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Formulation and Evaluation of Topical Liposomes Loaded with Antifungal Antibiotic Griseofulvin

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ABSTRACT
In order to perform the preformulation evaluation of the drug tests of identification such as physical appearance, melting point and FTIR spectroscopy were carried out. The solubility profile of drug in various solvent systems, incompatibility study by FTIR, partition coefficient and quantitative estimation of drug was also studied. The calibration curve of griseofulvin was constructed in methanol at concentration range of 10-60 µg/mL. The λmax was found to be 295 nm and was used for all the analysis of drug. The particle size and zeta potential were studied using Malvern zeta sizer and the particles were found to be having an average particle size of 158.1 nm to 268.4nm with a poly dispersity index of 0.417 to 0.521. The zeta potential of the formulation was found to be -17.1 to -17.9 mV. The entrapment efficiency ranged from 67.1 to 68.9 %. The maximum release was obtained in F3 (89.2%) while the lowest was found in F4 (78.4%) in 12 hours. The liposomal griseofulvin loaded formulation C3 & C4 released 77.3 and 75.1% griseofulvin in 12h. The antibacterial action of the liposomal cream formulation was compared to that of the pure drug solution and it was found that the liposomal formulations loaded with griseofulvin were able to exhibit comparable antibacterial activity against Staphylococcus aureus in the disc diffusion assay, as measured using the zone of inhibition.

Keywords: Antifungal, topical, liposome, griseofulvin, controlled release

I. INTRODUCTION

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 (Kumar et al., 2023). 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 (Lengert et al., 2020). 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 used orally to treat superficial fungal infections, primarily fingernail and toenail infections, but it does not penetrate skin or nails if used topically (Bavasrad et al., 2016)). 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 (drug bank, 2025). It a well-known fact that liposomes have the capacity to improve the stability of incorporated drug molecules (Al-Darraji et al, 2020). The objective of this project is to develop, optimize and characterize griseofulvin loaded liposomes, formulate them into cream preparation for topical use and to determine its antifungal efficiency and stability.

II. MATERIAL AND METHODS

Preformulation Studies (Chaurasia, 2016)

In order to perform the preformulation evaluation of the drug tests of identification such as physical appearance, melting point and FTIR spectroscopy were carried out. The solubility profile of drug in various solvent systems, incompatibility study by FTIR, partition coefficient and quantitative estimation of drug was also studied.

Calibration Curve

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 (Dash and Mishra, 2012). A calibration curve was prepared by analyzing a series of working standards at 295 nm using UV spectrophotometer.

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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 & 4 mg/ml) were dissolved in ethanol and griseofulvin (200 mg) was added to the organic phase (Table 1). Resulting organic phase was injected by means of a syringe pump to aqueous phase under magnetic stirring at 45 ± 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.

Table 1 Composition of liposome formulations

Formulation	Soy Lecithin (mg)	Cholesterol (mg)	Griseofulvin (mg)	Ethanol (mL)
GL1	10000	100	200	100
GL2	200	100	200	100
GL3	3000	100	200	100
GL4	1000	200	200	100
GL5	2000	200	200	100
GL6	3000	200	200	100

Characterization of Liposomes

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.

Zeta Potential

Zeta potential was measured by laser Doppler electrophoresis, using a Zetasizer Nano ZS90 analyser.

In vitro drug release

In-vitro drug release study of liposomal formulations was performed using franz diffusion cell. An egg membrane was placed between donor and receptor compartments. The receptor compartment contained phosphate buffer pH 6.8 was continuously stirred by magnetic bead and maintained at temperature of 37 ± 1ºC. One ml liposomal suspension was loaded on the donor compartment. The drug concentrations in aliquot were withdrawn at different time intervals and analyzed at 295 nm against appropriate blank.

Stability of Liposomes

Liposomal size and drug retention were used as parameters to preliminarily indicate the physical stability of liposomes. The protocol was adapted from Yang et al (2012) and Nkanga et al (2019).

Formulation of liposomal cream

Different formulations containing varying amounts of liposomes and other additive are shown in Table 2. Free griseofulvin, and griseofulvin liposomes were incorporated in cream base. An oil in water type of cream formulated. Firstly the desired concentration of oil phase i.e., stearic acid and lanolin were taken and heated in mineral oil at temperature not exceeding 70°C. The prescribed ratio of griseofulvin was dissolved in the oil phase. Separately, the water and triethanolamine were mixed together to prepare the aqueous phase. Both the phases were mixed together while triturating to obtain a consistent cream.

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Table 2 Liposomal cream formulation

S.No	Ingredients	Quantity of additives in various formulations			
		C1	C2	C3	C4
1	Griseofulvin	2 %w/w	2 %w/w	-	-
2	Griseofulvin liposome	-	-	2 %w/w	2 %w/w
3	Mineral Oil	10 g	10 g	10 g	10 g
4	Stearic acid	15 g	10 g	15 g	10 g
5	Lanolin	5 g	7 g	5 g	7 g
6	Triethanolamine	2 mL	2 mL	2 mL	2 mL
7	Water	48 mL	48 mL	48 mL	48 mL

Evaluation of cream (Panda and Ghosh, 2010; Purushottamrao et al., 2010)

pH of the formulation

Accurately weighed quantity of 5 g of each cream formulation was mixed separately with 45 mL of distilled water and the pH of the solution was determined with the help of digital pH meter.

Viscosity

The viscosity of each formulation was measured at 10 rpm by using Brookfield DV-1 viscometer employing a S94 spindle.

Spreadability

Spreadability of the formulations was determined using indigenously developed apparatus. The apparatus consisted of a wooden block provided with a pulley at a one end. A rectangular ground glass was fixed on the block. An excess of cream (3-5 g) was placed on this plate sandwiched using another glass plate having the dimensions as that of fixed ground plate. A 1 kg weight was placed on the top of the plates for 5 minutes to expel air and to provide a uniform film of the cream between the plates. Excess of the ointment was scrapped off from the edges. Weight of 80 g was hung on the hook of the top plate with the help of string attached to the hook and the time (in seconds) required by top plate to cover a distance of 10 cm was noted. Spreadability of the formulation was determined by the following formula:

$S = M * L/T$

In vitro release

In-vitro drug release study of cream formulations was performed using franz diffusion cell. An egg membrane was placed between donor and receptor compartments. The receptor compartment contained phosphate buffer pH 6.8 was continuously stirred by magnetic bead and maintained at temperature of 37 ± 1ºC. One gram cream was loaded on the donor compartment. The drug concentrations in aliquot were withdrawn at different time intervals and analyzed at 295 nm against appropriate blank.

Evaluation of antibacterial activity of cream

Lyophilized bacterial culture of Staphylococcus aureus was procured from Institute of Microbial Technology, Chandigarh. The lyophilized culture was revived using previously sterilized nutrient broth by incubation at 37°C for 24 h. The liposome cream was diluted in sterile distilled water to obtain a concentration of 100 µg/mL griseofulvin. 1mL of this solution was soaked in cellulose acetate circular paper disc for testing the antibacterial action. The sterilized media (nutrient agar) was cooled to 45°C and inoculated with the revived bacterial culture in a laminar air flow bench. This was poured in to sterile Petri dish and allowed to solidify and the test sample disc was carefully placed on the solidified media by using sterilized forceps. These Petri dishes were kept in the laminar air flow unit undisturbed for one-hour diffusion at room temperature and then for incubation at 37°C for 24 h in an incubator. The antibacterial action of the liposome was assessed by measuring the zone of inhibition of bacterial growth exhibited by the test sample disc.

III. RESULTS AND DISCUSSION

Preformulation Studies

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 3).

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Table 3 Organoleptic features and melting point of griseofulvin

S. No.	Characteristic	Observation
1	Color and appearance	Pale-cream; amorphous
2	Taste	Slightly bitter
3	Odor	Odorless
4	Melting Point	224-226°C
5	Solubility	Soluble in methanol, chloroform; slightly soluble in water and ethanol

The calibration curve of griseofulvin was constructed in methanol at concentration range of 10-60 µg/mL. The λmax was found to be 295 nm and was used for all the analysis of drug (Figure 1).

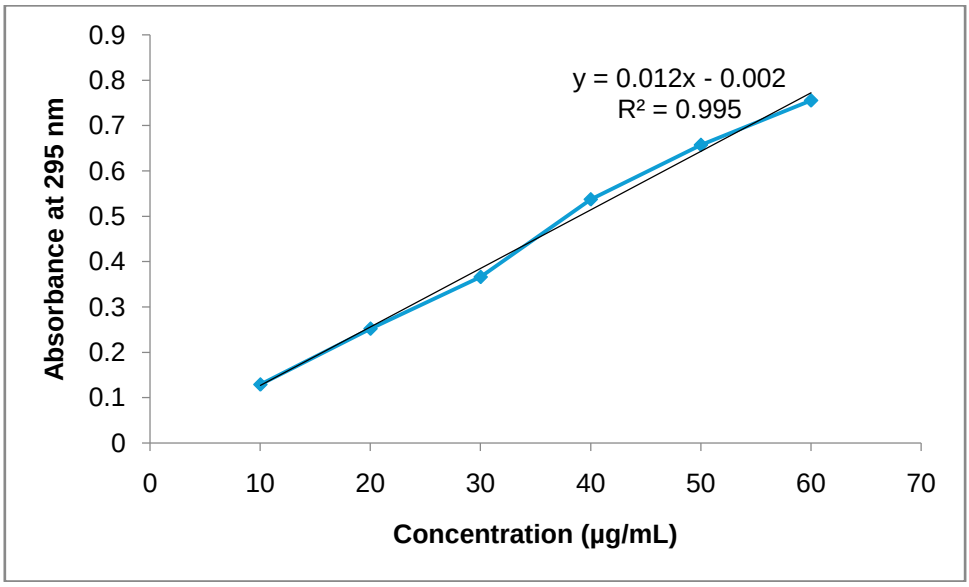

Figure 1 Calibration curve of griseofulvin in methanol

Liposomal formulation

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, the encapsulation efficiency and the in vitro drug release profile. Stability studies using particle size as an indicator of stability were also conducted for a 3-month period.

Particle size and zeta potential

The particle size and zeta potential were studied using Malvern zeta sizer and the particles were found to be having an average particle size of 158.1 nm to 268.4nm with a poly dispersity index of 0.417 to 0.521. The zeta potential of the formulation was found to be -17.1 to -17.9 mV. The high poly dispersity index of the particles could be attributed to the low zeta potential of the formulation (Table 4).

Table 4 Particle size, zeta potential and entrapment efficiency of liposomes

	Size (nm)	PDI	ZP (mV)	Entrapment Efficiency (%)
GL1	221.3	0.417	-17.5	68.2
GL2	189.7	0.513	-17.7	67.1
GL3	158.1	0.428	-17.9	68.4
GL4	192.4	0.456	-17.5	68.2
GL5	244.5	0.508	-17.4	68.5
GL6	268.4	0.521	-17.1	68.9

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It can be observed from the results that the particles size was very affected by the concentration of soy lecithin. The particles size was found to decrease on increasing concentration of lecithin and slightly increase by increasing the concentration of cholesterol in the formulations. Cholesterol is commonly added in liposomes to provide rigidity to the bilayer and improve the physical stability of liposomes. As the concentration of cholesterol increases more cholesterol molecules get distributed in the phospholipid bilayer, leading to an increase in the liposome mean size.

Entrapment Efficiency

The result of drug entrapment efficiency of liposomes (Table 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 67.1 to 68.9 %. The ability of cholesterol of hold the permeability of liposome was evident from the results of entrapment efficiency.

In vitrorelease

The in vitro release of griseofulvin from the liposomes was studies using Franz diffusion cell. The release was found to be affected by the amount of Lecithin as well as cholesterol in the formulation. While increasing the concentration of lecithin increased drug release, cholesterol was found to decrease the release. Formulations F4, F5 and F6 were found to exhibit slightly lower drug release throughout the study period. The maximum release was obtained in F3 (89.2%) while the lowest was found in F4 (78.4%) in 12 hours (Figure 2).

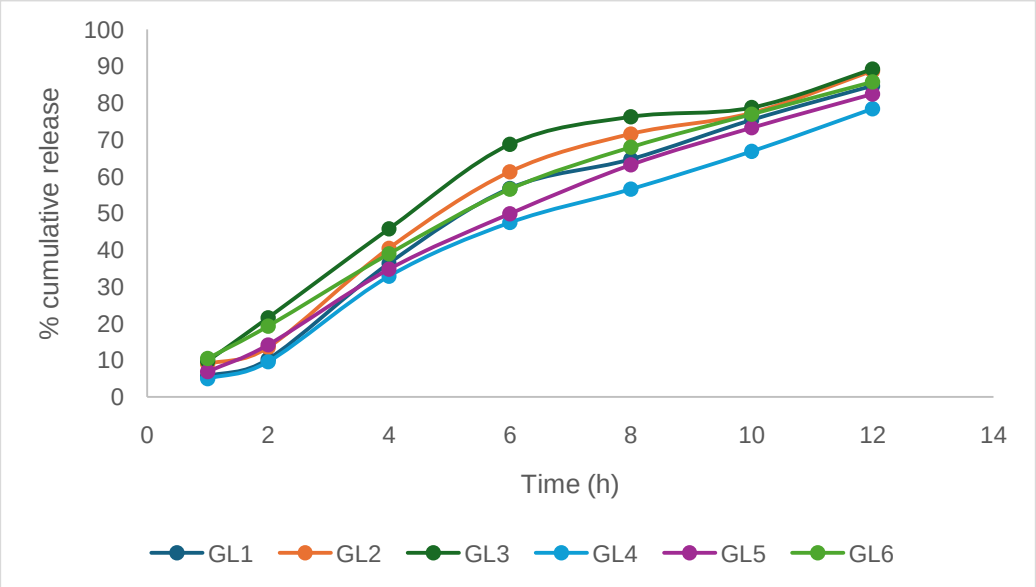

Figure 2 Drug release profile of griseofulvin from liposomes

Stability of liposomes

The change in particle size over a period of three months was considered to ascertain the stability of the liposomal formulation. No significant change in particle size was observed suggesting that the formulations were stable at the storage conditions.

Liposomal Cream Formulations

The data of the evaluated formulations LC1-LC4 are represented in Table 5; all the parameters were found to be in acceptable limits.

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Table 5 Physicochemical data of formulations LC1-LC4

Formulation	pH	Viscosity	Spreadability (g.cm/sec)
LC1	6.4	6100	18.25
LC2	6.3	6900	14.20
LC3	6.1	6300	17.70
LC4	5.9	7200	14.75

The consistency of the formulations was found to be proper in all the batches and the viscosity increased in formulations with higher amount of Lanolin. The in vitro release of griseofulvin from the liposomal cream was studied using Franz diffusion cell utilizing egg membrane as the skin simulating membrane (Figure 3).

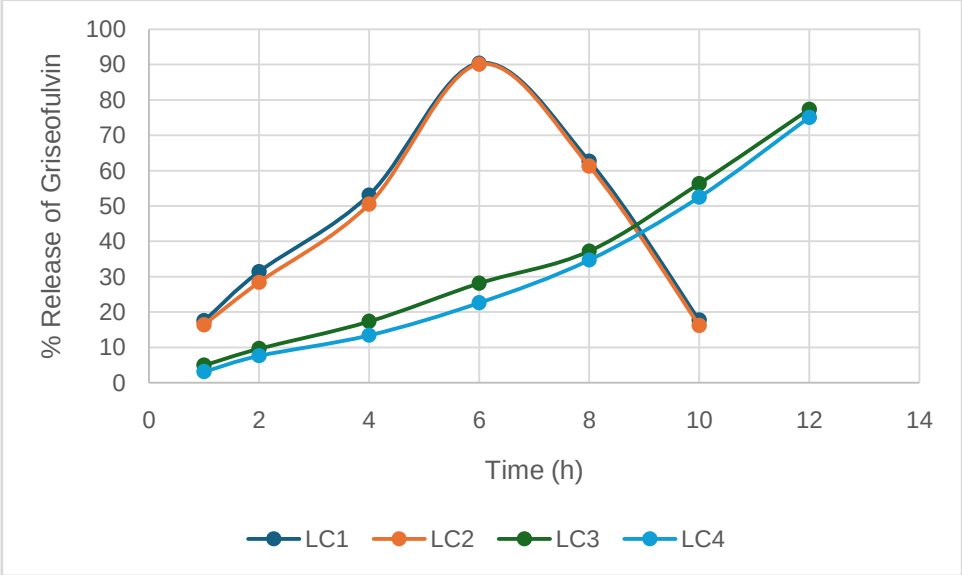

Figure 3 In vitro release profile of ketoconazole from gel formulations

The formulations loaded with griseofulvin released almost all the drug in 6 h (90.4 & 90.1% for LC1 & LC2 respectively) after which the concentration began to decrease suggesting degradation of the drug. No free griseofulvin could be detected in the 12th hour. On the other hand, the liposomal griseofulvin loaded formulation C3 & C4 released 77.3 and 75.1% griseofulvin in 12h. This suggests controlled release of griseofulvin making the formulations suitable for once-a-day topical application.

Antibacterial Activity of griseofulvin liposomal cream

The antibacterial action of the liposomal cream formulation was compared to that of the pure drug solution and it was found that the liposomal formulations loaded with griseofulvin were able to exhibit comparable antibacterial activity against Staphylococcus aureus in the disc diffusion assay, as measured using the zone of inhibition.

IV. CONCLUSION

The developed liposomal cream formulation presented a release of griseofulvin for at least 12 hours, suggesting an improved half-life, and bioavailability of the drug. The study helps in

establishing the liposomal cream as an effective way for topical application of griseofulvin in treatment of microbial infections.

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