Indonesian Journal of Pharmacy

VOL 35 (2) 2024: 211–218 | RESEARCH ARTICLE

Formulation and Evaluation of Luliconazole Nanosponge Gel Using Experimental Design

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Article Info

Submitted: 06-04-2023
Revised: 24-08-2023
Accepted: 28-08-2023

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ABSTRACT

Different approaches are being used for the topical application of antifungal drugs. Luliconazole (LUL) is one of the antifungal medications that are being used for the treatment of various superficial infections. The poor permeability of LUL is regarded to be a factor for its reduced efficacy. Hence, the current study aimed to develop a nanosponge hydrogel to improve dermal availability and permeability. A set of nanosponge formulations (L1-L17) were designed with the help of central composite design (Design Expert 13, state ease Inc., Minneapolis, MN, USA). L1-L17 was prepared by using the emulsion solvent evaporation technique. The nanosponges were characterized for drug-excipient compatibility (FTIR, P-XRD, and DSC), and particle size, polydispersibility index, zeta potential, entrapment efficiency (%EE), and *in vitro* drug release; further optimized. The optimized nanosponge formulation (OLF) was taken to produce six hydrogels (LF1-LF6) of LUL by varied proportions of the gelling agent. In this process, initially, the gel was constituted with Carbopol 934/ sodium CMC/HPMC. Later, hydrogel texture was evaluated for its viscosity, swelling, and membrane permeability, followed by *in vitro* drug release and antifungal efficacy. The nanosponge formulations (L1-L17) had an average particle size of 109±0.45 to 386±0.34 nm, entrapment efficiency of 35.45±0.46- 89.65±0.37 % with 84.67±0.54 - 99.65±0.48 % of drug release for 6 h. The formulation OLF was predicted with better particle size, %EE, and drug release responses at 378±0.25 nm, 84.65±0.45%, and 96.18±0.54% for 4 h. Out of six formulated nanosponge gels (LF1-LF6), LF2 showed an optimal viscosity (25.69 ±0.45 pa.S), pH (6.87±0.56) and % drug release (80.65 ±0.64%) in 4 h. Drug release was governed by non-fickian diffusion mechanisms and zero-order. This nanosponge hydrogel was found stable and had a high permeation rate with better retention, which can be compelling enough in topical applications.

Keywords: Luliconazole, Carbopol 934, Skin permeability, Central Composite Design.

INTRODUCTION

The delivery of medications to achieve a therapeutic effect in humans and animals is a regular practice (Tiwari *et al*., 2012). There is a variety of promising drug delivery systems, including topical, controlled, and conventional ways that can be employed to distribute a few medications and provide a desired therapeutic activity with a minimum of side effects. Targeted drug delivery is a novel method for administering medications at a specific *site* at a particular time with limiting side effects (Renu *et al*, 2020; Shivani *et al*, 2015). Modern medicine extensively uses nanotechnology to enhance drug delivery and prevent overdose. Nanosponge technology is one of the emerging strategies used to deliver various medications to a particular target site and then release the drug substance in a controlled manner. The preferred method of medicine delivery for local administration is topical. It provides benefits such as simple delivery, avoiding the first-pass effect, and improved patient compliance. Various topical formulations, including ointments, gels, creams, pastes, lotions, and foams, are produced by

combining excipients and active medicinal ingredients (Bansal & Jamil, 2018; Imran *et al*., 2011). Topical gels are typically regarded as the safest and most efficient medications for dermatological ailments. A cross-linked polymer structure called a gel has swelling capabilities and is comprised of a three-dimensional network of dispersed phase particles. Gels are a better choice than ointments and creams for topical drug administration because they are easier to apply and remove (Patil *et al*., 2019). Luliconazole (LUL) is a broad-spectrum antifungal medication effective against dermatophytosis, tinea pedis caused by Tinea rubrum, and tinea cruris (Aditya *et al*., 2021). LUL is an azole class of drug that works by obstructing ergosterol production. It inhibits the cytochrome P450, a 14-demethylase enzyme that blocks the formation of cell walls in fungi (Alhakamy *et al*., 2021).

LUL is a BCS class-II medication with restricted permeability, limited solubility, and bioavailability that is a barrier to topical delivery. Traditional topical cream formulations have various limitations, including limited penetration from the stratum corneum and low retention at the point of application. The poor solubility limits medication absorption through the skin during topical administration. It can be avoided by entrapping the drug in nano-carriers such as nanoparticles, and nano-emulsions have gained significance for delivering LUL. One of the crucial ways to produce LUL topical gel employs nanosponge technology, which has the advantages such as lower toxicity, better solubility, and permeation. The current research aims to design and develop the nanosponge formulations of LUL applying central composite design using eudragit L100 as a polymer to improve drug release properties and enhanced stability. The formulation was optimized for particle size, entrapment efficiency (%EE), and (Kumar *et al*., 2019) drug release in experimental design. The final formulation was produced using a variety of gelling agents (Rupal *et al*., 2010), including HPMC, Sodium CMC, and Carbopol 934, and evaluated for various parameters, including viscosity, swelling, drug diffusion, zone of inhibition (mm), skin irritation, etc.

MATERIALS AND METHODS

The API LUL was obtained from Maithri Drugs Pvt Ltd, Hyderabad. Eudragit L100 was obtained as a gift sample from Lee Pharma Ltd, Vizag. PVA, ethanol, DCM, HPMC, sodium CMC, carbopol 934, triethanolamine, polyethylene glycol 400, and propylparaben were procured from SD Fine Chemicals, Mumbai.

Experimental Design

The surface response curve method (RSM) in central composite design (CCD) was used to design nanosponge formulations of LUL. This approach is frequently used to assess the impact of the independent variables' optimal variable concentration. It enables the assessment of multiple-factor interactions and effects on multiple response variables (Aydar *et al*., 2018; Robert *et al*., 2002). The current study analyses the impact of independent factors such as polymer (Eudragit L100) (X1), surfactant (PVA) (X2), and string speed (RPM)(X3) concentrations on particle size (Y1), %EE (Y2), and drug release (Y3). To achieve an optimized formulation that meets all the parameters and critical quality attributes, all the combinations are considered utilizing levels -1 and +1 for three independent variables (Amer *et al*., 2020).

Formulation of nanosponges

Seventeen formulations (L1-L17) were prepared using various polymer ratios of Eudragit L100 and PVA and various stirring speeds (1000 to 1500 rpm) by emulsion solvent evaporation technique. The organic phase was prepared by dissolving the drug and rate retardant polymer (Eudragit L100) in DCM (10 mL). The organic phase was added dropwise to the aqueous phase (varying quantities of polyvinyl alcohol in distilled water (100 mL), followed by evaporation at room temperature using a magnetic stirrer for 2h. Filtered nanosponges, washed with distilled water, dried (at 40^{o}C), and stored in a desiccator (Solunke *et al*., 2019).

Formulation of nanosponge gel

After optimization, the optimized formulation of LUL nanosponge (OLF) was incorporated into a hydrogel to prepare a topical dosage form. Six formulations of hydrogels (LF1-LF6) were prepared by using varying amounts of gelling agents (Iriventi *et al*., 2020), i.e., HPMC, sodium CMC, and Carbopol-934 (Table I). These gelling agents were soaked using distilled water for 4-6 h of swelling. Disperse the required quantity of nanosponges (drug equivalent to 1%) in distilled water in the form of nanosuspension.

Table I. Formulation of LUL nanosponge gel

Ingredients	LUL nanosponge gel formulations					
	LF1	**LF2**	**LF3**	**LF4**	**LF5**	**LF6**
Nanosponges (g)	0.2	0.2	0.2	0.2	0.2	0.2
Carbopol (g)	1	1.5	-	-	-	-
HPMC (g)	-	-	1	1.5	-	-
Sodium CMC (g)	-	-	-	-	1	1.5
Triethanolamine (mL)	qs	qs	qs	qs	qs	qs
PEG4000 (mg)	150	150	150	150	150	150
Propyl paraben (mg)	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water (mL)	50	50	50	50	50	50

It was transferred into the gelling agent using constant stirring in a magnetic stirrer (500 rpm). The pH of the formulation was adjusted in the range of 5.5 to 7.0 with triethanolamine and made up the volume of 50 mL with distilled water (Nasir *et al*., 2019).

Optimization of the model

After the preliminary investigation, optimization of the dependent variables, such as particle size (Y1), %EE (Y2), and drug release (Y3), were attempted by applying a rotatable CCD experimental design (Haranath *et al*., 2021). All the experiments were conducted in random order and triplicate. The data was analysed by design expert-13 (state ease Inc., Minneapolis, MN, USA). The 2D and 3D response surface plots were drawn to investigate the interactions between the critical factors and responses. The validation of the design space was done by preparing the checkpoint formulations.

The polynomial models incorporating the interactive terms were fitted to the experimental results by the following equation:

$$Y = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 12X12 + \beta 22X22 + \beta 32X32 \quad \ldots\ldots\ldots\ldots (1)$$

Where 'Y' represented the measured response, 'βo' was the constant coefficient, and 'β1', 'β2', and 'β3'were interaction coefficients, respectively. X1, X2, and X3 were the variables. After that, using analysis of variance (ANOVA) analyzed the statistical significance ($p<0.05$) of the model coefficient.

Characterization of nanosponges

The characterization of drug-excipient compatibility was assessed by FTIR, P-XRD, and DSC. Particle size, PDI, and zeta potential were analysed by Malvern Zeta Sizer, etc.

The sample was diluted with distilled water in a clear zeta cell and measured for average particle size and dispersity index at 25±0.5°C. The size, PDI, and Zeta potential were determined using a Malvern Zeta sizer (Malvern Nano ZS Zeta sizer) (Rasmussen *et al*., 2020, Clogston and Patri, 2011, Abdullah *et al*., 2022). The sample was analyzed in triplicate to minimize the error.

SEM is used to measure the particle size, surface morphology and to determine pore size, shape, and size distribution of nanosponges of the particles in high vacuum mode, and images were observed at voltage 30KV. (Tayade and Kale, 2004)

DSC was used to measure the thermal properties of nanosponges. (Gill *et al*., 2010) Two samples of pure drug and formulations were heated up to 500°C at the heating rate of 10°C/min separately in an aluminium pan, using nitrogen as a purging gas by keeping the empty pan blank and observing the thermal changes of nanosponges.

XRD is used to determine the formation of a complex of drugs and polymers. XRD patterns were drawn for pure drugs and nanosponges using XRD-7000 by passing Cu K radiation at a 2θ rate from 5-60° (Singireddy & Subramanian, 2016).

Drug-excipient compatibility

FTIR studies were done using the KBr pellet method by mixing the sample with potassium bromide; pellets were prepared under a vacuum. The absorption bands for pure drug and prepared nanosponges were measured at 400-4000 cm^{-1} to find any interactions between the drug and polymers (Preetha *et al*., 2013).

Evaluation of LUL Nanosponges

Drug %EE measures the amount of drug entrapped in nanosponge formulation (Muqtader *et al*., 2021), essential to producing therapeutic efficacy and *in vivo* drug release at a specific site.

It was determined by dissolving 50 mg of the sample in a phosphate buffer (pH: 7.4) and was sonicated. The supernatant layer of the drug solution was analyzed using UV-visible spectroscopy at 299 nm, and the amount of drug entrapped in the formulated nanosponges was measured (Saima *et al*., 2021).

% DL is the amount of drug loaded per unit weight of the nanosponge. It indicates the percent mass of the nanosponge with an encapsulated drug. It was determined by dissolving the measured quantity of sample in the buffer; after filtration, the transparent sample was analysed in UV-spectrophotometer at 299 nm (Mihaliasa *et al*., 2020). Drug loading capacity was measured.

***In vitro* drug release study**

In vitro drug release was estimated using USP dissolution apparatus -II (Paddle method) at 100 rpm 35±0.5°C in 900 mL of the buffer. A measured quantity of nanosponges of the drug equivalent to 10 mg was used for drug dissolution. The weighed nanosponges were placed in a diffusion sachet in a buffer, and samples were collected at regular intervals of 0.5,1,2,3,4,5 and 6 h. Drug release was measured using spectrophotometry at 299 nm (Sundararajan *et al*., 2017).

Characterization of nanosponge gel

The pH of the gel formulations was measured using a Labtronics pH meter. The pH electrode was dipped directly into the gel formulation and allowed to stabilize for 2-3 min; after equilibrium, measure the pH using calibrated pH meter. Samples were analyzed in replicate, and the deviated values, if any, were adjusted to the skin using triethanolamine.

The viscometer using a T-bar spindle (Rheometer Merlin VR) was used to measure the rheological properties of the developed gel at room temperature. Viscosity was measured in Cp. (Aggarwal *et al*., 2016).

Two hundred mg of nanosponge gel was taken in a diffusion bag previously soaked in distilled water for 24 h. Then, allowed the gel to soak in 100 mL of distilled water for 3 h and observe the swelling capacity of the gel.

***In vitro* drug diffusion study**

Nanosponge gel was evaluated for drug diffusion studies to determine the amount of drug diffused through the membrane. The cellophane membrane was used as a diffusion membrane. Franz diffusion apparatus was used to assess drug diffusion with buffer. 2 g of nanosponge gel was placed carefully on the cellophane membrane attached to donor and receptor compartments. The receptor compartment was at a temperature of 37±0.5°C and under constant stirring at 50 rpm using a magnetic stirrer. Regular samples were withdrawn from the receptor compartment, and equal buffer volumes were replaced to maintain the persistent volume. Collected samples were analyzed using UV-visible spectroscopy at 299 nm (Shrishail *et al*, 2019).

***In vitro* drug release kinetics**

Drug release kinetics was used to find the mechanism of drug release. All the drug release results were fitted in Zero-order, First-order, Higuchi, and Peppas equations and observed drug release patterns.

***Ex vivo* permeation study**

A freshly cleaned rat skin membrane was used as a diffusion membrane. It was procured from the experimental animals from VIPER. IAEC committee approval was granted for the completion of the study (06/IAEC/VIPER/Ph.D./2021-22/II). Franz diffusion apparatus was used to determine drug diffusion with buffer. 2g of nanosponge gel was placed carefully on the membrane between the donor and receptor compartments. The receptor compartment was at a temperature of 37±0.5°C and under constant stirring at 50 rpm using a magnetic stirrer. Regular samples withdrawn from the receptor compartment and equal buffer volume should replace to maintain the persistent volume. Collected samples were analyzed using UV-visible spectroscopy at 299 nm (Sujitha and Muzib, 2019).

Skin irritation study

Skin irritation studies were performed to identify the skin reaction at the site of the application of nanosponge gel. Six Wistar rats were selected and isolated before 24 h of the test, maintained at a constant temperature of 25±0.5°C and under humid conditions. The hair on the dorsal part of the rat skin was shaved and cleaned with a saline solution. The rats are divided into groups: Group-I for control, Group-I for the optimized formulation, and Group III for the marketed product. The sample was applied to hair-free rat skin homogeneously covering 4 cm in three groups of rats and observed for skin reaction for 24, 48, and 72 h (Malkiet *et al*., 2020; Wang *et al*., 2017).

Table II: Optimized formulation (n=3)

Ingredients	OLF	Responses	Predicted	Observed
Eudragit L100 (mg)	444	Particle size	355nm	378nm±0.25
PVA (mg)	399	%EE	85.4%	84.64%±0.45
Stirring speed	1500	Drug release (at 6 h)	88.7%	96.13%±0.54

RESULTS AND DISCUSSION

Optimization of model

Prepared nanosponges were tested for the parameters like %EE, drug release, and particle size. The surface response curve approach was used to identify the responses dependent on the independent variables. This approach evaluated the data from evaluations for the best-fit model for the independent variables. The significance of quadratic, 2FI (Sequential sum of squares for the two-factor interaction), and linear polynomial models were developed and assessed by analysis of the variance. 3D surface plots identified the interaction between three independent variables. When the concentration of Eudragit-L 100 and PVA increases, the particle size and %EE increase. Drug release reduces when the RPM increases the particle size and %EE and reduces drug release.

The data of the CCD design depicted a linear model as the best fit for response 1(particle size), response 2(EE), response 3(drug release). The regression analysis summary indicates that *P*-value is 0.0001 and F-value is 148.56; hence the model was significant. The data from ANOVA for response 1i.e particle size, %EE and drug release depicted the linear model as the best fit. The equation generated by the software is.

Particle size =

$$257.06 + 109.64(A) + 35.49(B) - 16.20(C)$$

X1 and X2 were significant factors and had agonistic effects on the particle size. X3 has an antagonistic impact.

%EE=

$$65.99 + 16.61(A) + 9.03(B) + 1.52(C) - 2.82(AB) + 0.428(AC) - 1.38(BC)$$

X1, X2, and X3 were significant factors and had an agonistic effect on the %EE. It inferred that the polymer, surfactant concentration, and stirring speed positively affect the entrapment efficiency.

Drug release =

$$97.47 - 3.70(A) - 3.12(B) - 0.334(C) - 1.77(AB) + 0.056(AC) + 0.036(BC) - 4.97(A2) + 0.0512(B2) + 1.23(C2)$$

X1, X2, and X3 were significant factors that antagonistically affect drug release. Increasing polymer content slows the release of drugs by forming a dense matrix that prevents drug molecules from diffusing out of the formulation. The higher the polymer content, the greater the barrier effect and the potential for sustained or regulated drug release.

Optimizing nanosponge formulation aims to minimize particle size, maximize %EE and minimize drug release. The software provided 53 solutions, out of which one gave 0.541 desirability. So it was considered a batch OLF Formulation that would provide minimum particle size, maximize %EE and minimize the drug release. The statistically optimized formula OLF, the Optimized formula was evaluated for all parameters (Table II).

Characterization and evaluation of nanosponge

The particle size range for LUL formulations (L1-L17) is 109.4±0.45-386.7±0.34nm. The average particle size observed for OLF using the zeta sizer was 378±0.25 nm, suggesting that the formulation was in the nano-size range. The Polydispersity index for the best formulation OLF, was 0.492. and displayed a potential of -20.4 mV, indicating that the particles were not aggregated and were all separated by repulsive forces. SEM analysis revealed that different-sized nanosponges were generated. Each formulation had a homogeneous particle size, a structure resembling a sponge, and no drug crystals on the surface of the sponges (Figure 2). The DSC, XRD and FTIR showed that there was no drug excipient compatibility between the LUL and all excipients used in the nanosponge formulation.

The drug %EE of all formulations (L1-L17) was observed between 35.45±0.46 to 89.65±0.37, whereas for optimized formulation (OLF) was noticed as 84.65±0.45, which stated that an increase in the polymer concentration improves the %EE and %DL of all formulations was observed between 9.67±0.24 to 41.75%±0.57. By loading the drug into a nanosponge, its release rate can be controlled by allowing for more precise dosing and better therapeutic efficacy and drug release was in the range of 84.67±0.54-99.65%±0.48 for 6 hr for formulations L1-L17, and the optimized formulation showed the drug release was 96.18%±0.54.

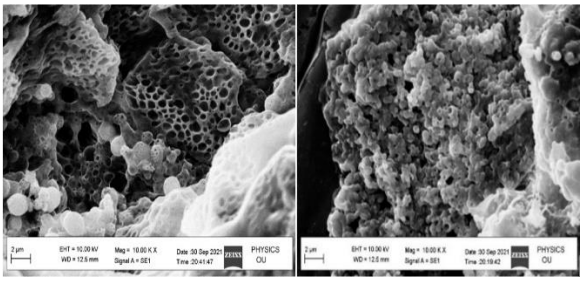

Figure 2. SEM images for nanosponges, L7 (a) and OLF (b)

Characterization and evaluation of nanosponge gel

The pH, viscosity of all formulations was tested. The pH of all formulations was between 6.84±0.75 to 7.64±0.24, and the best formulation, LF2, showed a pH value of 6.87±0.56 and viscosity for all formulations (LF1-LF6) was found in the range of 10.68±0.35 to 25.69±0.38Pa.S. LF2 had 25.69±0.38 Pa.S, which is considered the optimum viscosity for topical application (Table III).

The swelling behaviour of the gel was mainly due to many hydroxyl groups presented by permeation enhancer propylene glycol. Being a hydrophilic agent enhances the water uptake property of the gel. Prepared gels had good equilibrium swelling properties and were considered adequate for topical application (Table III).

Table III. Characterization of Nanosponge gels (n=3)

Formulation code	Viscosity (pa.S)	pH	Swelling index (%)
LF1	20.46± 0.45	7.64±0.24	32.12±0.24
LF2	25.69±0.38	6.87±0.56	36.58±0.35
LF3	15.48±0.65	6.84±0.75	23.43±0.63
LF4	17.89±0.34	7.34±0.53	29.45±0.22
LF5	13.56±0.25	6.88±0.68	19.34±0.43
LF6	10.68±0.35	7.34±0.34	21.56±0.39

In vitro drug diffusion was and observed using a for all formulations (LF1-LF6), drug release is between 80.65±0.64-99.54%±0.64. The drug release for LF2 was regarded in a controlled manner (99.54±0.64% at 4h) over other formulations. *Ex-vivo* permeation studies were done using a cleaned skin membrane for the best formulation LF2 was observed the permeation of the drug from the membrane was 81.76±0.41% (Figure 3).

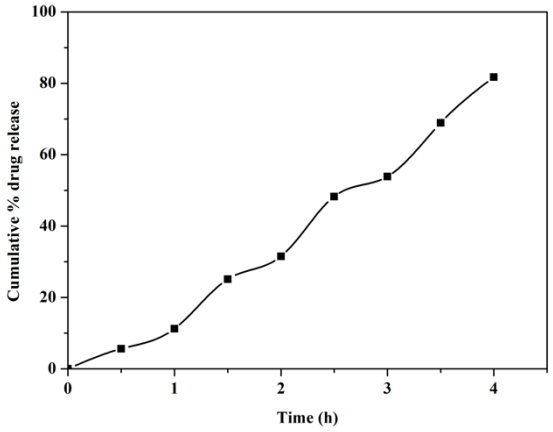

Figure 3. Ex-vivo Permeation study for LF2

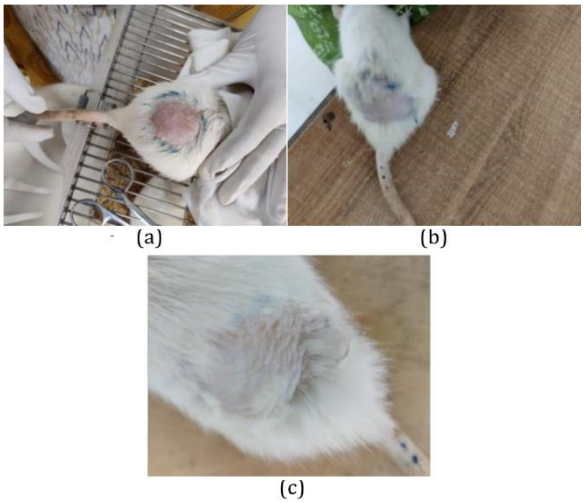

Figure 4. Skin irritation studies for LF2 nanosponge gel (a) At 24 h; (b) At 48 h; (c) At 72 h.

The skin irritation studies states that the formulation was safe for topical application. The present study concluded that the developed LUL nanosponge hydrogel could be the best approach and most effective than conventional gel for treating fungal infection.

The study utilized an experimental methodology to develop formulations of nanosponges, the central composite design (CCD) was used within the framework of the surface response methodology to assess the impact of the independent variables. The LUL nanosponges (L1-L17) were effectively synthesized with the emulsion solvent evaporation method. All formulations were subjected to characterization procedures to assess drug excipient compatibility,

particle size, encapsulation efficiency (%EE), and drug release study. The nanosponge formulations of LUL exhibited an optimal percentage of encapsulation efficiency and drug loading capacity, along with targeted drug release at specified experimental circumstances, surpassing the performance of previous formulations.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Principal and Management of the KL College of Pharmacy, KL Deemed to University, Vaddeswaram, for allowing us to perform this study. The authors are also appreciative to University College of Technology Osmania University, Hyderabad for providing the required facilities to get analytical characterisation data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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