liposomes) which have been shown to facilitate transport of drugs into and across skin.

While liposomes have been investigated for many years as parenteral drug carrier systems, particularly for the selective delivery of anticancer, antibiotic and antifungal agents, they have only for approximately one decade been considered for topical drug delivery, including ophthalmic, pulmonary and dermal/transdermal delivery.25,26

Due to their high degree of biocompatibility, liposomes were initially conceived of as delivery systems for intravenous delivery. It has since become apparent that liposomes can also be useful for delivery of drugs by other routes of administration. Liposomes are frequently used as vehicles in pharmaceuticals and cosmetics for their controlled and optimized delivery to particular skin layers. Liposomes are spherical vesicles whose membrane consists of amphiphilic lipids that enclose an aqueous core, similar to the bilayer membranes of living cells. Because liposomes offer an amphiphilic environment, they may encapsulate hydrophilic substances or drug in their aqueous core and lipophilic substances or drug in their lipid bilayer. This unique dual release capability enables the delivery of 2 types of substances once they are applied on the skin; each differs in its effects on skin permeability, which may enhance the desired therapeutic benefit.

**Structure and Composition of Liposome**

Among the variety of new drug delivery systems, liposomes seem to have the best potential to accommodate both water and lipid soluble compounds. To protect the liposome loaded drug from metabolic degradation and to act as a delivery mechanism, releasing active ingredients slowly and in a controlled manner.

Phospholipids, the cornerstone of the liposome lipid bilayer. Usually extracted from egg yolk or soyabean oil consists of a hydrophilic head portion covalently attached to two hydrocarbon tails representing the lipophilic portion. Aggregation in a bilayer structure occurs by orientation of the hydrophilic head groups towards the aqueous environment. While keeping the lipophilic hydrocarbon chains sequestered inside. Formation of such a configuration provides the vesicle with the lowest potential energy state through solvation of the polar head groups and hydrophobic interactions of the lipid chain.27,28

![Figure 3 A micrograph view of a liposome](image-url)

*Figure 3 A micrograph view of a liposome*
Natural phosphatidylcholine extracted from egg yolk or soybean oil or its semisynthetic derivatives represents the main constituent in various liposomal formulations. The chemical structure of naturally occurring phosphatidylcholine has a glycerol moiety attached to two acyl chains which may be saturated or nonsaturated. Each may have between 10 to 24 carbon atoms. Together forming the hydrophobic (lipophilic) portion of the molecule. The charged phosphate and choline moieties form the hydrophilic “head”. The fatty acid chains, depending on their length and degree of saturation, can exist in the gel phase in which the lipids are rigid, impermeable and easily aggregated upon storage. The temperature at which the gel phase converts to the liquid crystalline phase is known as the transition temperature. Cholesterol is frequently added in minute quantities to most of all liposomal formulations to increase the fluidity of the liposomal gel phase enhance the retention of hydrophilic particles and to stabilize the bilayer membrane in a manner similar to that of biological membranes.27, 29

Material & Methodology

Material

Econazole nitrate was a gift sample from FDC limited Mumbai, India. Phospholipon 90 G, 90 H was gifted by (Lipoid GmbH, Germany) and Methanol & Chloroform were obtained from (Merck Specialities Pvt Limited), Cholesterol was gifted by (high purity chemical Ltd. Mumbai, India). Carbopol 934 was gifted by (Corel chemical). All other ingredients and reagents were of analytical grade.

Method

Gel base was prepared by dispersing Carbopol 934 (Corel Chemical, Ahmedabad) in distilled water. The polymer was soaked in water for 2 hours and then dispersed in distilled water using a magnetic stirrer so as to obtain a homogeneous gel base of 1% w/w. For preparation of EN-loaded Liposomal gel, the liposomal suspension(s) was centrifuged at 2000 rpm for 20 minutes, suspended obtained were incorporated into the prepared gel base to get 1% w/w EN in the gel base. EN reference formulation (control; CG) was prepared by triturating EN with Carbopol 934 gel base.

Evaluation of formulation

pH measurement

The pH of Liposomal gel formulations was determined by using digital pH meter. One gram of liposome loaded gel was dissolved in 100 ml of distilled water and it was placed for two hours. The measurement of pH by calibrated digital pH meter. (Labtronics model LT 11 pH meter)

Viscosity measurement

The viscosity of the formulated batches was determined using a Brookfield Viscometer (Brookfield DV-2 + pro) with spindle S64. The formulation whose viscosity was to be determined was added to the beaker and was allowed to settle down for 30 min. at the assay temperature (25±1°C) before the measurement was taken. Spindle was lowered perpendicular in to the centre of liposomes loaded gel taking care that spindle does not touch bottom of the jar and rotated at a speed of adjusted rpm for 10 minutes. The viscosity reading was noted down.

Drug Content

Weigh accurately 1 g of Liposome loaded gel in volumetric flask and it was dissolved in 80 ml of Methanol and volume was made up to 100 ml using phosphate buffer pH 6.8. The volumetric flask was kept for 2 hours and shaken well in a shaker to mix it properly. The solution was passed through the filter paper and filtered. The absorbance was measured UV-spectrophotometer at 271nm. The drug content was determined using following formula

Drug content = (concentration *DF *Vol. taken) * conversion factor

In vitro Drug Release study

In vitro release studies were performed using kesari Chin cell. Dialysis membrane – LA- 390 (Hi-media laboratory) was placed between receptor and donor compartments. EN-loaded liposomal gel (eq. to 200 mg) was placed in the donor compartment and the receptor compartment was filled with phosphate buffer pH 6.8: methanol (20:80). The diffusion cells were maintained at 37 ± 0.5 oC with stirring at 100 rpm throughout the experiment. At fixed time intervals, 5ml of aliquots were withdrawn from receiver compartment through side tube and analyzed by UV-Visible Spectrophotometer at 271nm. Data obtained from in vitro release studies were fitted
to various kinetic equations to find out the mechanism of drug release from liposomal gel.

**Ex Vivo Permeation studies**

**Preparation of skin**

The abdominal hair of Wister male rats, weighing 150±25 g, was trimmed using trimmer 24 hr before treatment. After anesthetizing the rat with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable enzymes, the dermal side of the skin was in contact with a saline solution for 1hr before starting the diffusion experiment. All surgical and experimental procedures were reviewed and approved by the animal and ethics review CPCSEA committee, Shree Dhanvantri Pharmacy College, Kim, Surat. (SDPC/AFC/2013/242)

**Skin permeation study**

A system employing improved kesari chine cell was used for permeation studies. The excised rat skin was set in place with the stratum corneum facing the donor compartment and the dermis facing the receptor compartment. Econazole nitrate loaded liposomal gel (eq. to 200 mg) was applied to the skin surface in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 6.8 (24 ml). During the experiment, the diffusion cell was maintained at 37 ± 0.5 °C. After application of the test formulation on the donor side, at fixed time intervals, 2 ml of aliquots were withdrawn from receiver compartment through side tube and analysed by UV-Visible spectrophotometer at 271nm.

**Skin-retention studies**

After conducting the permeation study, the authors carefully removed the skin mounted on the Franz diffusion cells. The remaining formulation adhering to the skin was scraped with a spatula and then wiped with tissue paper. The cleaned skin piece deep in 10 mL of methanol and kept for 24 h for the complete extraction of the drug. The filtrate was removed and analyzed using UV spectrophotometry

**Kinetic study and Mechanism of drug release**

To find out the release mechanism of drug from emulgel, release study data were subjected to statistical analysis by Zero order, First order, Higuchi and Korsmeyer Peppas equations. Data obtained from in-vitro drug release studies were fitted to following kinetic models.

**Zero order release kinetics:** In many of the modified release dosage forms, particularly sustained or controlled release dosage forms, is zero-order kinetic.

\[ Q = K_0 t \]  

(1)

Where Q is fraction of drug released at time t & K0 is zero order release rate constant.

**First order release kinetics:** The drug release from most of the slow release tablets could be described adequately by apparent first order kinetics.

\[ \ln (1-Q) = -K_1 t \]  

(2)

Where Q is the fraction of drug released at time t. And K1 is the first order release rate Constant.

**Higuchi Model:** It defines a linear dependence of the active fraction released per unit of surface (Q) on the surface root of time. The dissolution pattern of the drug is dictated by water penetration rate (diffusion controlled) and thus the following relationship applies:

\[ Q = K_2 t^{1/2} \]  

(3)

Where, K2 is release rate constant.

A plot of the fraction of drug released against root of time will be linear if the release obeys first order release kinetics.

**Peppas & Korsemeyer Model (Power law):** In order to define a model, which would represent a better fit for the formulation dissolution data was further analysed by Peppas & Korsemeyer equation,

\[ M_t / M_\alpha = K t^n \]  

(4)

Where, Mt is the amount of drug released at time t and M á is the amount released at Time á, thus the Mt / Mα is the fraction of drug released at time t, K is the kinetic constant and n is the diffusional exponent. To characterize the mechanism for both solvent penetration and drug release n can be used as
abstracted. A plot between log of Mt \( M_t \) against log of time will be linear if the release obeys Peppas & Korsemeyer equation and the slope of this plot represents n value. The value of n indicates the drug release mechanism. For a slab the value \( n \leq 0.45 \) indicates Fickian diffusion, values of \( n > 0.45 \) and \( \leq 1.0 \) indicate non-Fickian.

**Antifungal activity**\(^{36} \)
The antifungal activity was evaluated by cup plate method with strains of Candida albicans. The fungal strains were spread over nutrient agar media in a Petri dish, which was then impregnated with the solution placed on the surface of the media inoculated with the fungal strains. The plates were incubated at 25°C for 24 hours. After incubation, the zones of growth inhibition around the disk were observed on an antibiotic zone reader.

**Sample:**
- A. Pure drug sample
- B. HCPG
- C. GCPG
- D. Marketed formulation

**Stability Study of Optimized gel HCPG**
Stability study of selected optimized batch HCPG was done at room temperature (40± 2° C / 75 ± 5% RH) for 1 month and formulation was finally evaluated for Colour, drug content and pH.

**RESULT & DISCUSSION**

**Fourier Transform infrared spectroscopy FTIR**

![Figure 4 Fourier Transform infrared spectroscopy FTIR](image)

**Differential scanning calorimetry (DSC)**
The DSC thermogram of econazole nitrate, blank liposomes prepared from Phospholipon 90 H and Phospholipon 90 G, and econazole nitrate loaded liposome prepared from same phospholipid. Thermogram were recorded on the differential scanning calorimeter as shown below.
The DSC thermogram of pure Econazole nitrate shows a single endothermic peak at 165.15 °C, due to melting of the drug. The measured melting endotherms for blank liposome prepared from Phospholipon 90 H and Phospholipon 90 G are observed at 77.41 °C and 78.13 °C respectively. In the DSC thermogram of ECN loaded liposome, profound modification in lipid and compatible with Econazole nitrate. It indicating that there are no changes in the lipid composition.

**Entrapment efficacy**

Vacuum pressure, hydrating medium, hydration time, flask rotation speed, and method of size reduction were optimized to prepare lipid vesicles of econazole nitrate. PBS at pH 6.8 was the best hydrating medium, with good stability and entrapment of the drug. Adjusting the evaporator’s rotation to 150 rpm increased the surface area for evaporation and was sufficient to form thin, uniform, and completely dried film.

### Table 1 Effect of Lipid Composition on Encapsulation Efficiency of Econazole Nitrate in liposomes using Phospholipon 90 H (PCS)

<table>
<thead>
<tr>
<th>Batch</th>
<th>Composition</th>
<th>Amount of drug entrapped ± SD (n=3)</th>
<th>% Entrapment Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>100:10:5</td>
<td>6.158±0.057</td>
<td>61.58%</td>
</tr>
<tr>
<td>H2</td>
<td>100:10:10</td>
<td>8.487±0.022</td>
<td>84.87%</td>
</tr>
<tr>
<td>H3</td>
<td>100:10:15</td>
<td>8.64±0.11</td>
<td>87.15%</td>
</tr>
<tr>
<td>H4</td>
<td>100:10:20</td>
<td>9.132±0.032</td>
<td>91.24%</td>
</tr>
<tr>
<td>H5</td>
<td>100:10:30</td>
<td>9.489±0.017</td>
<td>95.08%</td>
</tr>
<tr>
<td>H6</td>
<td>100:10:40</td>
<td>9.375±0.013</td>
<td>93.75%</td>
</tr>
</tbody>
</table>
Table 2 Effect of lipid composition on encapsulation efficiency of econazole nitrate in liposomes using

*Phospholipon 90 G (PCU)*

<table>
<thead>
<tr>
<th>Batch</th>
<th>Composition</th>
<th>Amount of drug entrapped ± SD (n=3)</th>
<th>% Entrapment Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>100:10:10</td>
<td>3.83±0.107</td>
<td>38.39 %</td>
</tr>
<tr>
<td>G2</td>
<td>100:10:20</td>
<td>4.004±0.093</td>
<td>40.04 %</td>
</tr>
<tr>
<td>G3</td>
<td>100:10:30</td>
<td>6.115±0.016</td>
<td>61.15 %</td>
</tr>
<tr>
<td>G4</td>
<td>100:10:40</td>
<td>7.129±0.01</td>
<td>71.29 %</td>
</tr>
<tr>
<td>G5</td>
<td>100:10:10</td>
<td>7.658±0.025</td>
<td>76.58 %</td>
</tr>
<tr>
<td>G6</td>
<td>100:10:20</td>
<td>8.76±0.041</td>
<td>87.65 %</td>
</tr>
<tr>
<td>G7</td>
<td>100:10:30</td>
<td>9.62±0.097</td>
<td>96.23 %</td>
</tr>
<tr>
<td>G8</td>
<td>100:10:40</td>
<td>9.465±0.087</td>
<td>94.65 %</td>
</tr>
</tbody>
</table>

Liposomes of econazole nitrate using the PCS and PCU were successfully prepared by dry thin film hydration method at different ration of lipid and cholesterol. An increase in CHOL concentration with the same econazole nitrate and PCS concentration (H1 to H5) led to an increase in entrapment levels of Econazole nitrate from 6.15 mg/105 mg to 9.48 mg/130 mg of total lipids. The increase in entrapment efficiency was attributed to the ability of CHOL to cement the leaking space in the bilayer membranes, which in turn leads to an enhanced drug level in liposomes (18).

A further increase in CHOL level with the phosphatidyl choline amount kept constant the entrapment efficiency, although the total lipid level also was increased. Same as that of PCS in phospholipid PCU found that increasing the PCU level increased the entrapment efficiency. At the lower level of PCU at 100 mg (G1), the entrapment efficiency was less. As the amount of PCU was increased to 125 mg (G7) and CHOL was kept at a constant level of 30 mg, the entrapment efficiency increased to 9.62 mg/155 mg of total lipid. Finally founds the ratio of Lipid:Cholesterol (100:30) of PCS and (125:30) of PCU have maximum loading entrapment efficacy. This occurrence indicates that Econazole nitrate entrapment is enhanced up to a certain level of PCS/PCU and CHOL and substantiates the importance of the appropriate proportions of PCS and CHOL to maximize the entrapment affinity of drug toward bilayer-forming phospholipids.

**Vesicle characterization**

Vesicle morphology and size distribution EN-loaded Liposome examined using a scanning electron microscope which shown image below. The particle size and zeta potential of liposomes were measured. The vesicle size of the liposomal system batch H5 and batch G7 were found 358 nm and 813 nm respectively. The zeta potential of the batch H5 and batch G7 were found to be -26.6 mV and -13.6 mV.

**Microscopy**

The prepared liposomes were viewed under a scanning electron microscope.
Zeta potential:
Here from the zeta potential data of both lipid concluded that because of a more negative charge of the PCS (-26.5 mV) than the PCH (-13.6 mV), the PCS more stable than the PCH phospholipid on storage. And they don't undergo any aggregation of the liposomes.

Drug leaking study
We conducted one month study of liposomal stability with respect to the liposomes’ ability to retain an entrapped drug during a defined time period. The G7 and H5 batch of liposomes were withdrawn from the study at room temperature because storage at these temperatures leads to a substantial loss of drug from the liposomes by the end of a one-month period. The amount of drug remain at the end of one month was found 91.20 and 95.68 respectively. Drug leakage at elevated temperatures may be a result of chemical degradation (oxidation and hydrolysis) of lipids in the bilayers, leading to defects in membrane packing. Thus, earlier reports of the low-temperature stability of liposomal products may be attributed to the deformation of gel-state lipid membranes that help hold Econazole nitrate molecules in place.

The drug-retention capacity of liposome H5 is higher than that of G7 in all conditions. The better stability of liposomes H5 compared with G7 may be attributed to the high phase transition temperature of PCS (56°C) relative to the low phase-transition temperature of PCU (28–30°C), the greater affinity of PCS toward the drug molecule, and the liposomes’ greater structural integrity. Both PCU and PCS have shown fairly high retention of drug inside the vesicles at room temperature for as long one months (~92%).

Figure 6 SEM of liposomal vesicle

Figure 7 Leaking test of liposome
Evaluation of liposome loaded gel formulation
pH
In the evaluation of gels, the pH of all gels was found between 5.5 and 6.54, thus lying in the normal pH range of skin, 4.5–7; hence the preparation will potentially be non-irritating.

Viscosity
Viscosity of EN-loaded liposomal gel batch H5 and Batch G7 were found 10,416 and 11,585 cps.

Drug content
Drug content of the liposomes loaded gel HCPG and GCPG was found to be 0.986 mg and 0.981mg respectively.

Drug release study
The in-vitro release profile is an important tool that predicts in advance how a drug will behave in-vivo. The in-vitro permeation of econazole nitrate using dialysis membrane from liposomal gel was compared with that of controlled gel and marketed formulation containing 1 % w/w of Econazole nitrate. The permeation of econazole nitrate was calculated in terms of the % cumulative drug released and flux (n=3) at each sampling time points during 12 hours study. Marketed formulation contain Econazole nitrate 1% cream (Ecodex, J. B. Pharmaceutical).

It can be concluded that cumulative permeation of econazole nitrate was significantly greater from CPLG and CPLH than from CG, CPG, and marketed formulation. Plot of % cumulative drug release vs. time (hours) is shown in figure 5.19. The release of econazole nitrate from liposomal gel is much better than from non liposomal formulations. Econazole nitrate from liposomes showed release of about 90-95% after 12 hours. The higher flux value of liposomal gel is suggestive of good permeation enhancer of econazole nitrate.

Table 3 Comparative % cumulative drug release

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Time (hr)</th>
<th>% Cumulative Drug Release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG</td>
<td>CPG</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>5.94 ± 0.87</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>8.75 ± 1.31</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>12.72±1.01</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>18.93±1.53</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>27.24±1.92</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>36.11±3.04</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>42.22±2.15</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>46.59±2.07</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>55.18±1.81</td>
</tr>
</tbody>
</table>

Figure 8 Comparative % cumulative drug release
Table 4 Comparative drug release and flux

<table>
<thead>
<tr>
<th>Sample of Gel</th>
<th>% Cumulative Drug Release</th>
<th>Flux (mg/cm²/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>55.18 ± 1.81</td>
<td>0.326 ± 0.01</td>
</tr>
<tr>
<td>CPG</td>
<td>70.04 ± 1.46</td>
<td>0.382 ± 0.006</td>
</tr>
<tr>
<td>GCPG</td>
<td>90.97 ± 0.55</td>
<td>0.458 ± 0.008</td>
</tr>
<tr>
<td>HCPG</td>
<td>94.65 ± 1.28</td>
<td>0.476 ± 0.003</td>
</tr>
<tr>
<td>Marketed Formulation</td>
<td>67.57 ± 1.27</td>
<td>0.378 ± 0.006</td>
</tr>
</tbody>
</table>

From the flux value of the econazole nitrate gel it can be concluded that CPG shows nearer to same flux value as that of marketed formulation. While compare to CPG gel that liposomal gel shows good flux value.

E. Kinetic Study and Mechanism of Drug Release

The correlation coefficient value (R²) of each formulation for zero order, first order, Higuchi model are shown in table.

Table 5 Kinetics and Release Mechanism of Optimized Batch HCPG

<table>
<thead>
<tr>
<th>Batch</th>
<th>R² Value</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Higuchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimized Batch</td>
<td>0.9084</td>
<td>0.9713</td>
<td>0.9829</td>
<td></td>
</tr>
</tbody>
</table>

Regression coefficient (R2) values of different kinetic models are shown in Table 5. This indicated that the release data of optimized batch (HCPG) follows Higuchi mode because the value of R2 is greater in this model. The mechanism of drug release is determined by Higuchi model where R2 is maximum 0.9829 hence the mechanism of drug release is Higuchi model for Optimized batch given in table.

From all the above data concluded that the HCPG has very good stability, drug release capacity and good flux rate so that the batch HCPG selected for the skin permeation study and skin retention study.

Ex vivo skin permeation study

![Figure 9 Cumulative Drug Release from the Drug](image)
Table 6 Drug release from the skin and flux of optimized batch

<table>
<thead>
<tr>
<th>Sample of Gel</th>
<th>% cumulative drug release</th>
<th>Flux (mg/cm²/hr)</th>
<th>Skin retention of drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimized batch</td>
<td>90.65 ± 1.28</td>
<td>0.4513 ± 0.004</td>
<td>0.85 mg</td>
</tr>
</tbody>
</table>

Antifungal studies
The in vitro antifungal activity, the decreasing order of zone of inhibition was pure drug, HCPG, GCPG, marketed formulation (i.e., 19, 18, 12, and 10 mm, respectively).

Figure 10 Comparative antifungal activity of Econazole Nitrate Loaded Gel

Where, A. Pure drug sample, B. HCPG, C. GCPG, D. Marketed formulation

The enhanced in vitro antifungal activity of tested HCPG liposomal gel may be attributed to enhanced diffusion of vesicles containing EN through fungal cell walls to inhibit ergosterol synthesis, because the B. liposomal gel showed maximum inhibitory activity as compared to other formulations.

CONCLUSION
Liposomes of econazole nitrate using the Phospholipon 90 H and phospholipon 90 G were successfully prepared by dry thin film hydration method at different ration of lipid and cholesterol, and it shows that the ratio of Lipid:Cholesterol (100:30) of PCS and (125:30) of PCU have maximum loading entrapment efficacy.

The FTIR studies confirmed no interaction between the drug and the lipid. And also, DSC studies confirm that there were no any interactions shown in the drug and liposome formulation. It was indicating that drug exists in the amorphous state, and that the lipid amount of lipid carrier used to solubilize the Econazole nitrate. From the particle size and zeta potential data of the both the lipid it was found that the PCS have very less particle size as compared to PCU so that it might improve the drug penetration of Econazole nitrate through skin and negative zeta potential shows good stability of vesicular formulation. Morphological features of liposome were studied by SEM. The SEM
photographic images of liposomes show the unequal spherical shape. The in vitro diffusion studies show that, cumulative permeation of econazole nitrate was significantly greater of CPLG and CPLH than from CG, CPG, and marketed formulation. The release of econazole nitrate from liposomal gel is much better than from nonliposomal formulations. Econazole nitrate from liposomes shows release of about 90-95% after 12 hours. The higher flux value of liposomal gel is suggestive of good permeation enhancer of econazole nitrate. From the flux value of the econazole nitrate gel it can be concluded that CPG shows nearer to same flux value as that of marketed formulation, while compared to that CPG gel liposomal gel shows higher flux value.

The results of the skin permeation and skin retention studies show that fairly high Econazole nitrate retention with liposomes. The higher permeation of drug across the skin and greater retention in the skin with liposomal systems clearly shows that liposomes can penetrate and form depots in skin layers. The liposomal phospholipids (natural constituent of skin lipids) proved better for generating and retaining the required physicochemical state of skin for enhanced permeation and retention. This characteristic can be attributed to phospholipids' ability to vesiculate independently because they carry two bulky nonpolar lipid chains and a polar-head group, which helps them spontaneously form into closed bilayered systems and produce long lasting effects.

The optimized HCPG batch was selected for 1-month stability study; there were no significant changes in colour, pH and % drug content which indicated the selected formulation is stable. The optimized formulation HCPG show better loading capacity and In-vitro drug release profile than marketed preparation. So, Econazole nitrate optimized liposomes loaded gel formulation can be used to treat the topical fungal diseases.

REFERENCE


How to cite this article:

Source of Support: Nil
Conflict of Interest: None declared

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