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Formulation and evaluation of luliconazole liposomal gel

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Abstract

This investigation presents an innovative formulation and evaluation of luliconazole liposomal gel, designed to effectively bypass first-pass metabolism, improve bioavailability, and enhance the drug release mechanism for targeted drug delivery. Utilizing the physical dispersion method with varying lipid concentrations in chloroform, we successfully developed liposomes that were converted into a gel, creating a superior transdermal drug delivery system. We generated eight distinct liposomal gel formulations, each undergoing comprehensive evaluation studies, including identification, drug-polymer compatibility, solubility, morphological characteristics, particle size, drug content, zeta potential, entrapment efficacy, spreadability, and pH assessments. The results demonstrated that our liposomes were uniformly spherical and free of incompatibility. The zeta potential measurements ranged from -42.5 mV to -32 mV, and the entrapment efficacy was impressive, ranging from 65.49% to 84.02%. Furthermore, the particle sizes varied between 262.58 nm and 654.06 nm. With a pH below 5.5 and a viscosity ranging from 42510 to 63768 cps, our liposomal gel showcased excellent spreadability. Notably, these formulations exhibited zero-order kinetics with non-Fickian diffusion, establishing a reliable and sustained drug release profile that underscores the potential of this innovative delivery system.

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INTRODUCTION

Both effective drug delivery and adequate drug selection are necessary for the best therapeutic results. The most ideal area of the human body for drug distribution is the skin. Over the past three decades, the pharmaceutical industry has placed a greater emphasis on developing appropriate drug delivery. The concentration of the drug at the site of action, which is dependent on the dose form and the amount of drug absorption at the site of action, determines the pharmacological response, which includes the necessary therapeutic effect

and the undesirable side effect of a drug [1]. About one-third of the blood that circulates throughout the body passes through the multilayered organ that is the skin, which occupies an area of about 2 m^2. The epidermis, which makes up the topmost layer of skin, is divided into morphologically separate areas called the spiny layer, stratum granulosum, stratum corneum, and basal layer. It is made up of lipid-membraned cells that are extremely cornified, or dead [2]. The composition of these extracellular membranes is distinct and includes free fatty acids, cholesterol, and ceramides. There are 1/1000 hair follicles and 200–250 worry ducts on each tetragon cm of the skin's surface. It is one of the human body's most easily accessible organs. It has been known for a few decades that the entire skin may serve as a drug administration harbour for the human body. However, the skin is a very difficult barrier to materials, allowing only minute amounts of a drug to pass through over time [3].

METHODOLOGY

Materials: The gift sample Luliconazole is from Yarrow chem. Products, Mumbai. Soya Lecithin, and Methanol from Merck specialities Pvt. Ltd., Cholesterol, Chloroform, Carbopol 934, Methyl paraben, Propyl paraben, and Propylene glycol are from Finar chemicals, Ltd, Ahemedabad.

Pre formulation studies:

The initial step in the rational development of a pharmacological substance's dosage forms is preformulation testing. It is the study of a drug substance's physical and chemical characteristics both by itself and in combination with excipients. [4] Preformulation testing's main goal is to produce data that will help formulators create safe, effective, and stable dosage forms. For the purpose of identification and compatibility research, preformulation tests were conducted on the drug samples that were collected.

Identification of Drug:

Infrared absorption spectral analysis was used to analyse the obtained sample, and the results were compared to the reference standard FTIR spectrum of luliconazole.

Method: IR Spectra of drug and drug-excipient blends were recorded on an IR spectrophotometer (Bruker, Germany) in the range of 4000-400 cm^{-1} using potassium bromide discs [5].

Compatibility Studies

Prior to formulation, it is crucial to ensure that the drug and polymers are compatible under experimental conditions [6]. Drug and excipient incompatibilities can change a drug's stability and bioavailability, which can impact its safety and/or effectiveness. An essential step in creating a stable solid dosage form is researching the compatibility of the medicine and the excipient. Testing for drug-excipient compatibility early on aids in choosing excipients that raise the likelihood of creating a stable dosage form [7].

Estimation of Luliconazole

a) Determination of λ max of Luliconazole in Methanol:

Luliconazole in methanol (100 mg in 100 ml) is the stock solution.
Scanning: Luliconazole 10µg/ml solution made in methanol solution was used as the stock solution, and it was scanned between 200 and 500 nm. For more research, the absorption peak at 427 nm was chosen [8].

a) Preparation of Luliconazole Liposomes:

Using varying ratios of lipids, liposomes were created using the physical dispersion method. Chloroform was used in this approach to dissolve the lipids. This lipid solution in chloroform was applied to a conical flask with a flat bottom. After that, the solution was allowed to evaporate at room temperature without being disturbed [9]. Aqueous medium phosphate buffer (pH 7.4) was used to hydrate the lipid film. This was accomplished by tilting the flask to one side, introducing the drug-containing aqueous medium down the side, and then gradually bringing the flask back to its upright position. For full expansion, the flask was left to stand at 37°C for two hours after the fluid was allowed to gently run over the lipid layer. Swirling the flask's contents produced a milky white solution, which was used to harvest the vesicles once they had swelled. Centrifugation was then applied to the formulations. Every batch of liposomes was made using the basic procedure mentioned above, and **Table 1** lists the lipid composition used in liposome creation [10].

b) Preparation of liposomal gels:

The dispersion approach can be used to create liposomal gels loaded with Luliconazole. This approach involves soaking Carbopol 934 in water for two hours and then stirring it with a magnetic stirrer for forty-five minutes to create a uniform slurry. After that, a 20% NaOH solution is added dropwise to neutralise the mixture and promote gel formation. After dissolving in ethanol, methyl and propyl parabens are added to the mixture mentioned above. The hydrogel bases are simultaneously supplemented with propylene glycol. To create Luliconazole liposomal gel, the afore-mentioned mixture is now combined with drug-dissolved liposome [11].

Table 1 Composition of Luliconazole loaded liposomes

Composition (%)	Formulation code							
	F1	F2	F3	F4	F5	F6	F7	F8
Luliconazole(mg)	100	100	100	100	100	100	100	100
Chloroform(ml)	5	5	5	5	5	5	5	5
Lecithin(mg)	120	150	180	210	240	270	270	270
Cholesterol(mg)	180	150	120	90	60	30	60	90
Phosphate buffer(ml)	10	10	10	10	10	10	10	10

Evaluation of Luliconazole Liposomal gel

Morphology:

The produced liposomes were subjected to morphological analyses through the use of optical microscopy. A small number of samples are spread out over a spotless slide. Images were taken using optical microscopy linked to Dewinter Microscopic camera software (Capture Pro 4 version) after the slide was focussed under optical light[12],[13],[14].

Particle size:

An optical microscope model was used to measure the liposomes' particle size. The size of each manufactured batch of liposomes was examined under a microscope. A tiny drop of liposomal dispersion was placed on each slide to quantify the size of the liposomal vesicles from each batch. The average size of the liposomal vesicles was then calculated.

Drug content:

The homogeneity of drug content was assessed in triplicate by dissolving the liposomes in methanol, centrifuging the dissolved liposomes for two hours at 3000 rpm, and then filtering them using Whatman filter paper (0.45 µm, Whatman, Maidstone, UK). After diluting the solution to Beer's range, it was examined in a UV spectrophotometer.

Entrapment Efficiency:

The centrifugation method was used to measure the liposomes' entrapment efficiency. A laboratory centrifuge (Remi R4C) was used to centrifuge 1 ml aliquots of liposomal dispersion for 90 minutes at 3500 rpm. The absorbance measured at 209 nm and non-entrapped Luliconazole were separated by carefully removing the clear supernatants. The absorbance of the sediment in the centrifuge tube was measured at 209 nm after it had been diluted with 100 ml of methanol. The total amount of Luliconazole in a 1 ml dispersion was determined by the amount of Luliconazole in the sediment and supernatant. The following formula was used to determine the percentage of drug entrapment.

$$\% \text{ Drug Entrapped} = \frac{\text{Total amount of drug} - \text{Unentrapped drug}}{\text{Total amount of drug}} \text{x100}$$

Zeta Potential:

Using a Zeta sizer, Nano ZS 90 (Malvern Instruments), dynamic light scattering was used to estimate the Z-average diameter of liposomes. Zeta potential was measured after 100µl of the formulation was diluted with the proper amount of phosphate buffer (pH 7.4).

Physicochemical Properties of Luliconazole loaded liposomal gel

The physicochemical characteristics of the liposome-enriched hydrogel, including colour, occlusiveness, pH, spreadability, extrudability, and viscosity, were assessed [15],[16].

Measurement of pH:

The digital pH meter was used to measure the pH of different gel compositions. After dissolving one gramme of gel in 100 millilitres of distilled water, it was left for two hours. Each formulation's pH was measured three times, and the average results were computed [17],[18].

Viscosity study:

A Brookfield viscometer was used to measure the created gel's viscosity. Viscosity was measured in Cps while the gels were rotated at a rate of 1.5 revolutions per minute.

Spreadability

A gel sample containing 0.1 g of each formula was sandwiched between two slides and allowed to sit for approximately five minutes. No more spreading was anticipated. The spread circles' diameters were measured in centimetres. They were interpreted as spreadability comparative values.

S = ML/T

Extrudability study

Filling the collapsable tubes with gel allowed for the determination of the gel compositions. The weight in grammes needed to extrude a 0.8 cm gel ribbon was used to calculate the gel formulation.

In-vitro drug release study

Franz diffusion cells were used to conduct the diffusion studies. A treated cellophane membrane was used to divide the donor compartment, which contained the liposomes, from the receptor compartment, which contained 100 millilitres of PBS pH 7.4. A magnetic stirrer was used to stir the receptor compartment during the procedure. At predetermined intervals, the aliquots were taken out, replaced with an equivalent volume of PBS H 7.4, and then examined at 427 nm [19].

Kinetics of drug release :

To examine the drug release kinetics and mechanism the cumulative release data were fitted to models of data treatment as follows [20]:

Cumulative percentage drug release Vs. Time (zero order rate kinetics)

Log cumulative percentage drug retained Vs. Time (first order rate kinetics)

Cumulative percentage drug release Vs. SQRT (Higuchis classical diffusion equation)

Log of cumulative percentage drug release Vs. log time (Peppas exponential equation)

RESULTS AND DISCUSSION

Luliconazole liposomes were successfully created using a variety of natural lipids. The impact of using natural lipid on formulations was evaluated. **Table 1** lists the composition of the eight formulations that were created for this study. Numerous physicochemical parameters were used to characterise the liposome formulation.

PREFORMULATION STUDIES OF PURE DRUG:

Identification of Luliconazole:

It was discovered that the pure drug's IR spectra resembled the conventional spectrum of luliconazole. The following functional groups are visible in Luliconazole's spectrum at the frequencies indicated in 3158.2, 3030, 3358.9, 3008, 1599, 1586, 2728.2, 2868, 1191, and 1107 cm-1.

Drug - polymer Compatibility Studies:

Before liposome formulation, compatibility investigations between lipids and the pure drug liconazole were conducted. **Figure 1** shows the IR spectra of lipids and pure drugs that were obtained. The spectra of Luliconazole showed all of its distinctive peaks at the appropriate wavelengths. This suggests that the medicine and polymers are compatible. It demonstrates that the drug's chemical integrity remained mostly unchanged.

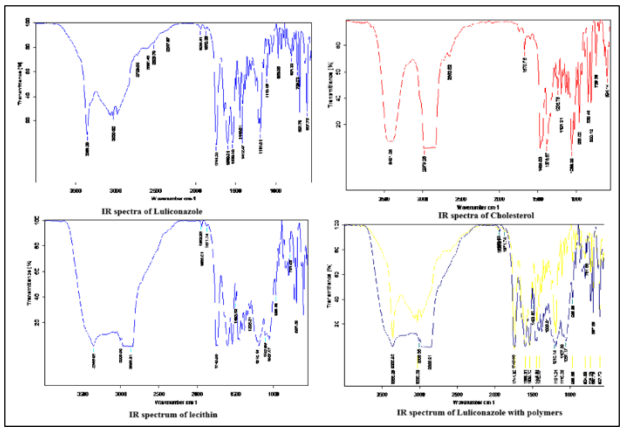

Figure 1 IR spectrum of Luliconazole with polymers

Characterization of prepared liposomes:

Morphology:

The developed liposomes were subjected to morphological analyses through the use of optical microscopy. A clean slide was covered with a small number of samples. Images were taken using optical microscopy linked to Dewinter Microscopic camera software (Capture Pro 4 version) after the slide was focussed under optical light. All vesicle types appeared to have a comparable spherical or oval form based on morphological evaluation analysis (**Figure 2**). It's possible that the liposomes' distortion during sample processing produced these oval-shaped vesicles.

Figure 2 Morphology of Liposomes

Particle size:

According to particle size analysis, the diameters of the various formulations ranged from 252.19 nm to 654.56 nm (**Table 2**), suggesting that all of these vesicles were tiny. This demonstrates that larger vesicles are the result of more cholesterol.

For this outcome, a number of ideas might be considered. Steroids have been demonstrated to increase the range of the gel state of liposomes by eliminating the phase transition temperature of soy lecithin. As a result, the vesicles stay in a solid form and the bilayers are not partially diluted. This may have resulted in larger liposome vesicles with higher cholesterol content.

Drug Content

Drug content uniformity was determined as triplicate by dissolving the liposomes in methanol and dissolved liposomes were undergone Centrifugation at 3000 rpm for 2 h and filtered with Whatman filter paper (0.45 µm, Whatman, Maidstone, UK).

The solution was diluted to Beer's range and observed in UV-Spectrophotometer.). The values range from 79.49% to94.3%.

Zeta potential:

A liposome preparation's zeta potential can be used to forecast the liposomes' in vivo destiny. In every instance, the zeta potential remained on the negative side. Increases in cholesterol content were associated with higher zeta potential values, while increases in soy lecithin were associated with lower values. Despite the fact that the influence of luliconazole content on zeta potential was modest and constant, it appears that higher luliconazole content also exhibited an adverse relationship with zeta potential values. According to published reports, liposomes loaded with luliconazole exhibited a very negative zeta potential value. Due to the partial ionisation of luliconazole at pH 7.4, this indicates a pharmacological interaction with the bilayer surface. Furthermore, there is a correlation between vesicular size and zeta potential. The surface charge (negative) is reduced when the liposomal size increases since it reduces the effective surface area.

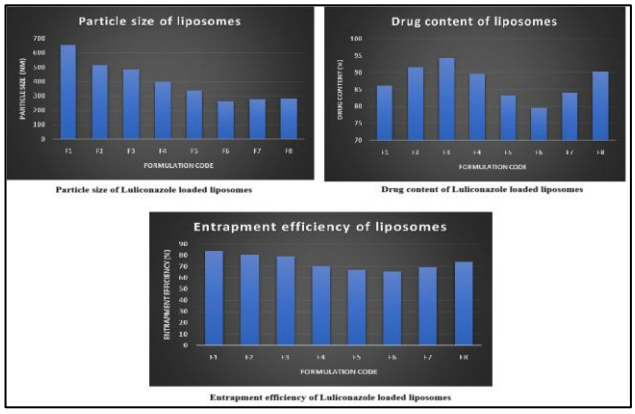

Figure 3 Particle Size, Drug Content and Entrapment efficiency of Luliconazole loaded liposomes

Entrapment Efficiency:

The range of the entrapment efficiency is 65.49% to 84.02%. The drug entrapment efficiency and particle size of various formulations are displayed in **Table 2**. According to these findings, particle size grew and entrapment efficiency dropped

when cholesterol concentration rose, but soy lecithin concentration increased entrapment efficiency at the same time. The amount of cholesterol injected during film formation was discovered to have an impact on the percentage of luciconazole entrapment efficiency. Luliconazole entrapment (%) in the liposome increased as cholesterol levels rose. This could be because a larger quantity of cholesterol in the lipid bilayer makes the bilayer more stiff, which increases stability and reduces the permeability of the liposomal membrane, which improves drug entrapment.

Characterization of Luliconazole loaded liposomal gels

The macroscopic features and attributes of the liposomal gel formulation, including colour, aspect, and scent, were evaluated. Every gel formulation was white, transparent, uniform, and had a smooth texture. Since the carbopol was made with a pH between 5 and 5.5, which is sufficient to achieve a satisfactory viscosity and clarity of the

Table 2 Entrapment efficiency, Particle size, and Zeta potential of all Formulations

S.No.	Formulation Code	Particle size (nm)	Entrapment efficiency (%)	Drug content	Zeta Potential(mV)
1	F1	654.06	84.02	86.09	- 32.0
2	F2	515.84	80.66	91.57	-35.6
3	F3	483.18	78.7	94.3	-37.4
4	F4	398.31	70.25	89.65	-37.7
5	F5	337.62	67.14	83.32	-39.1
6	F6	262.58	65.49	79.49	-42.5
7	F7	279.67	69.28	84.14	-38.1
8	F8	282.13	74.01	90.34	-36.9

Table 3 Characterization of Luliconazole loaded liposomal gel formulations

Formulation Code	Macroscopic properties	pH	Spreadability (%)	Viscosity (cps)
F1	Good and clear	5.5	86.09	42510
F2	Good and clear	5.3	81.57	63604
F3	Good and clear	5.3	83.20	45732
F4	Good and clear	5.5	81.79	59281
F5	Good and clear	5.4	83.26	57020
F6	Good and clear	5.4	80.46	63768
F8	Good and clear	5.3	82.10	58135
F9	Good and clear	5.5	87.91	50017

Table 4 In-vitro diffusion data for formulation F1 to F4

Time (Hrs)	%Cumulative drug release							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	10.76	10.92	14.48	11.97	13.46	12.58	12.17	12.41
2	19.55	19.45	20.96	20.14	22.84	21.79	20.01	21.07
3	24.96	25.33	26.41	25.60	28.72	28.56	26.99	27.57
4	29.23	29.88	31.35	30.52	34.02	35.04	33.39	31.91
5	38.33	38.97	35.26	38.72	43.42	47.96	43.28	37.62
6	43.75	44.87	41.25	46.10	51.66	54.14	51.73	44.13
7	48.04	50.24	48.79	53.22	58.15	60.95	56.13	51.20
8	52.62	54.28	54.01	59.53	63.48	67.45	61.69	55.84
12	58.34	61.26	64.41	70.74	74.37	79.18	73.06	67.23
24	66.61	70.91	76.89	81.70	85.57	89.08	83.28	79.18

gel, it was expected that the pH values of the liposomal gels would fall between 5.3 and 5.5. These pH readings indicated that there was little chance of skin irritation from the liconazole liposomal gel. Liposomal gels were thought to have a low spread of time, indicating strong spreadability. The distribution of gels determines their therapeutic effectiveness. In order to ensure that the gel is applied evenly to the skin, the prepared gels must be able to spreadability and meet the necessary standards for topical application. Additionally, it is thought to be a significant determinant in treatment compliance. Every formulation of liposomal gel coated with luliconazole was easily extruded. In general, consistency is reflected in gel formulation viscosity. Because of their low flow resistance when used under high shear circumstances, these gels' viscosity lowers as the rate of shear increases, a phenomenon known as non-Newtonian flow (shear thinning). The viscosity falls with potential pseudoplastic behaviour seen in the formulations, confirming the high spreadability characteristic caused by the viscosity decreasing when a specific force is applied while also having the ability to stay at the application site without draining.

In vitro Diffusion Studies of Luliconazole loaded Liposomal gel

All of the luliconazole liposome formulations performed in vitro diffusion tests using phosphate buffer with a pH of 7.4. Over the course of the 24-hour trial, the cumulative percentage of drug release was computed at various points in time. Variability in composition had a notable impact on the release of luliconazole from liposomes. Luliconazole release was significantly impacted by and inversely linked with the liposomal vesicles' cholesterol content. The release rate was lowered by doubling the cholesterol ratio. The release of liposomes with reduced cholesterol was the fastest of all liposomal dispersions with different cholesterol levels. Because cholesterol works as a strengthen in liposomal formulation, it enhances the integrity of the vesicles. Additionally, cholesterol in the lipid bilayer above the phospholipid phase transition temperature changes the fluidity of the membrane by restricting the movement of relatively mobile hydrocarbon chains, lowering membrane permeability, and lowering the efflux of the entrapped drug, which leads to drug retention. Consequently, when cholesterol levels rise, drug release decreases.

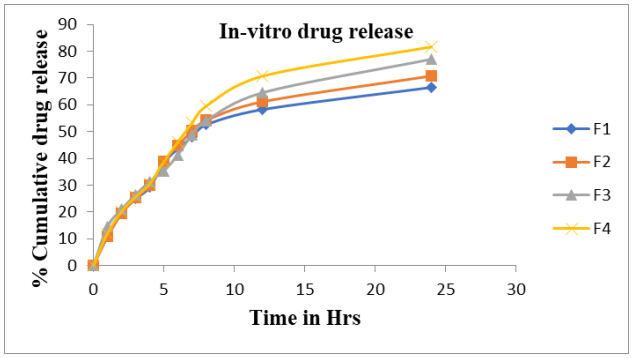

Figure 4 In-vitro diffusion studies for formulations F1 to F4

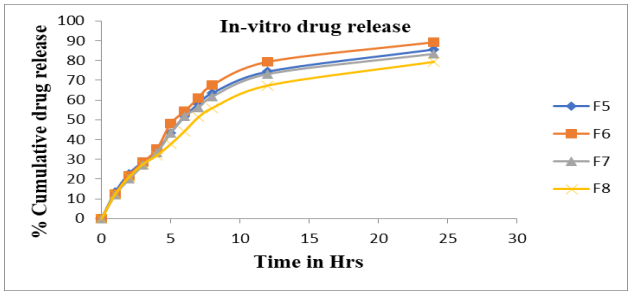

Figure 5 In-vitro diffusion studies for formulations F5 to F8

Table 5 Release Kinetics Data of the Formulations F1 to F8

Formulation code	Zero order R^2	First order R^2	Higuchi's R^2	Korsemeyer Peppa's	
				n	R^2
F1	0.722	0.835	0.928	0.712	0.983
F2	0.743	0.869	0.936	0.729	0.986
F3	0.816	0.943	0.968	0.622	0.990
F4	0.793	0.935	0.948	0.745	0.991
F5	0.771	0.941	0.945	0.719	0.990
F6	0.756	0.943	0.932	0.781	0.989
F7	0.764	0.926	0.939	0.765	0.990
F8	0.803	0.942	0.963	0.692	0.995

To determine the drug release mechanism and kinetics of the drug release from the liposomes, the in vitro release data was submitted to zero order, first order, Higuchi's, and Korsemeyers-Peppas models. High R2 values and linearity were observed when Higuchi's model was applied to the in vitro release data, indicating that the drug release from the liposomal gel followed a diffusion mechanism. The Korsemeyer-Peppas model was used to determine the precise mechanism in order to create the ideal model that would better reflect the in vitro release data. This model showed good linearity with high R2 values. All of the formulations had n values greater than 1.0, indicating that non-Fickianan diffusion governed the drug release.

CONCLUSION:

Liposomes offer a simple and practical approach to circumvent first pass metabolism, increase bioavailability, and modify drug release profiles essential for sustained, site specific and localized drug action. A total of eight liposomalgelscontained Luliconazole were formulated efficaciously by physical dispersion method for extended release and assessed for parameters like entrapment efficiency, zeta potential, vesicle size, diffusion studies. So, Luliconazole loaded into Liposomes, used for the treatment of fungal infections can produce sustained effect besides lowering the ill-effects associated with the conventional therapy. Thereby frequency of drug administration can be reduced, and it can be economic for patients

Author Contribution

All authors made substantial contributions to the conception, design, acquisition, analysis, or interpretation of data for the work. They were involved in drafting the manuscript or revising it critically for important intellectual content. All authors gave final approval of the version to be published and agreed to be accoun for all aspects of the work, ensuring its accuracy and integrity.

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