

# Formulation And Characterization of Griseofulvin Liposome Loaded Topical Film

Prashant Kumar Mishra<sup>1</sup>, Dr. Kaushelendra Mishra<sup>2</sup>,  
Dr. Mehta Parulben D<sup>3</sup>

<sup>1</sup>Research Scholar, Department Of Pharmaceutics, Lakshmi Narain College Of Pharmacy, Bhopal

<sup>2</sup>Professor, Department Of Pharmaceutics, Lakshmi Narain College Of Pharmacy, Bhopal

<sup>3</sup>Professor & Director, Lakshmi Narain College Of Pharmacy, Bhopal

## Abstract

This research paper explores the formulation and characterization of a liposome-loaded topical film containing griseofulvin, an antifungal drug. Liposome-based drug delivery systems offer enhanced drug concentration, prolonged residence time, and reduced side effects. This study aims to improve the efficacy of griseofulvin by incorporating it into liposomes and formulating it into a topical film for transdermal delivery. Liposomes were used as carriers to encapsulate Griseofulvin and incorporated into a topical film for ease of application. The paper reviews the principles of liposome-based drug delivery, transdermal film technology, and the potential benefits of this approach. The work plan includes preformulation studies, liposome preparation and evaluation, formulation of liposome-loaded topical films, and evaluation of film properties. The results demonstrated that the developed formulation has the potential to improve the delivery and efficacy of Griseofulvin in the treatment of cutaneous fungal infections.

**Keywords:** Griseofulvin, liposomes, topical film, transdermal delivery, drug delivery, skin penetration, antifungal, formulation, characterization.

## 1. Introduction

The concept of drug targeting originated ever since Paul Ehrlich (1906), first envisaged the use of ‘magic bullets’ for eradication of diseases [1]. This report described targeted drug delivery as an event where a drug-carrier complex is able to deliver drug exclusively to the preselected target cell. Targeting of drugs to specific tissues or cells using novel drug delivery systems has been described by Gregoriadis as ‘old drug in new cloths’ [2-4].

Drug delivery systems (DDSs) have revolutionized pharmaceutical research by enhancing drug efficacy, minimizing side effects, and improving patient compliance. Nano-sized drug carriers like liposomes have shown promise in improving drug pharmacokinetics, biodistribution, and therapeutic index. Liposomes are lipid vesicles with high biocompatibility and versatility, making them a suitable choice for targeted drug delivery. In this study, we focus on formulating and characterizing a liposome-loaded topical film containing griseofulvin, an antifungal drug [5].

Griseofulvin is effective against dermatophytic fungal infections but has limited bioavailability due to low water solubility and poor absorption. To overcome these challenges, we propose incorporating

griseofulvin into liposomes and formulating it into a topical film. This approach aims to improve drug absorption through the skin, enhancing its therapeutic potential.

Liposomes are spherical, closed structures composed of phospholipids in the colloidal size range of 5–200 nm and contains one or more concentric/non-concentric membranes, of around 4 nm thickness [8]. The liposomes consist of amphiphilic phospholipids with hydrophilic head and hydrophobic tail, which aids in unique characteristics such as self-sealing of liposomes in aqueous media. The size of liposomes ranges from 30 nm to the micrometer scale, with the phospholipid bilayer being 4–5 nm thick [6].

A transdermal patch is a medicated adhesive patch placed on skin to deliver a time released dose of medication through the skin for treating topical or systematic illness. Since early 1990, this dosage form of transdermal therapeutic system has been available in the pharmaceutical market [7].

In addition transdermal dosage form is user-friendly, convenient, painless, and offers multi-day dosing, it generally leads to improved patient compliance [8].

It offers many important advantages over oral drug delivery, e.g., gastrointestinal and hepatic first pass metabolism, reduces variation in delivery rates, avoids interference due to presence of food, controls absorption rate, suitable for unconscious patients, and enables fast termination of drug delivery, if needed [9].

## 2. Proposed Research

Until recently, chemotherapy of fungal infections has lagged far behind chemotherapy of bacterial infections. This lack of progress has resulted, in part, because the most common fungal infections in humans have been relatively superficial infections of the skin and mucosal membranes and potentially lethal deep-seated infections have been quite rare. Because most humans with a normally functioning immune system are able to ward off invading fungal pathogens with little difficulty, the demand for improvements in antifungal therapy has been small.

Oral antifungal drugs are reserved for extensive or severe infection for which topical antifungal agents are inappropriate or ineffective, because of high cost, potential side effects and drug interactions.

Griseofulvin is a widely acknowledged antifungal drug used orally to treat superficial fungal infections, primarily fingernail and toenail infections, but it does not penetrate skin or nails if used topically. The lower aqueous solubility and absorption causes the dose of griseofulvin to be very high (500 mg/kg, twice a day in adults). On the other hand only 25-50% of the drug is absorbed and with around 42% drug excreted unchanged in urine after 4 hours thereby decreasing its half-life [10].

As the absorption of drug is low, it was envisioned that formulating griseofulvin as liposome and incorporating the liposome into topical film (patch) would be helpful to improve efficacy and in turn its topical absorption and bioavailability.

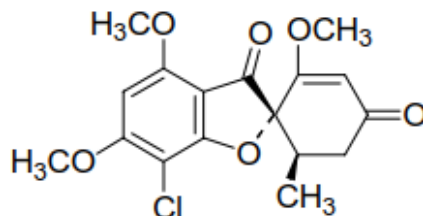
## 3. Objective

The objective of this work was :

- a. To formulate liposomes loaded with griseofulvin using reported procedure
- b. To incorporate the griseofulvin liposomes into transdermal patches
- c. To evaluate the transdermal patches for various parameters

#### 4. Drug Profile [11,12]

- Name - Griseofulvin
- Chemical structure -



- Molecular Weight - 352.8
- Chemical Formula-  $C_{17}H_{17}ClO_6$
- IUPAC Name - (1S,6'R)-7-chloro-2',4,6-trimethoxy-6'-methylbenzofuran-2-spiro-1-cyclohex-2'-ene-3,4'-dione
- Description - An antifungal antibiotic griseofulvin may be given by mouth in the treatment of tinea infections.
- Indication - For the treatment of ringworm infections of the skin, hair, and nails, namely: tinea corporis, tinea pedis, tinea cruris, tinea barbae, cradle cap or other conditions caused by Trichophyton or Microsporum fungi.
- Pharmacodynamics - Griseofulvin is a mycotoxic metabolic product of Penicillium spp. It was the first available oral agent for the treatment of dermatophytoses and has now been used for more than forty years. Griseofulvin is fungistatic with in vitro activity against various species of Microsporum Epidermophyton, and Trichophyton.
- Mechanism of Action - Griseofulvin is fungistatic, however the exact mechanism by which it inhibits the growth of dermatophytes is not clear. It is thought to inhibit fungal cell mitosis and nuclear acid synthesis. It also binds to and interferes with the function of spindle and cytoplasmic microtubules by binding to alpha and beta tubulin. It binds to keratin in human cells, then once it reaches the fungal site of action, it binds to fungal microtubes thus altering the fungal process of mitosis.
- Absorption - Poorly absorbed from GI ranging from 25 to 70% of an oral dose. Absorption is significantly enhanced by administration with or after a fatty meal.
- Metabolism - Primarily hepatic with major metabolites being 6-methyl-griseofulvin and its glucuronide conjugate.
- Half life - 9 to 12 h
- Elimination - Less than 1% of a dose is excreted as unchanged drug in the urine whereas approximately 36% of griseofulvin is excreted unchanged in the feces.
- Commercial availability - Griseofulvin is prescribed in the form of 250 and 500 mg tablets

#### 5. Excipient Profile

##### 5.1 Hydroxypropyl Methylcellulose [13]

- **Synonyms**  
Cellulose, hydroxypropyl methyl ether, HPMC, methocel, metolose, and pharmacoat
- **Empirical Formula**  
HPMC is a partially o-methylated and o-(2-hydroxypropylated) cellulose. It is available in several grades, which vary in viscosity and extent of substitution.

- **Properties**

Hypromellose is an odorless and tasteless, white or creamy white fibrous or granular powder. Ph of HPMC 5.5-8.0 for a 1% w/w aqueous solution.

- **Functional Category**

Coating agent, film-former, stabilizing agent, suspending agent, tablet binder, viscosity-increasing agent.

- **Viscosity**

A wide range of viscosity types are commercially available. Aqueous solution are most commonly prepared, it may also be dissolved in aqueous alcohol such as ethanol and propan-2-ol. Dichloromethane and ethanol mixture may also used to prepare viscous solution. Solution prepared using organic solvents tend to be more viscous.

- **Solubility**

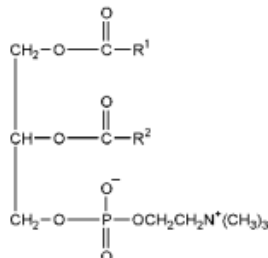
It is soluble in cold water, forming a viscous colloidal solution and practically insoluble in chloroform, ethanol and ether, but soluble in mixture of ethanol and dichloromethane, and mixture of methanol and dichloromethane.

## 5.2 Lecithin [14]

- **Synonyms**

Egg lecithin, ovolcithin, Phospholipon 100, Phosal 53 MCT

- **Structural Formula**



- **Functional Category**

Emollient; emulsifying agent; solubilizing agent.

- **Pharmaceutical Applications**

Lecithins are mainly used in pharmaceutical products as dispersing, emulsifying, and stabilizing agents and are included in intramuscular and intravenous injections, parenteral nutrition formulations, and topical products such as creams and ointments

## 5.3 Cholesterol [15]

- **Synonyms**

Cholesterin; cholesterolum.

- **Chemical Name**

Cholest-5-en-3b-ol

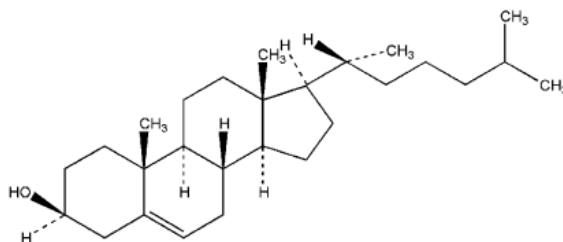
- **Empirical Formula**

C<sub>27</sub>H<sub>46</sub>O

- **Molecular Weight**

386.67

- Structural Formula



- **Functional Category**

Emollient; emulsifying agent.

- **Pharmaceutical Applications**

Cholesterol is used in cosmetics and topical pharmaceutical formulations at concentrations of 0.3–5.0% w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity.

## 6. Materials And Methods

### 6.1 Materials

The material required for the formulation of solid dispersion and gel were procured from various sources and used as obtained.

Table 6.1 : List of material and procurement source

| S. No. | Ingredient                             | Source                         |
|--------|--|--------------------------------|
| 1      | Griseofulvin (API)                     | Yarrow Pharmaceuticals, Mumbai |
| 2      | Lecithin                               | HiMedia, Mumbai                |
| 3      | Cholesterol                            | HiMedia, Mumbai                |
| 4      | Hydroxy propyl methyl cellulose (HPMC) | Oxford Fine Chemicals, Mumbai  |
| 5      | Ethyl cellulose (EC)                   | Oxford Fine Chemicals, Mumbai  |
| 6      | DMSO                                   | CDH, New Delhi                 |
| 7      | Sodium hydroxide                       | Oxford Fine Chemicals, Mumbai  |
| 8      | Methanol                               | Oxford Fine Chemicals, Mumbai  |
| 9      | Ethanol                                | Jiangsu Huaxi, China           |
| 10     | Distilled Water                        | Freshly prepared in laboratory |

The equipments used for performing the present study were used without performing the calibration study.

Table 6.2 : Equipment used for the study

| S.No. | Equipment          | Make              |
|-------|--------------------|-------------------|
| 1     | Electronic Balance | Wensar, India     |
| 2     | Magnetic Stirrer   | Labtronics, India |

|   |                      |                     |
|---|----------------------|---------------------|
| 3 | Vernier Caliper      | Excel Instruments   |
| 4 | Franz diffusion cell | Locally fabricated  |
| 5 | UV Spectrophotometer | Labtronics, India   |
| 6 | Heating Mantle       | Bio-Technics, India |

## 6.2 Methods

### • Preformulation Studies

Organoleptic Evaluation :

The color, odor and taste of the obtained drug sample were observed with the help of the sensory organs.

Solubility :

Solubility was observed in different solvents like water, HCl, ethanol and acetone by shaking a small unmeasured amount of drug in 1 mL of solvent and observing for undissolved particles, if any.

Identification Test :

FT-IR spectrum of the sample of Griseofulvin was obtained and examined for the presence of characteristic peaks and matched with that of the reference spectra in databases for confirmation of the identity of the drug.

Melting point determination :

Melting point was determined by open capillary method and is uncorrected. A small quantity of powder was placed into fusion tube and placed in the melting point apparatus. The temperature of the apparatus was gradually increased and the temperature at which the powder started to melt and the temperature at which all the powder got melted was recorded.

Compatibility analysis :

The FTIR spectra of the pure drug and a physical mixture of the drug and the excipients under study were obtained and observed for deletion of the characteristic peaks of the drug.

Loss on drying :

It was determined by drying the pure drug in an oven at 100°C to 105°C for 3 h. The percent loss of moisture was calculated by the difference between the initial and final weight of the drug.

Partition Coefficient :

The partition coefficient of the drugs was performed by using octanol as oil phase (10 mL) and water as aqueous phase (10 mL). Both the phases were mixed by shaking vigorously in a separating funnel and then 5 mg of drug was added. The drug was allowed to dissolve in both the phases by shaking and allowing for equilibration. Both phases were taken in a conical flask and then analyzed against their respective blank solution and the partition coefficient was calculated.

Determination of  $\lambda_{\max}$  :

Accurately weighed 5 mg of Griseofulvin was dissolved in 5 mL of methanol in a 10 mL volumetric flask. 1 mL of this solution was taken in to a 10 mL volumetric flask and volume made up to the mark with methanol [16]. The resulting solution was then scanned between 200-400 nm using UV spectrophotometer. The  $\lambda_{\max}$  was found to be 295 nm. The solution was stored for 3 days at room temperature and rescanned to observe any changes in wavelength.

- **Preparation of Calibration Curve in methanol**

Accurately weighed 10 mg of Griseofulvin was taken in 10 mL volumetric flask and dissolved in methanol to the mark resulting in a stock solution of 1000 µg/mL. 1 mL of the above stock solution was taken in another 10 mL volumetric and volume was made up with methanol to mark resulting in a solution of 100 µg/mL. Aliquots of 1-6 mL of stock solution were taken into a series of 10 mL volumetric flask and volume was made up to the mark using methanol and were analyzed at 295 nm using UV spectrophotometer. A standard curve was constructed against absorbance and concentration.

- **Preparation of Liposomes**

Drug loaded liposomes were prepared by a modified ethanol injection method. Required amounts of phospholipids (20, 40, 60 mg/ml) and cholesterol (2 mg/ml) were dissolved in ethanol and griseofulvin (200 mg) was added to the organic phase (Table 6.1). Resulting organic phase was injected by means of a syringe pump to aqueous phase under magnetic stirring at  $45 \pm 2$  °C. A spontaneous formation of liposome occurred as soon as the ethanolic solution was in contact with the aqueous phase. Liposome suspension was then kept under stirring for 1h at room temperature to remove the traces of solvent. The unloaded drug was removed by ultracentrifugation of liposome suspension at 10,000 rpm for 1 hour and stored at 4°C [17].

All liposome dispersions were characterized immediately after preparation.

Table 6.3 : Composition of liposome formulations

| Formulation | Soy Lecithin (mg) | Cholesterol (mg) | Griseofulvin (mg) | Ethanol (mL) |
|-------------|-------------------|------------------|-------------------|--------------|
| GL1         | 200               | 20               | 200               | 100          |
| GL2         | 400               | 20               | 200               | 100          |
| GL3         | 600               | 20               | 200               | 100          |

- **Characterization of Liposomes**

The characterization of the liposomes was carried out by estimation of encapsulation efficiency, and particle size.

**Entrapment Efficiency :**

5 ml of liposome formulation was taken and transferred to a 100 ml volumetric flask containing 25 ml of phosphate buffer (skin pH 6.8), and sonicated using an probe sonicator for 6 minutes at 35% impulse and 1 min cycles and filtered through a 0.45µm membrane filter. The filtrate was finally diluted with phosphate buffer (pH 6.8) and absorbance was recorded by UV visible spectrophotometer at 295 nm.

**Particle Size Determination :**

The particle size of the microspheres was determined by using microscope, employing the calibrated eye piece and stage micrometer method. Size of liposomal vesicles was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles was determined.

- **Formulation of transdermal patches**

Griseofulvin liposome loaded transdermal patches were formulated utilizing the solvent casting method using a petridish of area 38.46 cm<sup>2</sup>. Polymers were accurately weighed and dissolved in 10 mL of water-ethanol (1:1) solution, stirrer for 30 min on a magnetic stirrer and kept aside to form clear solution (Table

6.2). Griseofulvin liposome equivalent to desired amount of griseofulvin was accurately weighed and was dissolved in the above solution and mixed until clear solution was obtained. Polyethylene glycol 400 (30% w/w of total polymer) was added to be used as plasticizer and propylene glycol (15% w/w of total polymer) was added as the permeation enhancer. The resulted uniform solution was cast on the petri dish, which was lubricated with glycerin and dried at room temperature for 24 h. An inverted funnel was placed over the petridish to prevent fast evaporation of the solvent [18]. After 24 h, the dried patches were taken out and stored in a desiccator for further studies.

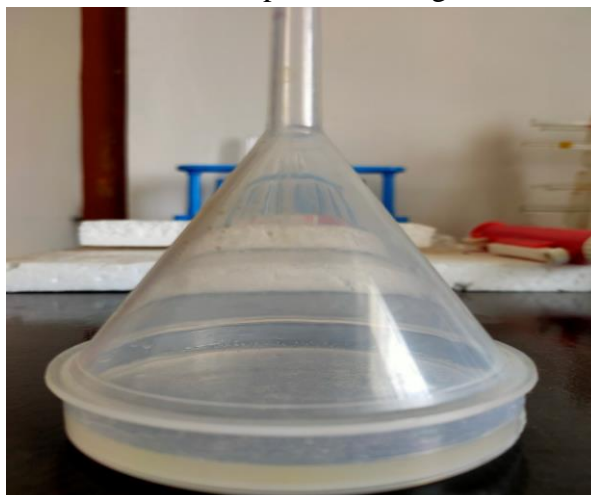
Table 6.4 : Formula for Griseofulvin liposome loaded transdermal patches

| <b>Ingredients</b>                        | <b>GLP1</b> | <b>GLP2</b> | <b>GLP3</b> | <b>GLP4</b> |
|---|-------------|-------------|-------------|-------------|
| <b>GL equivalent to griseofulvin (mg)</b> | 225         | 225         | 225         | 225         |
| <b>HPMC (mg)</b>                          | 100         | 150         | 200         | 250         |
| <b>EC (mg)</b>                            | 100         | 100         | 100         | 100         |
| <b>PEG-400 (%w/w)</b>                     | 30          | 30          | 30          | 30          |
| <b>Propylene Glycol (%w/w)</b>            | 15          | 15          | 15          | 15          |

Calculation of dose [19]

Area of petridish = 38.465 cm<sup>2</sup>  
 No. of films of 4 cm<sup>2</sup> in whole plate = 9  
 Amount of drug in each film = 25 mg  
 Total amount of drug required = 225 mg  
 Label claim of films = 25 mg

Figure 6.1 : Solvent evaporation using inverted funnel





- **Evaluation of Transdermal Patches [20]**

**Physical appearance :**

The formulated patches were evaluated for homogeneity, transparency, clarity, color, and smoothness.

**Uniformity of weight test :**

The patches were subjected to mass variation by individually weighing each formulated patch and checking the weight of patch against the average weight of the formulated patches. Measurement of patch weight was carried out using a calibrated analytical balance. The determination was carried out for each formulation in triplicate.

**Thickness :**

The thickness of each patch was measured by the use of vernier caliper at six different positions of the patch and the average was calculated.

**Surface pH :**

The surface pH of the transdermal patches was measured using a calibrated pH meter. In a test tube, 1 mL of distilled water and a 1 cm<sup>2</sup> portion of transdermal patch was kept at room temperature (25 ± 2°C) for 2 h. The water from the test tube was decanted and the wet patch was used for surface pH analysis. The pH electrode was placed at three different places at the swollen part of the patch for calculating the average pH.

**Folding endurance :**

Folding endurance was determined by repeatedly folding one patch from the same place till it cracked or broke. The number of times the film could be folded from the same place without breaking/cracking represented the value of folding endurance.

**Drug content test :**

Three pieces of 4 cm<sup>2</sup> were collected by cutting off zones from different parts of patch from each patch. These pieces were dissolved in 10 ml ethanol and were placed on vortex shaker for 1 hr to dissolve completely the patches. The resultant solutions were filtered through the whatman paper and then 0.1 mL solution was withdrawn into another volumetric flask (10 mL) and dilution was made up to 10 mL. The absorbance of this solution was observed at 295 nm using UV-Visible spectrophotometer and the drug content was calculated.

**Percent moisture content :**

The prepared transdermal films were weighed individually and kept in desiccators containing fused calcium chloride at room temperature for the duration of 24 hours. After 24 hours, the films were re-weighed and the percentage moisture content was determined by the given formula

$$\text{Percentage of moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

**In-vitro permeation study :**

In-vitro permeation studies of the transdermal patches were carried out by using Franz diffusion cell with a receptor compartment capacity of 30 ml. The formulated patch of surface area of 4 cm<sup>2</sup> was placed in between the dialysis membrane and the donor compartment and then dialysis membrane was mounted between the donor and receptor compartment of diffusion cell. The receptor compartment of diffusion cell was filled with phosphate buffer saline pH 7.4. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was constantly and continuously stirred magnetic beads at 50 rpm; the temperature was maintained at 37±0.5°C. The 1 ml aliquots were withdrawal at different time intervals (0, 2, 4, 6, 8, 12 and 24 h) and analyzed the drug content by UV at 295 nm by appropriated

dilution with ethanol. The receptor phase was replenished with an equal volume of phosphate buffer (37°C) at each sample withdrawal, the cumulative amount of drug permeated per square centimeter of patches were plotted against time.

Figure 6.2 : Franz diffusion cell assembly



## 7. Results and Discussion

- Physical evaluation, melting point and solubility

The sensory organ (eye, tongue, skin and nose) have been used to perform the organoleptic evaluation of griseofulvin. The melting point has been determined using open capillary method and the result of the same is reported uncorrected for environmental factors (Table 7.1).

Table 7.1 : Organoleptic features and melting point of griseofulvin

| S. No. | Characteristic       | Specification [21]   | Observed   |
|--------|----------------------|--|--|
| 1      | Color and appearance | White to pale-cream; crystalline   | Pale-cream; amorphous  |
| 2      | Taste                | Tasteless  | Slightly bitter  |
| 3      | Odor                 | Odorless   | Odorless   |
| 4      | Melting Point        | 220°C  | 224-226°C  |
| 5      | Solubility           | Soluble in DMF; slightly soluble in ethanol, methanol, chloroform, acetone, acetic acid, benzene & ethyl acetate; practically insoluble in water | Soluble in methanol, chloroform; slightly soluble in water and ethanol |

The octanol-water partition coefficient of the griseofulvin sample was calculated to be 1.21 whereas the loss on drying was obtained to be 0.61%.

• **Compatibility study by FTIR**

The FTIR spectrum of griseofulvin (figure 7.1a) exhibited significant peaks of C-N stretch, C=O stretch, C-O-C stretch, N-H and O-H stretch and the peaks were compared to the standard spectra available at NIST [22]. No deletion of the characteristic peaks of griseofulvin was found in the FTIR spectrum of the physical mixtures of drug and polymer (figure 7.1b, 7.1c).

Figure 7.1a : FTIR spectra of griseofulvin

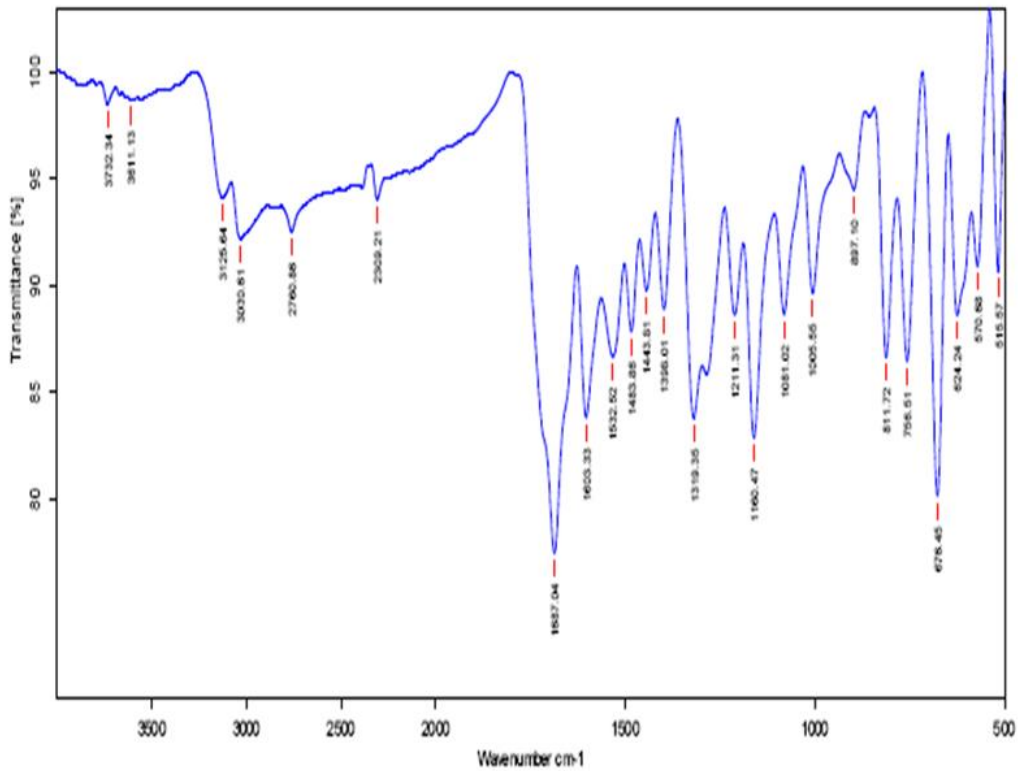


Figure 7.1b: FTIR spectrum of lecithin, cholesterol & griseofulvin

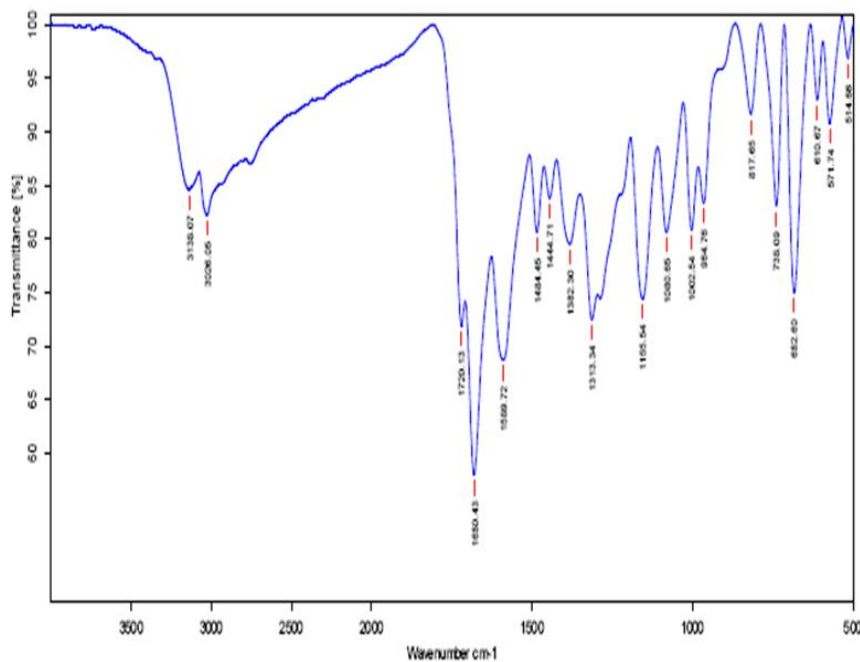
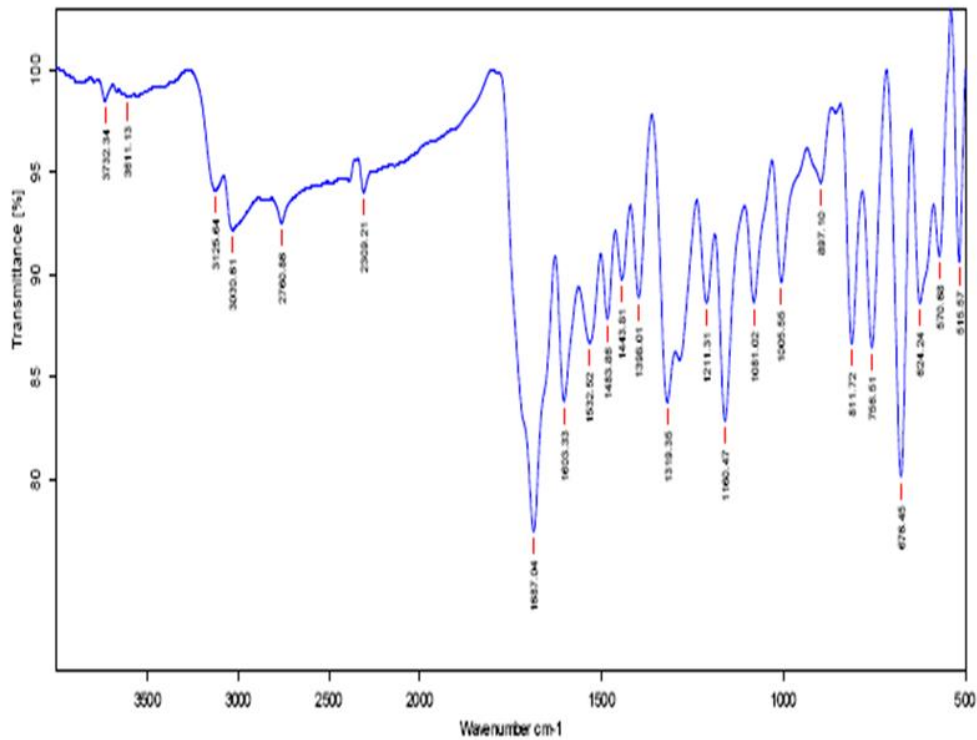


Figure 7.1c : FTIR spectrum of HPMC, EC & griseofulvin



• **Construction of calibration curve**

The calibration curve of griseofulvin was constructed in methanol at concentration range of 10-60 µg/mL. The  $\lambda_{max}$  was found to be 295 nm and was used for all the analysis of drug.

Figure 7.2 : UV spectrum of griseofulvin

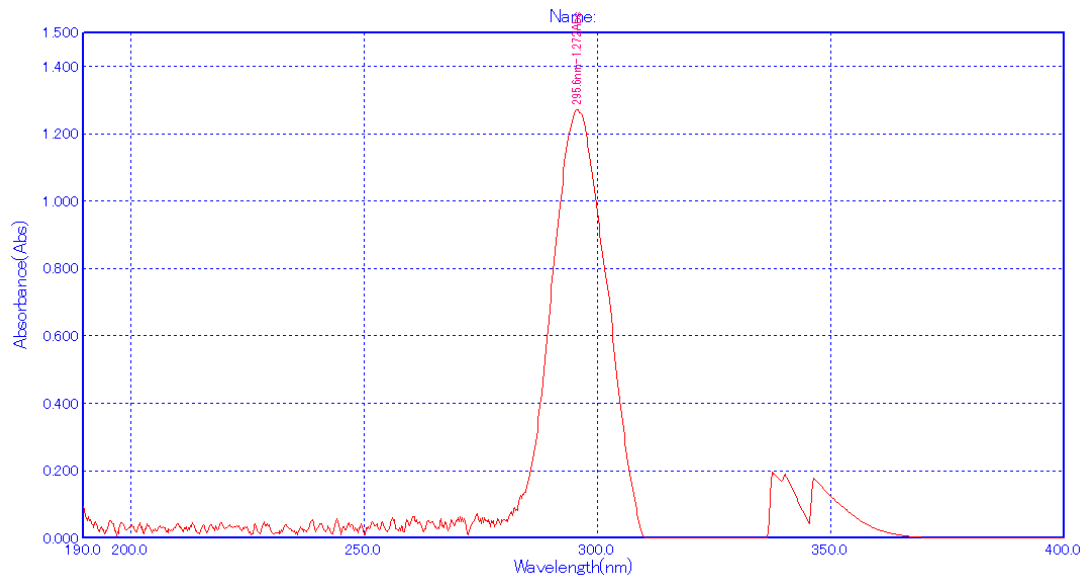
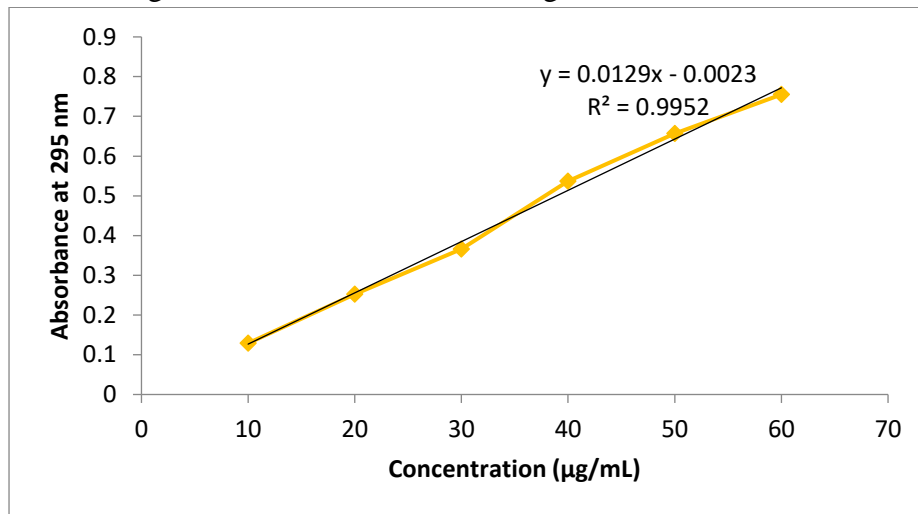


Table 7.2 : Absorbance of griseofulvin by UV spectrophotometer

| Concentration (µg/ml) | Mean absorbance ±SD (n = 3) |
|-----------------------|-----------------------------|
| 10                    | 0.129 ± 0.003               |
| 20                    | 0.252 ± 0.005               |
| 30                    | 0.366 ± 0.004               |
| 40                    | 0.537 ± 0.005               |
| 50                    | 0.657 ± 0.008               |
| 60                    | 0.755 ± 0.004               |

Figure 7.3 : Calibration curve of griseofulvin in methanol



• **Griseofulvin Liposomes**

The process and formulation and parameters strongly affect the properties of drug-loaded liposomes. The parameters used to characterize the liposomes in the preliminary experiments included particle size and the encapsulation efficiency.

Particle size :

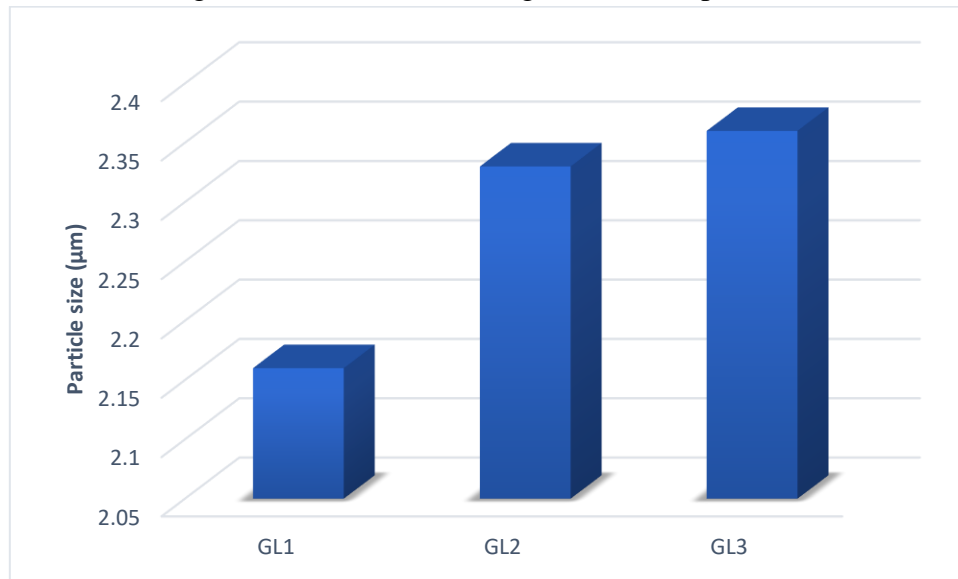
Table 7.3 shows the average size of griseofulvin liposomes prepared using various combinations of cholesterol and soy lecithin. All values are reported as mean ± standard deviation.

A total of around 200 particles of all the formulations were observed under the microscope and the particle size of each particle was calculated using the calibrated eye piece. The particle size of the formulations ranged from  $2.16 \pm 6.0274$  to  $2.36 \pm 6.6146$  µm.

Table 7.3 : Particle size of liposomes

| Formulation | No. of Particles counted | Average Particle Size (µm) |
|-------------|--------------------------|----------------------------|
| GL1         | 212                      | $2.16 \pm 6.0274$          |
| GL2         | 208                      | $2.33 \pm 6.4835$          |
| GL3         | 211                      | $2.36 \pm 6.6146$          |

Figure 7.4 : Particle size of griseofulvin liposomes



The particles size was found to slightly increase by increasing the concentration of lecithin in the formulations. Cholesterol is commonly added in liposomes to provide rigidity to the bilayer and improve the physical stability of liposomes.

Entrapment Efficiency :

The result of drug entrapment efficiency of liposomes (Table 7.4) indicates that as the concentration of lecithin increases, drug entrapment efficiency of liposomes decreases which may be due to the saturation of lipid bilayer. The encapsulation efficiency of liposomes is governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Cholesterol improves the fluidity of the bilayer membrane and improves the stability of bilayer membrane in the presence of biological fluids such as blood/plasma. The entrapment efficiency ranged from 27.37 to 49.35%.

Table 7.4 : Entrapment efficiency of griseofulvin in liposomes

| Formulation | Entrapment Efficiency (%) |
|-------------|---------------------------|
| GL1         | 27.37 ± 0.1202            |
| GL2         | 34.44 ± 0.4030            |
| GL3         | 49.35 ± 1.4283            |

The liposomal griseofulvin formulation with highest entrapment was used for incorporation in to transdermal patches.

• **Transdermal films (patch)**

Transdermal patches were prepared using Hydroxy propyl methyl cellulose (HPMC) as the hydrophilic matrix and ethyl cellulose (EC) as the lipophilic component. The elasticity of the patches was attained using PEG-400 (30% polymeric weight) as the plasticizer and propylene glycol (15% polymer weight) was used as the permeation enhancer to assist permeation of drug into the dermis. Solvent casting method is the most widely used and the simplest method for formulation of transdermal patches. The use of inverted funnel allows for controlled evaporation of the solvents from the patch. The patches were

prepared by varying the ratio of the hydrophilic and the lipophilic matrix. EC and HPMC were used in 4 different ratios (1:1, 1:2, 1:3, 1:4) to obtain the most optimized formulation

Physical evaluation of patches :

All the prepared patches were subjected to visual inspection for examining the physical appearance. The physical appearance of the patches gave satisfactory results. All the prepared patches were found to be smooth, non-sticky, opaque, homogeneous, and flexible in nature.

Physicochemical properties of prepared patches :

The evaluation of the patches was done for various physical parameters as per procedure and the results are reported in Table 7.5.

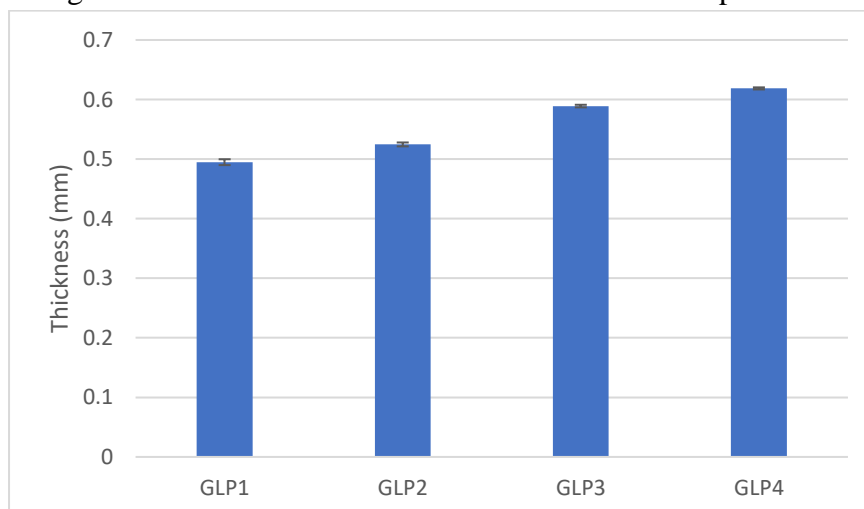
Table 7.5 : Characteristics of griseofulvin patches

|             | Thickness (mm) | Average weight (mg) | Moisture loss (%) | Drug content (%) | Folding Endurance | Surface pH    |
|-------------|----------------|---------------------|-------------------|------------------|-------------------|---------------|
| <b>GLP1</b> | 0.494 ± 0.0049 | 28.95 ± 0.1715      | 7.45 ± 0.3535     | 94.43 ± 0.4163   | 65.0 ± 2.3094     | 5.27 ± 0.0057 |
| <b>GLP2</b> | 0.524 ± 0.0032 | 33.29 ± 0.200       | 7.75 ± 0.0707     | 95.56 ± 0.2516   | 67.5 ± 2.5166     | 5.37 ± 0.01   |
| <b>GLP3</b> | 0.589 ± 0.0021 | 38.49 ± 0.0152      | 10.35 ± 0.2121    | 96.46 ± 0.2081   | 74.5 ± 1.5275     | 5.58 ± 0.0115 |
| <b>GLP4</b> | 0.618 ± 0.0017 | 43.70 ± 0.0152      | 12.1 ± 0.2828     | 97.13 ± 0.2516   | 77.5 ± 2.0816     | 5.61 ± 0.0152 |

As shown in the table the pH levels of the patches ranged between 5.27 to 5.61 suggesting their suitability of human use and possibly suggesting that no skin irritation would be produced on application of the patches.

The thickness of the transdermal patches ranged from 0.494 ± 0.0049 mm to 0.618 ± 0.0017 mm. This difference in the thickness could be attributed to the nature and concentrations of polymers, i.e., an increase in the concentration of the hydrophilic polymer HPMC led to an increased thickness of the transdermal patch (Figure 7.5).

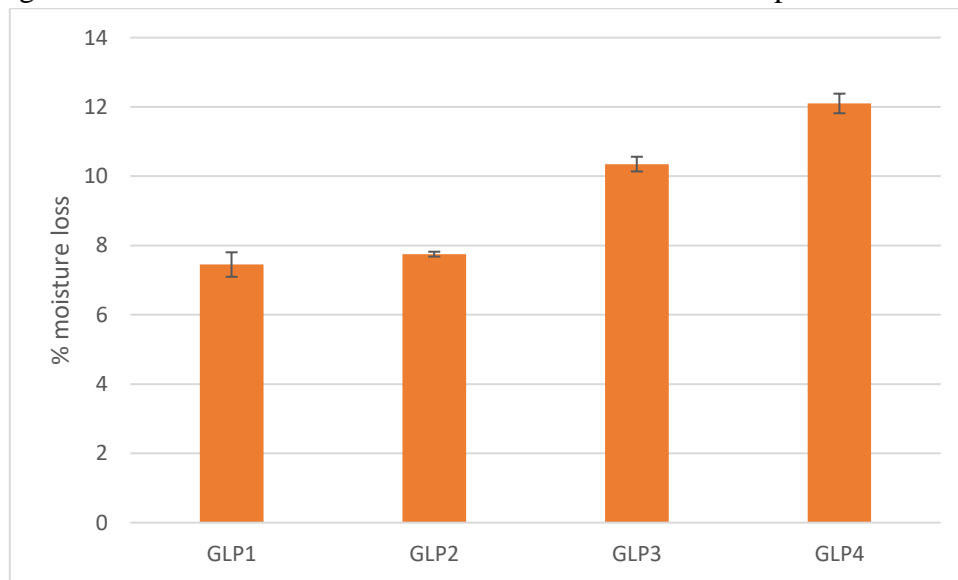
Figure 7.5: Variation in thickness of the transdermal patch formulations



The formulated transdermal patches displayed weight variations between  $28.95 \pm 0.1715$  mg and  $43.70 \pm 0.0152$  mg. It was revealed from the weight variation data that the increase in the concentration of HPMC resulted in an increased weight of patches. This might be due to the fact that HPMC possesses a greater affinity for water and greater moisture uptake, causing an increased patch weight. The HPMC polymer is more hygroscopic in nature in comparison to EC; it might cause water retention in the patches, thereby resulting in increased weight of patches.

The moisture content of the formulated transdermal patches varied from  $7.45 \pm 0.3535\%$  to  $12.10 \pm 0.2828\%$ . Once again, the formulations containing greater amounts of HPMC resulted in an increase in moisture content. As HPMC is hydrophilic and it can cause absorption, as well as retention, of water in transdermal patches (Figure 7.6).

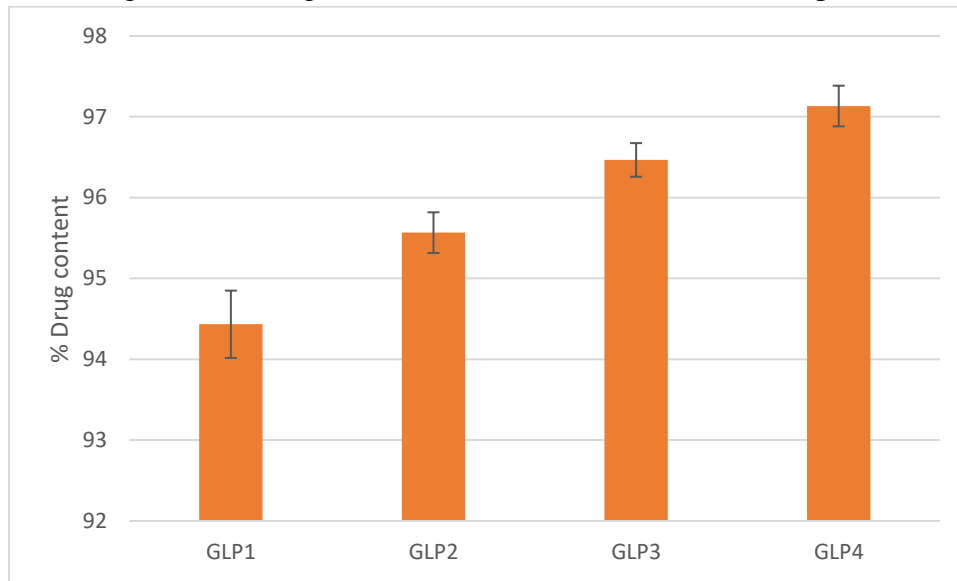
Figure 7.6 : Variation in moisture content of the transdermal patch formulations



Folding endurance is of utmost importance for patches because greater folding endurance prevents patches from being easily broken or damaged, and patches are considered to meet good quality. All the formulated transdermal patches exhibited high folding endurance (>60 times). This reveals that all transdermal patches meet the standard patch requirements. Different concentrations of the polymers (HPMC and EC) did not considerably affect the folding endurance of the transdermal patches though higher HPMC content increased the folding endurance. PEG400 was used as a plasticizer for obtaining flexible patch formulation. All the transdermal patch formulations exhibited uniform drug content and with a minimum variability within the batch. The drug content ranged from  $94.43 \pm 0.4163\%$  to  $97.13 \pm 0.2516\%$  (Figure 7.7). This drug content range is deemed suitable for transdermal application.



Figure 7.7 : Drug content in the formulated transdermal patches



From the above figure it was very clear that increase in the concentration of HPMC caused an increase in drug loading in the patch.

**In-vitro permeation study :**

The amount of drug that permeated or released from the transdermal patches was determined using Franz diffusion cell.

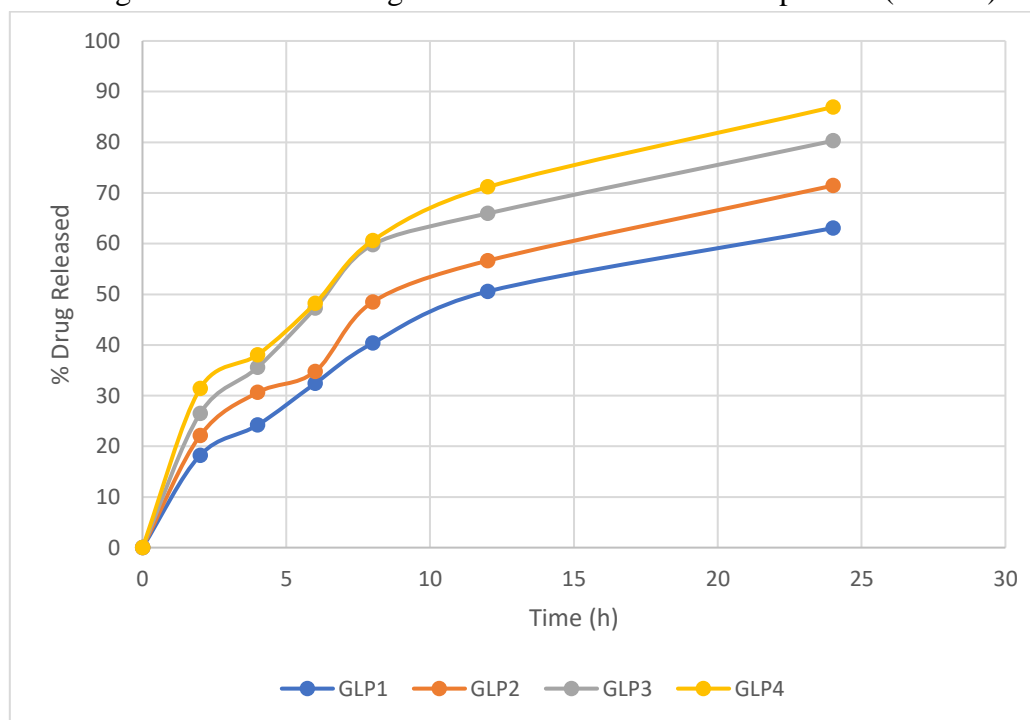
The in vitro drug release study depicted that the highest amount of drug was released from GLP4 ( $86.56 \pm 1.145$ ) while the lowest was released from GLP1 ( $63.06 \pm 1.793\%$ ) at the end of 24 hours of release study. Faster drug release was observed from formulated patches containing greater amounts of the hydrophilic polymer, HPMC. The study also depicted an increase in hydrophilic polymer that resulted in an increase in burst effect, as well as drug release in the formulation (Table 7.6, Figure 7.8).

Table 7.6 : In vitro release of Griseofulvin from transdermal patches

| Formulation | Drug Release (%) |                     |                     |                     |                     |                     |                     |
|-------------|------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|             | 0                | 2                   | 4                   | 6                   | 8                   | 12                  | 24                  |
| GLP1        | 0                | 18.21<br>±<br>0.995 | 24.18<br>±<br>1.342 | 32.40<br>±<br>0.608 | 41.34<br>±<br>0.773 | 50.56<br>±<br>1.105 | 63.06<br>±<br>1.793 |
|             | 0                | 22.08<br>±<br>2.718 | 30.62<br>±<br>0.986 | 38.26<br>±<br>3.011 | 48.47<br>±<br>0.626 | 56.63<br>±<br>0.040 | 71.44<br>±<br>1.520 |
| GLP3        | 0                | 26.44<br>±<br>1.245 | 35.57<br>±<br>1.042 | 47.23<br>±<br>0.706 | 59.79<br>±<br>1.194 | 65.96<br>±<br>0.097 | 80.28<br>±<br>0.436 |

|      |   |                     |                     |                     |                     |                     |                     |
|------|---|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| GLP4 | 0 | 31.37<br>±<br>0.566 | 38.05<br>±<br>0.486 | 48.19<br>±<br>0.385 | 60.58<br>±<br>0.714 | 71.17<br>±<br>0.652 | 86.95<br>±<br>1.145 |
|------|---|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|

Figure 7.8 : Release of griseofulvin from transdermal patches (in vitro)



## 8. Summary

In the present study the prime objective was to develop transdermal patches loaded with griseofulvin liposomes in order to improve its bioavailability by sustaining the drug release.

Griseofulvin encapsulated liposomes were prepared by a modified ethanol injection method using soy lecithin (20, 40, 60 mg/ml) and cholesterol (2 mg/ml) as the lipids require to prepared the liposomes and maintain its stability. The characterization of the liposomes was carried out by estimation of encapsulation efficiency and particles size of the liposomes.

The particle size of the formulations ranged from  $2.16 \pm 6.0274$  to  $2.36 \pm 6.6146$   $\mu\text{m}$  and the entrapment efficiency ranged from 27.37 to 49.35 %.

Transdermal patches were prepared using Hydroxy propyl methyl cellulose (HPMC) as the hydrophilic matrix and ethyl cellulose (EC) as the lipophilic component. The elasticity of the patches was attained using PEG-400 (30% polymeric weight) as the plasticizer and propylene glycol (15% polymer weight) was used as the permeation enhancer to assist permeation of drug into the dermis. The patches were prepared by varying the ratio of the hydrophilic and the lipophilic matrix. EC and HPMC were used in 4 different ratios (1:1, 1:2, 1:3, 1:4) to obtained the most optimized formulation

All the prepared patches were subjected to visual inspection for examining the physical appearance. The physical appearance of the patches gave satisfactory results. All the prepared patches were found to be smooth, non-sticky, opaque, homogeneous, and flexible in nature.

The pH levels of the patches ranged between 5.27 to 5.61 suggesting their suitability of human use and possibly suggesting that no skin irritation would be produced on application of the patches.

The thickness of the transdermal patches ranged from  $0.494 \pm 0.0049$  mm to  $0.618 \pm 0.0017$  mm. This difference in the thickness could be attributed to the nature and concentrations of polymers, i.e., an increase in the concentration of the hydrophilic polymer HPMC led to an increased thickness of the transdermal patch.

The formulated transdermal patches displayed weight variations between  $28.95 \pm 0.1715$  mg and  $43.70 \pm 0.0152$  mg. It was revealed from the weight variation data that the increase in the concentration of HPMC resulted in an increased weight of patches. The moisture content of the formulated transdermal patches varied from  $7.45 \pm 0.3535\%$  to  $12.10 \pm 0.2828\%$ .

All the formulated transdermal patches exhibited high folding endurance ( $>60$  times). This reveals that all transdermal patches meet the standard patch requirements.

All the transdermal patch formulations exhibited uniform drug content and with a minimum variability within the batch. The drug content ranged from  $94.43 \pm 0.4163\%$  to  $97.13 \pm 0.2516\%$

The in vitro drug release study depicted that the highest amount of drug was released from GLP4 ( $86.95 \pm 1.145$ ) while the lowest was released from GLP1 ( $63.06 \pm 1.793\%$ ) at the end of 24 hours of release study.

## 9. Conclusion

The hypothesis to be verified in the study was that as the absorption of griseofulvin is low, formulating griseofulvin as liposome and incorporating the liposome into topical film (patch) would be helpful to improve efficacy and in turn its topical absorption and bioavailability. The liposomes were effectively prepared using ethanol injection method and the patches using solvent evaporation method. The release study revealed that the drug was released up to 24 h. Thus it could be concluded from the study that incorporating liposomes in transdermal patch could be an effective method to improve the bioavailability of poorly soluble drugs.

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