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Passive and iontophoretic delivery of bifonazole loaded Liposomal formulation: A comparative study

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***Abstract*---**Fungal infections are one of the most common dermatological conditions worldwide. The goal of this study was to create and test a liposomal version of the antifungal medication Bifonazole. The thin film approach was used to make nine batches of Bifonazole liposomal gel, each with a different ratio of cholesterol, soya lecithin, and stearyl amine. It was investigated how formulation variables affected entrapment efficiency, particle size, surface charge, and drug release behaviour. The addition of a positively charged surfactant like stearyl amine improves the entrapment effectiveness of Bifonazole into liposomes considerably. When compared to the other formulations, the LFG8 formulation had the highest drug-loading capacity (83.210.69%). After 24 hours, the total amount of medication released from the Bifonazole liposomal gel ranged from 51.520.67% to 83.210.69%. The molar ratio of soya lecithin in the formulation rose, delaying drug release. This may be due to Bifonazole's interaction with the vast surface area of the liposome's lipid bilayer membrane and the thickness and fluidity of the soya lecithin layer. In an ex-vivo skin permeation investigation, it was discovered that iontophoretic administration had superior penetration than passive diffusion. Iontophoresis dramatically enhances the amount of medication that is

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absorbed through the skin. Because of electrochemical polarisation in the skin, direct application of electric current (0.3mA/cm2, 0.4mA/cm2, and 0.5mA/cm2) in a continuous mode improved Bifonazole penetration. Finally, a stable Bifonazole loaded liposomal formulation for topical application was developed.

***Keywords*---**Bifonazole, Liposome, soya lecithin, iontophoresis, passive diffusion and fungal infection.

Introduction

One of the most intriguing areas in medication delivery systems is non-invasive biopharmaceutical delivery. Because the stratum corneum is thought to be an effective physical barrier to the permeation of high molecular weight biopharmaceuticals, numerous approaches for overcoming this difficulty and improving their penetration have been investigated.[1] Drug carriers such as microemulsions, nanoemulsions, solid lipid nanoparticles, and liposomes are one method for improving transdermal administration. By altering the physicochemical properties of the entrapped substance, these carriers can aid drug penetration.[2] Liposomes are used as possible medication carriers to improve biopharmaceutical percutaneous absorption. Enhancement of medication delivery, solubilization of poorly soluble pharmaceuticals, decrease of side effects or incompatibilities, or as rate-limiting barriers for regulation of systemic absorption are all important applications of liposomal drug formulations for transdermal and topical delivery.[3] Although the mechanism of action is unknown, this delivery strategy has been studied for the topical distribution of various hydrophobic medicines. Hydrophilic medicines, on the other hand, have been proven to have low encapsulation capacity and skin partitioning.[4]

Iontophoresis is another approach for increasing the permeability of ionic or charged substances through biomembranes by utilising electric potential. New drug delivery systems that combine liposomes and the iontophoresis technique are fast gaining popularity and offer additional benefits.[5] It is feasible to distribute neutral medications by encasing them in a charged carrier, and iontophoresis can improve their flow. As a result, combining liposomes and iontophoresis can be an effective and long-lasting method for transdermal penetration of biopharmaceuticals.[6]

A fungus causes fungal skin diseases. Because fungi feed on keratin, a protein that makes up skin, hair, and nails, they mainly harm the skin. Rashes, which can be red, scaly, or dry, are common indications of a fungal infection. Antifungal medications were used to treat fungal infections.[7]

Bifonazole is an azole antifungal that is used to treat tinea, Athlete's foot (tinea pedis), and body ringworm. Bifonazole is applied topically once a day (100 mg/day) for 2-3 weeks to treat Athlete's foot, although it has a very low absorption rate after being applied topically with Bifonazole gel.[8] Two techniques have been made to find a better way to transport medications into the skin: single topical

treatment utilising various liposomes and iontophoresis combined with topical application of ionic liposome.

Materials and Methods

Materials:

Yarrow Chem Products, Mumbai, India, provided the bifonazole and cholesterol. The study also used stearyl amine (Sigma-Aldrich, USA) and triethanolamine and soya lecithin (Himedia, Mumbai, India). All of the other chemicals and reagents were of analytical quality.

Methods:

Preparation of Bifonazole Liposome:

A thin film hydration approach was used to make liposomes. In a nutshell, varied amounts of soya lecithin and cholesterol were dissolved in a chloroform-methanol (1:1) solution, and 1 percent Bifonazole was added to the mixture. The drug solution was transferred to a 250 mL round bottom flask, which was then coupled to the rotary vacuum evaporator. The flask was kept in a thermostat water bath at 40°C and spun at around 100 rpm. The process was continued until all of the liquid in the solution had evaporated and a dry lipid layer had formed on the flask's wall. The thin film was hydrated with phosphate buffer of pH 6.8 after it was created in the round-bottomed flask. The suspension was agitated by vortex for 15 minutes and then sonicated for one hour.[9] Formulation chart of Bifonazole hydrogel is shown in table 1.

Bifonazole-enhanced the gel basis used was Hydroxy propyl methyl cellulose K100M (HPMC K100M). For all batches, the concentration of HPMC K100M was kept constant at 1 percent w/w. HPMC K100M powder was disseminated in distilled water with a glass rod and allowed to hydrate at room temperature for 24 hours for swelling. Then, using a mechanical stirrer, liposomal gel formulations containing Bifonazole were produced and blended into the HPMC K100M gel (100 rpm, 15 minutes). Preservatives included methyl paraben (0.2%) and propyl paraben (0.02%). The dispersion was neutralised using 0.5 percent w/w triethanolamine. A bifonazole hydrogel was also made and used in the penetration test.[10]

Table 1: Formulation design of Bifonazole loaded liposome

Formulation	**Bifonazole (%w/w)**	**Soya lecithin (mg)**	**Cholesterol (mg)**	**Stearylamine (mg)**	**Chloroform: Methanol (ml)**
LFG1	1.0	250	37.5	--	5:5
LFG2	1.0	250	50	--	5:5
LFG3	1.0	250	100	--	5:5
LFG4	1.0	250	150	--	5:5
LFG5	1.0	250	200	--	5:5

LFG6	1.0	250	250	--	5:5
LFG7	1.0	250	150	50	5:5
LFG8	1.0	250	150	100	5:5
LFG9	1.0	250	150	150	5:5

Evaluation of Bifonazole Liposome:

Determination of surface morphology:

Optical and scanning electron microscopy were used to investigate the surface morphology of Bifonazole-loaded liposomes (SEM). The vesicles were studied with an optical microscope and photographed with a fitted camera at a magnification of 10. Using a scanning electron microscope, the morphology of the optimised formula was examined. This was accomplished by dropping one drop of optimised liposome onto a collodion-coated copper grid and leaving it for roughly 2 minutes to allow drying and collodion adherence. A drop of uranyl acetate solution was then applied to a grid for 1 minute. After drying the material, it was examined with a scanning electron microscope.[9-10]

Encapsulation Efficiency (%EE):

Bifonazole encapsulation efficiency was calculated and compared to the original amount applied. Centrifugation at 15,000 rpm and 25°C for 30 minutes separated free Bifonazole from liposomes using an ultracentrifuge (Remi, India). Liposome pellets and a clear supernatant solution were obtained. To facilitate lysis of the liposomally encapsulated Bifonazole, the pellet containing only liposomes was disturbed with 0.1 percent Triton X-100 and vortexed for 5 minutes. To release the medicine, the vesicles were shattered, and the drug content was estimated.[9-10] The absorbance of the drug was noted at 254 nm. The entrapment efficiency was then calculated using following equation:

EE% = [Amount of entrapped Bifonazole /Total amount of Bifonazole added] × 100

Determination of particle size and zeta potential:

The Malvern Zetasizer was used to assess particle size, zeta potential, and polydispersity index (PDI) for all Bifonazole loaded liposomes (Malvern, UK). Before being exposed to measurements, the Bifonazole-loaded liposomal colloidal dispersion was diluted with filtered water.[9-10]

***In-vitro* drug diffusion studies:**

*I*n-vitro Bifonazole release from a liposomal gel was measured utilising a Franz diffusion cell with a dialysis membrane and an 8000 Dalton molecular weight cut-off. The water jacketed recipient compartment held 25 mL and had two arms, one for sampling and the other for the thermometer. The donor chamber had a 2 centimetre interior diameter. The donor compartment was positioned so that it only came into contact with the diffusion medium in the receptor compartment. The receptor compartment was filled with pH 6.8 phosphate buffer saline (PBS) with Tween 80 (1%) as the receptor media (buffer solution), which was kept at

37°C±1. The donor compartment was then coated with 1 gramme of liposome containing 1% (w/w) Bifonazole. At 1, 2, 4, 6, 8, 10, 12, and 24 hours, aliquots (1 ml) of the medium were removed and replenished with the same amount of buffer to maintain sink condition. All samples were filtered using Whatman filter paper (No. 2) and examined using a Shimadzu-1800 UV spectrophotometer at 254 nm.[8-9]

***In-vitro* passive permeation studies:**

The approach used in the release study was followed to transport Bifonazole over the skin from the tested gel formulations. The excised rat skin was mounted between Franz donor and receiver cells, with the stratum corneum facing the donor cell and the dermal side of the skin in contact with pH 6.8 phosphate buffer saline (PBS) with Tween 80 (1%) in the receiver compartment. The cells were clamped, and the gel formulation (1 gramme) was evenly applied to the skin's epidermal surface. To keep the volume of the release medium constant, 1 ml aliquots were removed from the receptor compartment every 24 hours and quickly replaced with an equivalent volume of fresh medium. The rate of drug transport through the skin was assessed by measuring the absorbance of the removed samples at 254 nm.[11]

***Ex-vivo* drug permeation study with iontophoresis:**

The Dual Channel Pocket Transcutaneous Electrical Nerve Stimulation TENS Iontophoretic equipment (Heera Surgicals, Mumbai) used a 500 mA A.C. adaptor and two copper electrodes for this study. The iontophoretic diffusion cell was made up of three glass pieces that were joined together to form a vertical diffusion cell. The receiving chamber was somewhat large (25 ml), with two parallel ports on top and a sample port on the side. Two top ports served as support for the donor and return electrode compartments and were actually cylindrical extensions of the receiving chamber. Two independent compartments (donor and return electrode chambers) were established with skin affixed to the bottom of two small cylindrical glass tubes inserted into these ports. Glass joints kept the inner and outer tubes connected. Each chamber had one electrode, and skin connected to both chambers touched the receptor fluid at the same depth (anode and cathode). The anodal chamber received bifonazole, while the cathodal chamber received a similar amount of water. The donor and return electrode chambers were connected through the skin to the receiver fluid. To keep the temperature at 37±0.5 °C, the assembly was placed in a magnetic water bath. Current flowed from anode to cathode as soon as the power supply was turned on, establishing electromigration and electroosmosis forces. Three different current densities, 0.3, 0.4, and 0.5 mA/cm2, were used in the experiment for a total of 24 hours. Bifonazole concentration was evaluated spectrophotometrically at various time intervals.[12]

Data Analysis:

The steady-state flow was determined by plotting the cumulative amount of medication penetrated vs time. Permeability coefficient was calculated using the following formula:

$$Kp=\frac{Jss}{Cd}$$

Where, Kp represents permeability coefficient, Jss steady-state flux, Cd concentration of drug in donor compartment. Flux enhancement was calculated by dividing iontophoretic steady-state flux with the corresponding passive steady-state flux.[12]

Results and Discussion

Skin fungus infection is a typical dermatological condition nowadays. Physicians have a variety of therapy options, including solid, semisolid, and liquid dose formulations. Liposomes have been studied extensively in both cosmetics and pharmaceuticals as a topical formulation. Liposomes and other types of lipid vesicles have been studied extensively for their potential to improve transdermal absorption and local medication administration of a variety of drugs. When liposomal formulations are employed instead of free-drug formulations, skin penetration is often improved. Despite years of investigation, no mechanism for liposome-enhanced dermal absorption of medicines by intact skin has been discovered. By altering the lipid composition, manufacturing technique, and surfactant inclusion, liposomes of various sizes and qualities can be created, and these various parameters can alter the transdermal transport of the encapsulated medicines. Thin-film hydration was used to make Bifonazole liposomes, which were then tested for various criteria.

Surface morphology of Liposomes:

Optical and scanning electron microscopy were used to examine the morphology of the liposome vesicles in order to validate their vesicular features. The particle size of liposomes generated was determined using scanning electron microscopy. The optical microscopy and SEM study demonstrated that the particles had almost spherical and uniform shapes and did not aggregate to each other (figure 1 and 1).

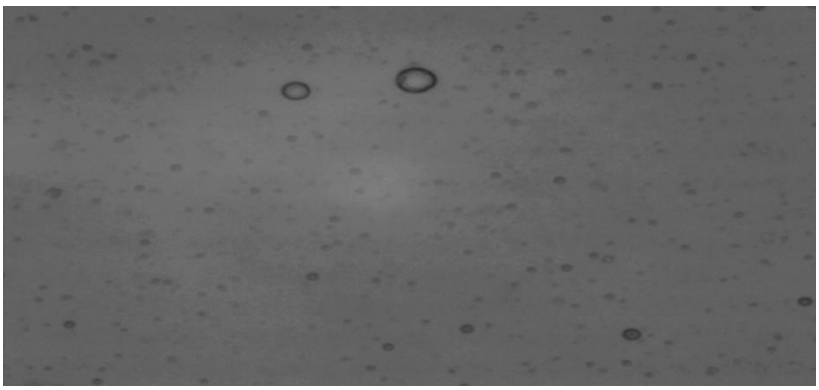

Figure 1: Optical microscopy image of Bifonazole loaded liposome

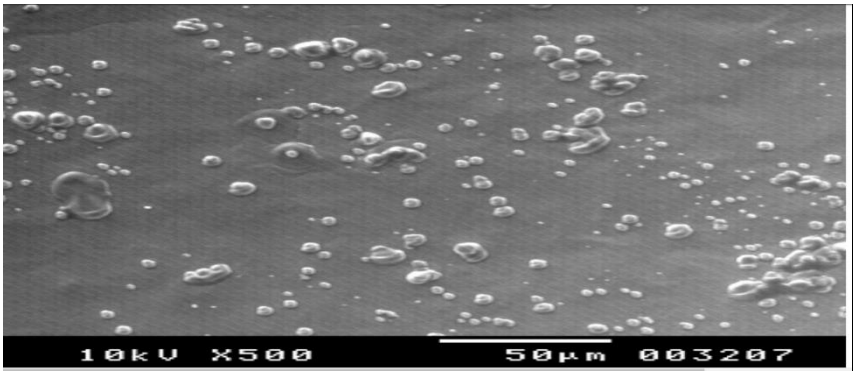

Figure 2: Scanning electron microscopy of Bifonazole loaded liposome

Encapsulation Efficiency (%EE):

Centrifugation was used to test the encapsulation effectiveness of Bifonazole-loaded liposomes. Entrapment efficiency is measured as a percentage of the initial pharmacological dosage. The entrapment effectiveness of all liposomes ranged from 48.85% to 94.34 percent 3.09. Bifonazole entrapment efficiency varied with lipid composition and cholesterol content, as seen in Table 2. By increasing the cholesterol content, the % entrapment efficiency of Bifonazole rose.

Table 2
Formulation design of Bifonazole loaded liposome

Formulation	**% Entrapment efficiency (n=3, ±SD)**
LFG1	48.85±3.74
LFG2	57.39±2.49
LFG3	65.20±3.62
LFG4	84.27±3.36
LFG5	78.59±2.90
LFG6	71.43±3.67
LFG7	89.32±3.41
LFG8	94.34±3.03
LFG9	90.22±3.19

Determination of particle size and zeta potential:

The most frequent approach for liposome preparation is thin-film hydration. The squeezing process most likely took place by allowing the lipids to flip swiftly at the extremities, as cholesterol has a hard ring and an ultra-smooth face in its structure. As a result, lipids with a high lateral mobility and packed acyl chains are produced. As a result, the packing factor influences the intensity and duration of van der Waals forces in liposomes, which contributes to size variation.When the cholesterol concentration was increased, the size of the drug-loaded liposome expanded dramatically from 68.0 nm to 164.6 nm, according to the results of a

study. The smallest particle size was found in formulation LFG1, and the largest particle size was found in formulation LFG6 (164.6 nm).

At room temperature, the zeta potential was determined via dynamic light scattering. To regulate the intensity, liposome dispersions were diluted with phosphate buffer before measurement. Because of the net charge of the lipid content in the nano-formulations, the zeta potential values of liposomal formulations (LFG1-LFG6) were discovered to be negative, ranging from -18.6 mV to -28.1 mV. Liposome LFG6-LFG9 had a positive surface charge, ranging from 21.9 to 26.2 mV. Charged liposomes were found to be more resistant to aggregation and fusion than uncharged liposomes in general. All developed formulations have a high enough zeta potential to prevent coagulation and provide vesicle stability. Results of particle size, PDI and zeta potential are showed in table 3.

Table 3
Evaluation of Bifonazole loaded liposome

Formulation	**Particle size (nm)**	**PDI**	**Zeta potential (mV)**
LFG1	68.0	0.190	-23.4
LFG2	122.1	0.231	-20.5
LFG3	129.0	0.229	-23.1
LFG4	134.5	0.204	-24.0
LFG5	147.2	0.166	-26.6
LFG6	164.6	0.199	-18.6
LFG7	137.4	0.237	26.9
LFG8	182.6	0.219	29.5
LFG9	195.0	0.213	34.22

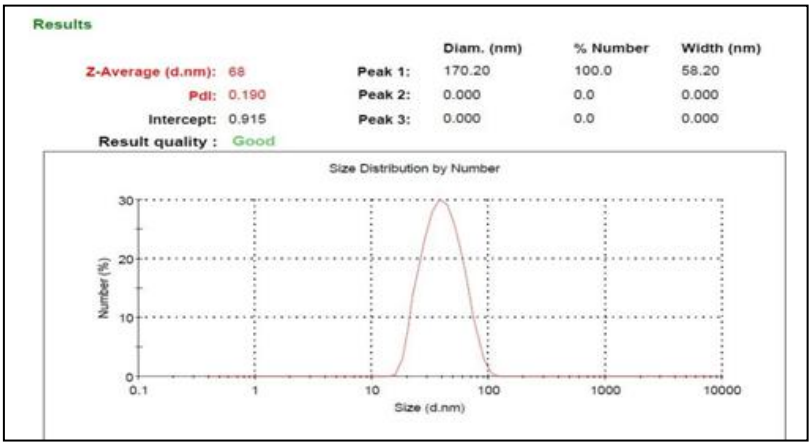

Figure 3: Particle size distribution of Bifonazole loaded liposome

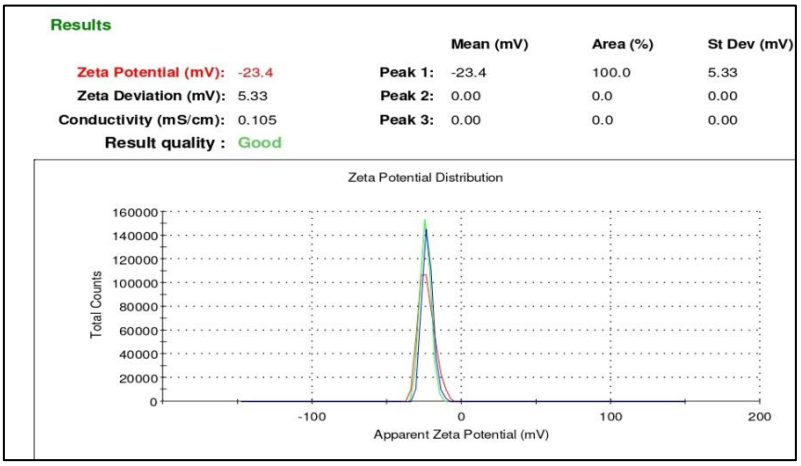

Figure 4: Zeta potential determination of Bifonazole loaded liposome

***In-vitro* drug release studies:**

Figure 5 depicts the drug release characteristics of Bifonazole liposomal gel. For 24 hours, the in-vitro release profile of Bifonazole from various formulations was measured in phosphate buffer solution (pH 6.8) containing 1.0 percent Tween 80. After 24 hours, the percent cumulative drug release from several batches of liposomal gel formulations ranged from 51.520.67% to 83.210.69%. Furthermore, after 24 hours, the release from liposome formulations ranged from 57.250.27% to 93.210.64%. In-vitro release of liposome and liposomal gels was compared, and the slower, sustained release of Bifonazole from the liposomal gel was found.The difference in bilayer compositions, as well as the fact that the effective diffusion double barrier consisted of both gel and vesicular lamellae, may explain the delayed release from liposomal gel. The increased release of Bifonazole from liposomes could be owing to the bilayer membrane's greater fluidity and reduced permeability; otherwise, liposomal vesicles could have been disrupted.

The viscosity of the liposomal gel formulation may also have an impact on drug release by reducing the rate of drug diffusion from the vesicles. The viscosity of different gels was found to have a negative connection with the total amount of medication released.When compared to liposomes, the liposomal gel formulation containing HPMC K100M 1.0 percent w/v showed sustained cumulative drug release. The researchers discovered a reciprocal relationship between the viscosity of liposomal gel formulations and the total amount of medication delivered.

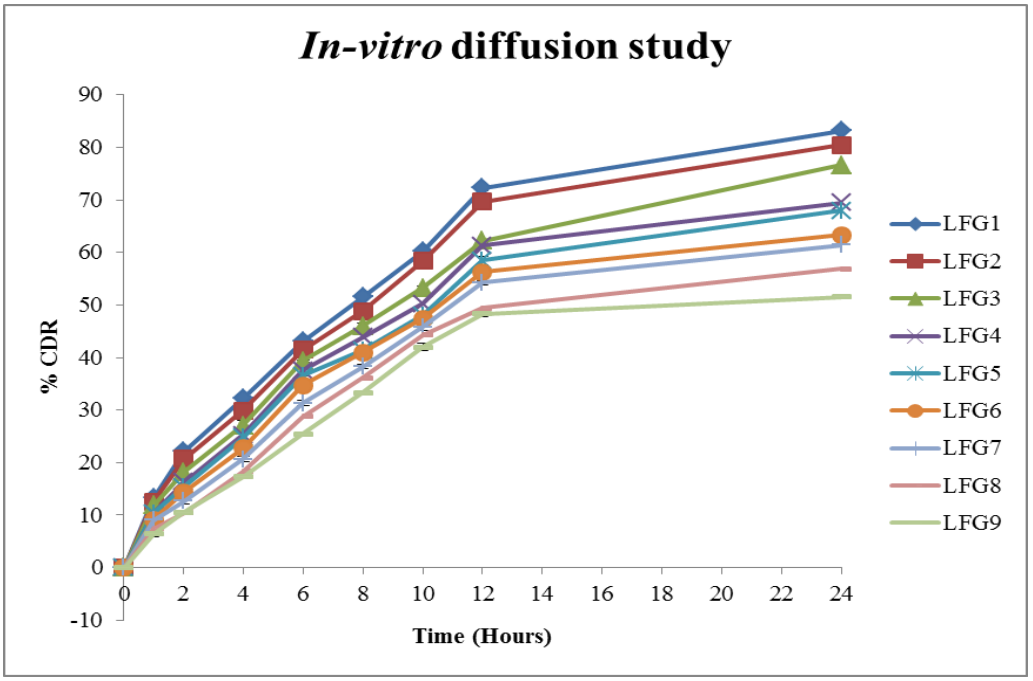

Figure 5: Comparative *in-vitro* drug release profile of Bifonazole liposomal gel

***Ex-vivo* skin permeation study:**

The formulations LFG8 and LFG9 were chosen for an ex-vivo skin permeation research based on the in-vitro drug release profile results. The passive diffusion analysis demonstrated that Bifonazole release from hydrogel formulations and Liposomal gel was similar, with the greatest percentage penetrated after 24 hours not exceeding 76 percent. Because Bifonazole has a pKa of 6.29 and the pH of the gel base is between 6.8 and 7.4, the majority of the medication will be ionised, resulting in a decreased partition coefficient.Because the unionised species of the drug is more lipophilic at this pH, it would have permeated the skin via the transcellular pathway; however, because this form of the drug is present to a lesser level at pH 7.4, penetration through the skin was minimal. Table 4 shows the penetration profile of Bifonazole from hydrogels during passive permeation testing. When iontophoresis was used, however, the proportion of medication penetrated from all formulations increased significantly. Because the molecule was discovered to penetrate rapidly enough due to electrochemical polarisation in the skin, direct application of electric current (0.3mA/cm2, 0.4mA/cm2, and 0.5mA/cm2) in a continuous mode improved Bifonazole penetration.

Iontophoresis increased the amount of Bifonazole penetrating through the skin, as shown in Table 4. Because the current functions as the driving force for the passage of ions across the skin, the amount of drug delivered to the receptor compartment from all gels increased significantly when iontophoresis was utilised. The observed values of percentage drug penetrated at the end of 24 hours, as reported in Table 4, demonstrate this impact.

It has been claimed that extended exposure to current causes the release of heat energy, which can make skin lipids more fluid, altering the integrity of the skin structure and, as a result, altering the skin's permeability. This explains why drug permeability during iontophoresis is higher than for passive diffusion. Increases in current density are also likely to increase electroosmotic volume flow, resulting in an increase in drug flux. The current density appears to promote drug penetration from hydrogel and liposomal gel formulations. As a result, increasing the current density from 0.3 to 0.5mA/cm2 increases drug penetration into nonionisable polymers like HPMC. Table 4 shows the computed permeability coefficient, steady-state flux, and enhancement factor for passive and iontophoretic Bifonazole transport for various current densities. All formulations demonstrate an increase in flow during iontophoresis. At 0.5mA/cm2 current density, there was a higher flow in liposomal gel LFG8.

Liposomes were used to encase bifonazole. The addition of stearyl amine provided a positive charge to the surface. The considerable increase in transport during iontophoresis of a liposomal formulation compared to a hydrogel formulation could be owing to the larger driving force of electro repulsion of positively charged liposomes rather than the hydrogel formulation's electroosmosis.

This improvement in liposomal formulation appears odd, because now larger particles (vesicles) are given instead of a little medication in hydrogel. Liposomes that are intact have not been shown to cross the skin, but it is thought that they fuse with the surface and carry their cargo across the stratum corneum. During iontophoresis, the liposomes are pulled toward the epidermis due to their surface charge (electro repulsion). They fuse with the surface layers once they come into touch with the epidermis and distribute the Bifonazole. Because the main driving factor (electro repulsion) was present, electroporation alone was not as effective. Not only was the driving force present in the instance of electroporation followed by iontophoresis, but the skin was also permeable, resulting in higher transport values. It's possible that electroporation-induced structural changes in the skin result in intact liposomes being transported through the epidermis, however this was not the goal of this work. The findings show that electroporation, when combined with iontophoresis, has the potential to significantly improve the transdermal transport of nonionized or neutral medicines encapsulated in positively charged liposomes.

Table 4: Comparison of calculated permeability parameters of hydrogel and liposomal gel formulations (LGF8 & LGF9) at 24 hours

Formulation	Mode of Permeation	Flux value (J) (μg/cm²/hr)	Permeability coefficient (K_p) (cm/h)	Enhancement ratio
Hydrogel	Passive diffusion	35.61 ± 2.17	0.0115	--
	Iontophoresis (0.3mA/cm²)	42.20 ± 2.63	0.0128	1.20
	Iontophoresis (0.4mA/cm²)	56.93 ± 3.88	0.0174	1.60
	Iontophoresis (0.5mA/cm²)	97.32 ± 3.51	0.0241	2.80
LFG8	Passive diffusion	41.09 ± 2.57	0.0126	--
	Iontophoresis (0.3mA/cm²)	72.55 ± 3.60	0.0205	1.76
	Iontophoresis (0.4mA/cm²)	96.42 ± 3.89	0.0326	2.34

	Iontophoresis (0.5mA/cm^2)	136.52 ± 3.92	0.0584	3.32
LFG9	Passive diffusion	39.40 ± 3.11	0.0189	--
	Iontophoresis (0.3mA/cm^2)	63.36 ± 3.44	0.0210	1.60
	Iontophoresis (0.4mA/cm^2)	85.07 ± 3.52	0.0318	2.16
	Iontophoresis (0.5mA/cm^2)	118.49 ± 3.17	0.0431	3.01

Conclusion

Due to the skin barrier qualities, skin illnesses represent substantial hurdles for formulation scientists. According to extensive study, treating skin problems requires accurate identification, estimate, and therapy. Topical medicine delivery looks to be a good alternative for skin problems thus far. The main disadvantage of commercially available topical drug delivery formulations is systemic drug absorption, which leads to systemic toxicity. Due to these disadvantages, nanotechnology was developed, which is a simple and non-invasive technology. Liposome drug delivery systems have been the subject of recent formulation development advancements.

Liposomes are small vesicles that contain aqueous compartments and are used to deliver hydrophilic and lipophilic medicines. Liposomes are one of the most effective drug delivery technologies for delivering drugs to target organs while limiting drug distribution to non-target tissues. Liposomes are becoming more widely recognised as a medication delivery vehicle, and when compared to traditional dosage forms, they distribute pharmaceuticals in a more regulated manner. The present study used the thin film approach to make Bifonazole-loaded liposomes. By altering the ratio of cholesterol, soya lecithin, and stearyl amine, nine distinct liposome compositions were created. All batches had the same quantity of soya lecithin in them.

The centrifugation method was used to test the encapsulation efficiency of Bifonazole-loaded liposomes. The use of a positively charged surfactant like stearyl amine improves Bifonazole entrapment efficiency substantially. When compared to the other formulations, the LF8 formulation had the greatest drug-loading capacity. The drug release profile of a bifonazole-loaded liposomal gel was extended. In an ex-vivo skin permeation investigation, iontophoretic administration outperformed passive diffusion. These findings show that the developed liposomal formulation's merits as an effective and safe treatment for fungal infection in patients outweigh its potential to improve the drug's efficacy and safety.

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