Ind. J. Pharm. Edu. Res., 2024; 58(1s):s187-s195.
https://www.ijper.org

Original Article

Formulation, Development and Characterization of PLGA-Luliconazole Nanoparticles Loaded Gel System for Topical Fungal Treatment

Santosh S. Chhajed[1,*], Umesh D. Laddha[1,*], Sandeep S. Sonawane[1], Mrunal Pekhale[1], Prajakta Thorat[1], Sanjay J. Kshirsagar[1], Jaipraksh N. Sangshetti[2]

[1]MET's Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nasik, Maharashtra, INDIA.
[2]Y. B. Chavan College of Pharmacy, Aurangabad, Maharashtra, INDIA.

ABSTRACT

Background: Fungal infections are the primary cause of skin diseases. Topical application of antifungal drugs is the most preferred treatment which is associated with key limitations viz. need for high doses and frequency of application. By considering the limitations of current topical treatment, the present study involves the formulation and characterization of luliconazole nanogel. **Materials and Methods:** The luliconazole nanoparticles were prepared by using biodegradable polymer PLGA 50:50 and dispersed in polymeric dispersion prepared by Carbopol 940 and HPMC K4M. For optimization of nanoparticles as well as their polymeric dispersion a factor-three level (3^2) design was implemented. Optimized nanoparticles were characterized for various parameters viz. particle size, PDI, entrapment efficiency, XRD, SEM, whereas the nanogel was investigated for viscosity, cumulative drug release, spreadability, extrudability, skin irritation test, and antifungal activity. **Results:** The nine batches of nanoparticles as well as nanogel were prepared. Nanoparticles prepared by using 100 mg PLGA at 6 HPH cycles showed size, PDI, and % entrapment as 406 nm and 87% respectively was the desired batch. The XRD and SEM study ensured the encapsulation of drug with satisfactory surface morphology. Nano-gel formulated with 0.3% Carbopol and 1.0% HPMC was selected as optimized; it showed viscosity and % cumulative drug release as 3650 cP and 54% respectively. Nanogel showed significant sustained luliconazole release (54.12%) in comparison to simple nanoparticles (65.22%). The formulation showed improved antifungal activity than standard treatment without any skin irritation signs. **Conclusion:** Prepared nano-gel is a promising alternative to the current treatment.

Keywords: Nanoparticles, Nano-gel, Optimization, Antifungal, Skin test.

Correspondence:
Dr. Santosh S. Chhajed, Ph.D.
Associate Professor, MET's Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik-422003, Maharashtra, INDIA.
Email: chhajedss@gmail.com

Dr. Umesh D. Laddha
[1]Assistant Professor, MET's Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik-422003, Maharashtra, INDIA.
[2]Regeron Inc., Chuncheon-si, Gangwon-do, SOUTH-KOREA.
Email: umeshladdha698@gmail.com

Received: 18-02-2023;
Revised: 01-03-2023;
Accepted: 03-11-2023.

INTRODUCTION

Infections caused by fungal species are the major skin disease burden, which on an average result in more than 150 million cases annually. Furthermore, about 1.7 million deaths have been reported per year because of fungal infections.[1] At an early stage, fungi attack on the skin surface and start to invade the different layers of skin if they remain untreated.[2,3] Depending on the skin layer infected by fungi, infection can be categorized as cutaneous mycoses and subcutaneous mycosis. Additionally; COVID-19 has further increased the mobility and mortality of fungal infection.[1]

In practice most of infections to the skin are being known treated by topical application of suitable agent instead of oral and systemic application to avoid undesired effects. Advantages topical therapy includes the targeted application directly on the desired site with limited systemic side effects, absence of pre-systemic metabolism, along with ease of application which increases the patient compliance.[4] Despites the several advantages of topical delivery of antifungal drugs, the adverse skin reaction, itching, need of high dose, poor retention on skin, high frequency of application and need of high dose has placed limitations on topical drug delivery of antifungal drugs.[5-7] A topical antifungal Luliconazole inhibits the enzyme lanosterol demethylase which is assumed to be the cause of luliconazole's antifungal effect, while the precise mechanism of action is still unknown. Ergosterol, a crucial component of the fungus cell membranes, is produced through the action of lanosterol demethylase. In order to surmount above mentioned undesired effects/characteristics of conventional therapy the novel approach that is drug delivery system is envisaged. Nano-carriers loaded with anti-fungal drugs known to exhibit promising results.

DOI: 10.5530/ijper.58.1s.18

With this understanding in the present study, preparation of PLGA nanoparticles of luliconazole and further loaded in gel prepared by carbopol and HPMC. In order to optimize process parameters, a 3^2 factorial design approach has been employed successfully. At the initial stage of nanoparticles were optimized and characterized for particulate dimension, PDI, percent entrapment, zeta potential, XRD, SEM and DSC. In the lateral stage, the optimized lyophilized nanoparticles were dispersed in the polymeric structured vehicle (gel) prepared by using Carbopol and HPMC. Nanogel was optimized and further evaluated for various parameters. Antifungal activity was confirmed by using the 'zone of inhibition method'. The suitability of the prepared formulation was confirmed by using a skin irritation study on an animal model.

MATERIALS AND METHODS

Materials

Luliconazole and PLGA 50:50 was gifted by Blue Cross Pharmaceuticals (Nashik) and Evonik Pvt. Ltd. (Mumbai) respectively.

Methods

Preparation of Luliconazole nanoparticles

Nanoparticles were obtained by incorporating the organic phase (PLGA+Drug+DMSO) gradually in aqueous phase (PVA+Polysorbate 80) under the probe sonication to obtain the O/W emulsion (single emulsion solvent evaporation method). The prepared crude emulsion was sheared through a high-pressure homogenizer with different cycles at a fixed pressure of 10 kpsi. The organic phase was vaporized by placing the sample over a magnetic stirrer for 10 hr. Prepared nanoparticles were investigated for size, PDI, and entrapment. The optimized batch was lyophilized by standard protocol.[8]

Experimental design (optimization)

The effect of Polymeric concentration (PLGA) and X2: HPH cycles on the Y1: size, Y2: PDI, and Y3: % EE was tested by using 3^2 factorials designs. The X1 and X2 were investigated at three levels and the resulting data was statistically analyzed by using Design Expert Software V13.

Characterization of Nanoparticles

Particle size and Polydispersity Index (PDI)

Prepared nanoparticles were investigated for size and PDI by using Zetasizer (HORIBA SZ 100 Z-Type Ver 2.40) as both the parameters are strongly related to stability and efficacy. Furthermore; for better topical uptake, the nanoparticles should possess a size below 500 nm which also relates to dose reduction.[8]

Entrapment efficiency

A supernatant obtained after the 20 min of cold centrifugation (4°C, 15000 rpm) of prepared nano-dispersion was evaluated for the free drug concentration by UV-spectrophotometer at 299 nm and entrapment was calculated by following formula;[8]

$$\%\,EE = \frac{Total\ amount\ of\ drug - amount\ of\ free\ drug}{Total\ amount\ of\ drug}\ X\ 100$$

Zeta potential

Charges on the shear plane of colloidal particles and its magnitude define the stability of dispersion by maintaining enough repulsion between particles. Zeta potential±20 mV or above indicates the good stability. However; in absence of desired value of zeta potential, formulation can be stabilized by structured vehicle like Carbopol, HPMC etc.[9]

Drug loading (DL), product yield and drug content

Nanoparticles obtained after Lyophilization was tested for product yield and luliconazole loading efficiency by using following formula;

$$\%\,DL = \frac{Total\ amount\ of\ drug - amount\ of\ free\ drug}{Weight\ of\ freeze\ dried\ nanoparticles}\ X\ 100$$

$$\%\,Yield = \frac{Weight\ of\ freeze\ dried\ nanoparticles}{Drug + Polymer\ weight + dispersing\ agent}\ X\ 100$$

The drug content was determined by dissolving 100 mg of nano-particles in DMSO and further volume was adjusted to 10 mL by phosphate buffer (pH 5.8). The system was analyzed at 299 nm (UV-Shimadzu 1700, Japan) to investigate the drug concentration. Evaluation of these parameters plays crucial role in calculation of weight of nanoparticles equivalent to required drug dose.[9]

Surface morphology of NPs

The nanoparticles were further subjected for SEM (scanning electron microscopy) testing to determine the surface morphology.

X-ray Diffraction studies (XRD)

Prepared nanoparticles, drug and polymer was subjected for X-ray diffraction study (3-80° angle) to confirm the entrapment of drug.

Preparation of nanoparticles loaded gel

To prepare the nanoparticle loaded gel, required quantity of Carbopol 940 and HPMC K4M was soaked in 25 mL of water for 12 hr. Nanoparticles equivalent to 50 mg of drug was added in polymeric mixture. Weight of formulation was adjusted to 100 g by further addition of water. Prepared mixture was placed on magnetic stirrer (20 rpm) and pH was adjusted by gradual addition of Triethanolamine to obtain the gel. Prepared gel was used for further investigation.[10]

Experimental design optimization

To optimize the concentration of Carbopol 940 (X1) and HPMC K4M (X2), 3^2 factorial design was implemented. Viscosity (Y1) at 20 rpm and cumulative percent drug release (Y2) at the end of 5 hr were the responses. The X1 and X2 were analyzed ate three levels and obtained data was further statically examined by design expert software v13.

Characterization of Nanogel Formulation

Appearance and pH

All prepared nanoparticles laded gel were subjected for visual inspection against black and white background to confirm the clarity and presence any contaminant particles. The pH of Nanogel was measured in triplicate by using a calibrated digital pH meter.[11]

In vitro Drug Release Study

A Franz diffusion cell was used to perform i*n vitro* drug release of nanogel by using cellophane membrane which was soaked for 12 hr in diffusion medium prior to study. A phosphate buffer (pH 6.4) was placed in receptor compartment and membrane was clamped between the donor and receptor compartment. Nanogel (1 g) was transferred in donor compartment and the system was placed on magnetic stirrer (37±0.5°C, 50 rpm). The 1 mL sample was withdrawn at intervals time interval of 60 min for 5h and drug concentration was measured by using UV-spectrophotometer at 299 nm. Study was performed in triplicate. Sink condition was maintained while performing the experiment. Data obtained was analyzed for release kinetics. Similarly, simple nanoparticles were also investigated for the drug diffusion behavior with same experimental condition.[10]

Viscosity

One of the major factors that governs the residence of semisolid preparation on the targeted site is the viscosity. All batches were subjected for viscosity testing at 20 rpm by using a Brookfield viscometer.[10]

Spreadability study

Prepared nanogel (1 g) was placed at the center of a glass slide and another glass slide was covered on it to determine the spreadability. For the next 10 min, 1 kg weight was placed over the slide and the diameter of the gel was measured in g.cm/sec.[12]

Extrudability

Extrudability test involves the measurement of the number of semisolids that get expelled upon application of weight on the tube. A nanogel (10 gm) was filled in the aluminum collapsible tube with 5 mm opening tip. The tube was further clamped in glass slides and a weight of 1 kg was placed. The quantity of gel extruded was collected and weighed and percent extrusion was calculated (Grades +++: excellent; ++: good; and +: fair).[12]

Antifungal Activity

The anti-fungal activity of prepared nano-gel was confirmed by the zone of inhibition method against *Candida albicans*. The zones of inhibition of a simple drug, a marketed formulation, and an optimized nano-gel were compared using the agar disc diffusion approach. The prepared plates were incubated at 35°C for 24 hr.[13-15]

Skin irritation test

Optimized nano-gel was evaluated for dermal irritation study on Albino Wister rats as per OECD -404 guidelines. Rats (n=6) of weight 200-250 gm of either sex were placed on normal food and water intake for 24 hr prior to study. Skin was shaved carefully and formulation was applied. Skin was observed for any sign of irritation as redness, erythema and edema.[16]

RESULTS AND DISCUSSION

Development of drug loaded nanoparticles

The nine batches of nanoparticles were fabricated and optimized batch was subjected for the lyophilization in order to obtain maximum yield.

Experimental design (optimization)

The optimization was carried out by using 3^2 factorial designs in which independent variables were investigated at three levels as shown in Table 1 in their actual and coded form.

Impact of independent variables was studied on responses Y1: Particle size, Y2: PDI and Y3: % Entrapment efficiency which was found to in the range of 367-670 nm, 0.345-0.548 and 81-94% respectively as depicted in Table 1.

Data was subjected to optimization by Design Expert v13 software. The model suggested by software and regression analysis for responses are represented in Table 2. The suggested model was found to be suitable since the probability value was less than 0.05.

Software suggested polynomial equations;

$$Y1 = 417.7+142.33*X1-28.83*X2+22.00*X1 X2+98.33*X1^2+27.83*X2^2 \quad 1$$

$$Y2 = 0.4432-0.0250*X1-0.0928*X2 \quad 2$$

$$Y3 = 87.67+4.17*X1+2.33*X2 \quad 3$$

From the above equation, it was observed that PLGA concentration (X1) has a positive impact on particle size as well as on entrapment i.e., increasing the concentration of polymer increases the size of nanoparticles as well as the entrapment of the drug. At the same time, it leads to a drop in the PDI. On the other

Table 1: Experimental design for fabrication of nanoparticles.

Sl. No.	Batch No	Conc. Of Polymer (mg) (X1)	HPH Cycles (X2)	Particle Size (nm) (Y1)	PDI (Y2)	Entrapment Efficiency (%) (Y3)
1	F1	50	4	436	0.548	81
2	F2	50	6	372	0.481	84
3	F3	50	8	367	0.396	86
4	F4	100	4	512	0.549	85
5	F5	100	6	406	0.429	87
6	F6	100	8	396	0.470	88
7	F7	150	4	670	0.512	90
8	F8	150	6	672	0.418	92
9	F9	150	8	689	0.345	94

Table 2: Summary of results of regression analysis for responses.

Response	Model	*p*-value	*F*-value	Adjusted R^2
Particle size	Quadratic	0.0121	24.81	0.9370
Polydispersity index	Linear	0.0001	54.28	0.9177
Entrapment Efficiency	Linear	<0.0001	351.86	0.9887

hand, HPH cycles showed a negative impact on the particle size and PDI, whereas the increased HPH cycles showed a positive impact on entrapment.

The impact of X1 and X2 on responses can be further confirmed by using 3D surface response plot (Figure 1).

The desirability search approach was used to obtain the optimized batch. The selection of optimized batch was done on the basis of moderate particle size, less PDI, and good entrapment of drug with minimum HPH cycles. The batch F5 (particle size 406 nm; PDI-0.429, %EE-87%) with desirability 1 was selected as the optimized batch (Figure 2). Optimized batch was lyophilized and used for further analysis.

Characterization of nanoparticles

Particle Size, PDI and zeta potential

Particle size and PDI can relate with the stability of nano-formulation. For topical application, particle size below 500 nm is one of the important requirements to improve the penetrability through various barriers. Optimized batch showed 406 nm particle size and 0.429 PDI which fulfils the requirement of targeted site (Figure 3). Zeta potential measures the charges on particles. Optimized batch showed zeta potential value as -1.7 mV (Figure 4). Zeta potential±20 mV and above indicates the satisfactory repulsion between the dispersed particles which prevents their aggregation. Even though for prepared nanoparticles the charges are less negative, these nanoparticles have been stabilized in lateral stage by use of polymeric structured vehicle.

Drug Loading (DL), product yield and drug content

Lyophilized nanoparticles were evaluated for drug loading, product yield and drug content which was found to be 8.92, 83.20% and 92.30% respectively. Results indicate maximum entrapment of drug in polymer with minimum loss.

Scanning Electron Microscopy (SEM)

The lyophilized nanoparticles were tested for surface morphology by SEM analysis. Results are illustrated in Figure 5. Particles were found to be needle shaped which is characteristics of lyophilized material.

X-ray Diffraction (XRD)

The drug and nanoparticles were subjected for X-ray diffraction study. The characteristic sharp peaks were recorded in drug X-ray diffractogram (Figure 6) which were vanished in diffractogram of nano-particles (Figure 7). Results confirms the encapsulation of drug by PLGA.

Preparation of nanoparticle loaded gel

After confirmation of desired properties of lyophilized nanoparticles, the nano gel was formulated.

Experimental design

The optimization was carried out by using 3^2 factorial designs in which independent variables were investigated at three levels as shown in Table 3 in their actual and coded form.

Impact of independent variables was studied on responses Y1: Viscosity at 20 rpm, Y2: % drug release at 5 hr which was found

Table 3: Experimental design for development of nano-gel.

Sl. No.	Batch No	Conc. Of Carbopol 934 (%) (X1)	Conc. HPMC K4M (%) (X2)	Viscosity at 20 rpm in cP (Y1)	% Drug release at 5 hr (Y2)
1	F1	0.15	0.5	2010	69
2	F2	0.15	1.0	2867	61
3	F3	0.15	1.5	3532	58
4	F4	0.3	0.5	3025	57
5	F5	0.3	1.0	3650	54
6	F6	0.3	1.5	4050	50
7	F7	0.45	0.5	3850	47
8	F8	0.45	1.0	4062	45
9	F9	0.45	1.5	4542	40

Table 4: Summary of results of regression analysis for responses.

Response	Model	*p*-value	*F*-value	R^2
Viscosity	2FI	0.0082	17.96	0.9898
% Drug release	Linear	<0.0001	165.12	0.9822

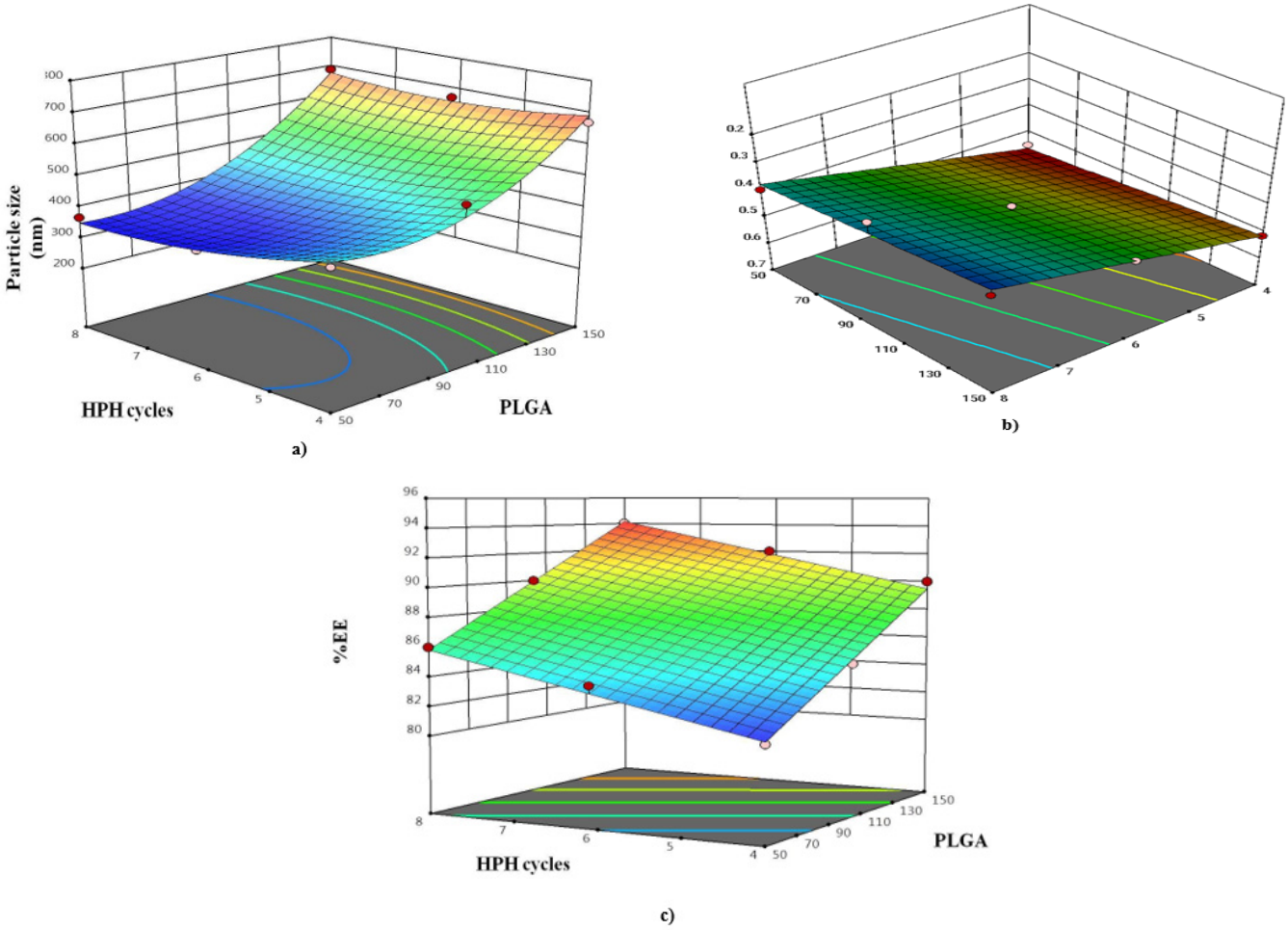

Figure 1: Three dimensional (3 D) response surface plot for response a) Particle size, b) Polydispersity index (PDI), c) % Entrapment Efficiency (EE).

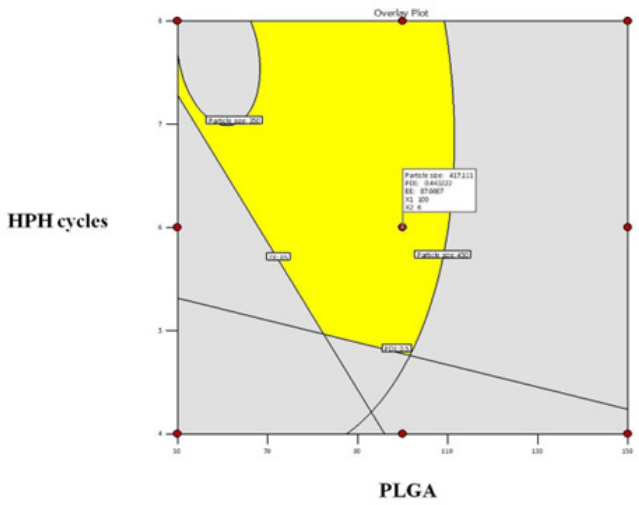

Figure 2: Desirability plot.

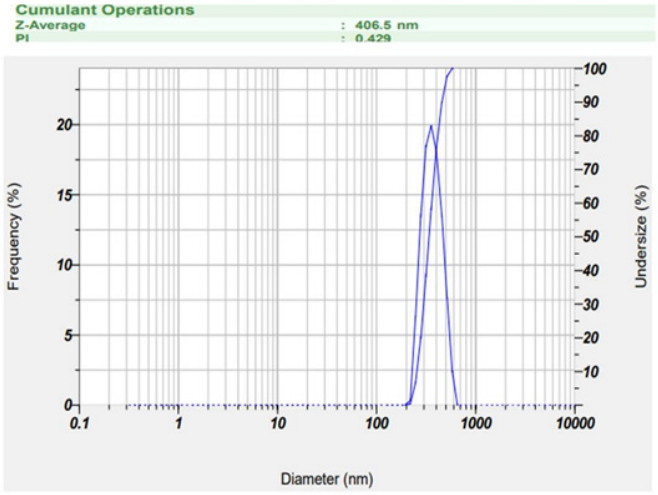

Figure 3: Particle size of optimized batch.

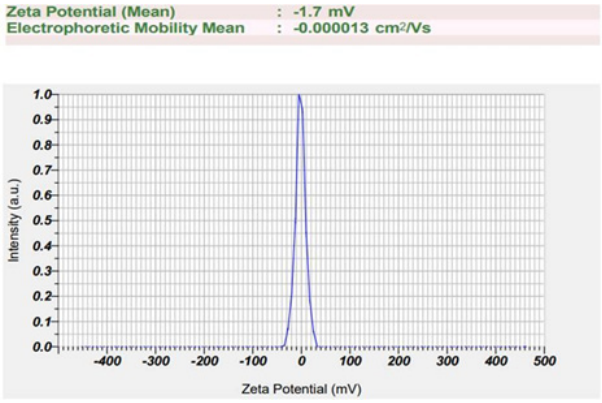

Figure 4: Zeta potential of optimized batch.

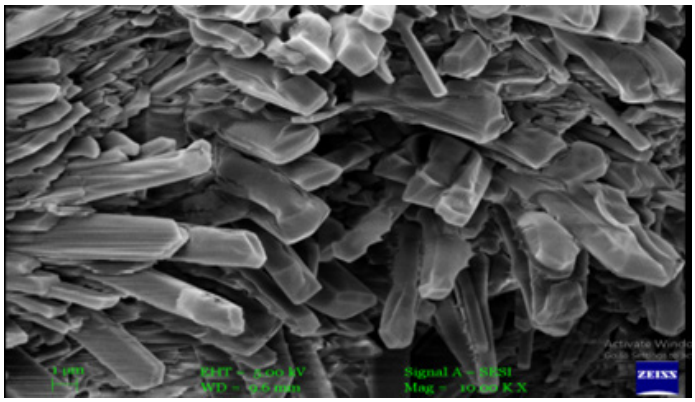

Figure 5: Surface morphology by SEM analysis.

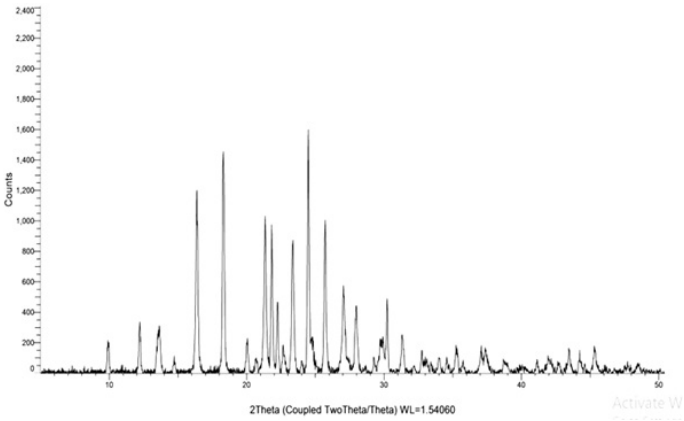

Figure 6: XRD of Luliconazole.

to in the range of 2010-4542 cP nm and 69-40% respectively as depicted in Table 3.

Data was subjected for optimization by Design expert v13 software. Model suggested by software and regression analysis for responses are represented in Table 4. As the probability value is less than 0.05 for each model that confirmed the significance of model for responses.

Software suggested polynomial equations;

$$R1=+3509.78+674.17\ X1+539.83\ X2-207.50\ X1X2 \quad 4$$

$$R2=+53.44\ -9.33\ X1-4.17\ X2 \quad 5$$

Form the equation 4 it was observed that the concentration of Carbopol as well as HPMC has the positive impact on viscosity i.e., as the concentration of polymer increases the viscosity also increases. Equation 5 indicates the negative impact of both variables on % drug release i.e., increases in polymer concentration showed decreased drug release.

The impact of X1 and X2 on responses can be further confirmed by using 3D surface response plot (Figure 8).

Selection of optimized batch was done by desirability search approach and batch F5 containing 0.3% carbopol and 1.0% HPMC which was selected as the optimized batch and used for further analysis.

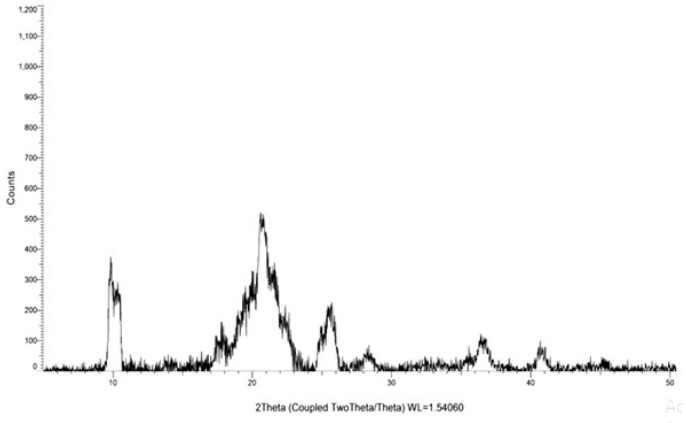

Figure 7: XRD of drug loaded nanoparticles showing molecular dispersion of drug.

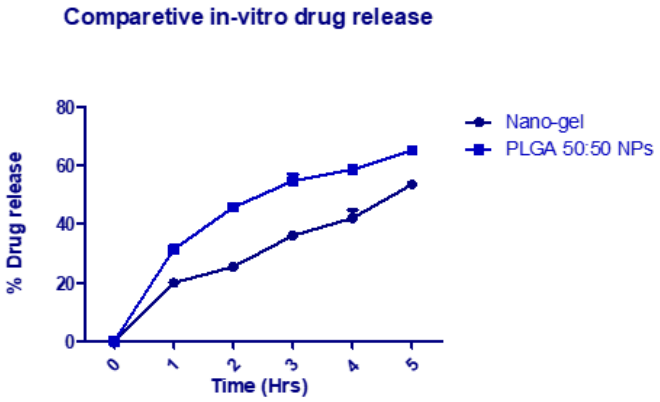

Figure 9: Percentage drug release from nanoparticles and nano-gel (*n*=3, mean±SEM).

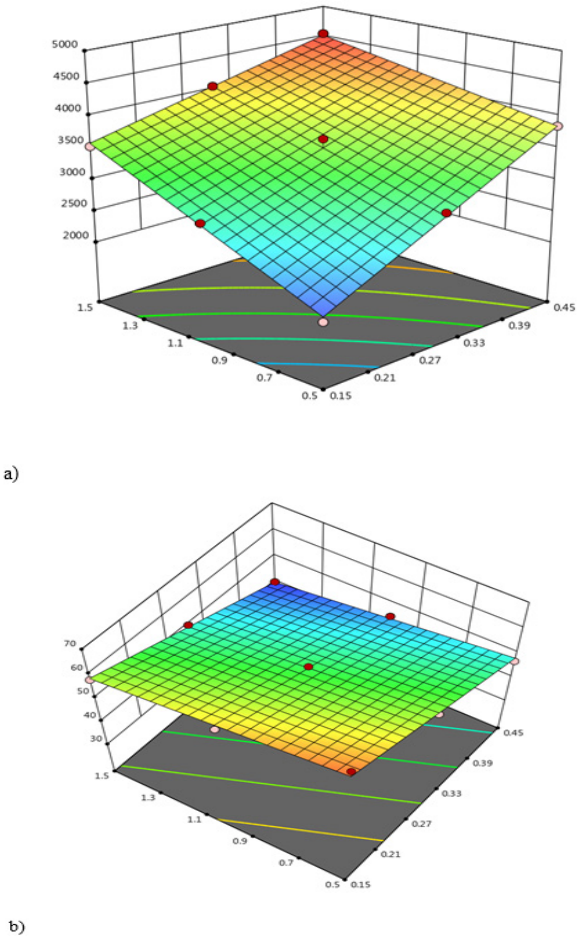

Figure 8: Three dimensional (3 D) response surface plot for response a) Viscosity, b) % Cumulative Drug Release (%CDR).

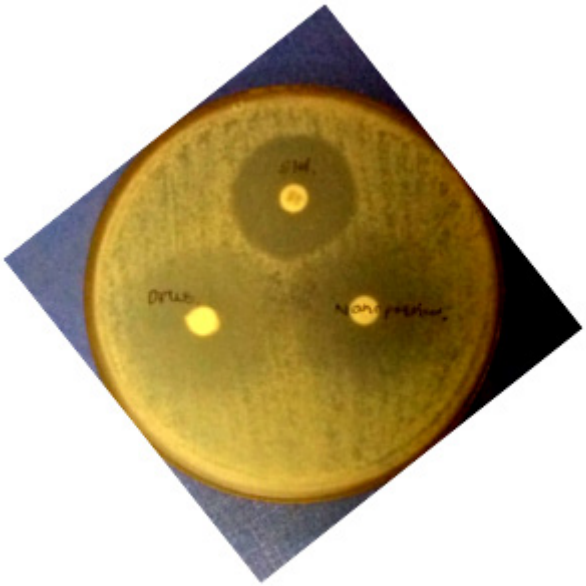

Figure 10: Comparative zone of inhibition.

Figure 11: Skin irritation test.

Characterization of nano-gel

Clarity and pH

All prepared batches were visually inspected against dark and white background. Formulations were clear, homogeneous and free from grittiness. The pH of all batches was found in the range

Table 5: Model fitting for release profile.

Formulation	Coefficient of determination (R^2)				Best fit model
	First order	Zero order	Higuchi	Hixon-Crowel cube root	
Nanoparticles	0.9188	0.9562	0.9779	0.9662	Higuchi
Nps loaded in-situ gel	0.9281	0.9044	0.9889	0.9548	Higuchi

Table 6: Korsmeyer-Peppas drug release kinetics.

Formulation	R^2	n value	Mechanism
Nanoparticles	0.9688	0.505	Non-Fickian
NPs loaded in-situ gel	0.9699	0.459	Fickian

of 6.38 to 6.56 which fall in the normal range of the skin and makes formulation compatible with skin.

Determination of Viscosity

Viscosity is one of the important factors associated with semisolid preparation which can be correlated with spreadability, extrudability, retention on skin and ultimately the patient compliance. All batches were tested for viscosity by using Brookfield viscometer at 20 rpm. Viscosity was found to be in the range of 2010-4542 cP. Optimized batch showed viscosity 3650 cP which ensures the ease of movement over skin upon application.

In vitro drug diffusion study

Prepared nanoparticles and nano-gel were subjected for comparative *in vitro* drug diffusion study. Nanoparticles showed 65.22% drug release at the end of 5 hr, whereas nano-gel showed more sustained drug release as 54.12% at the end of 5 hr (Figure 9). Results clearly indicate more prolog release pattern from nano-gel than simple nanoparticles. This difference is due to the two-step movement of drug from nano-gel as nanoparticles to gel-matrix and then from gel to diffusion medium.[10]

Obtained data was subjected for to determine release kinetic profile. From the slop, the release constant was calculated, and the regression coefficient was determined (Table 5). Higuchi model was found to be the suitable to describe the drug release kinetics from nano-gel as well as the simple nanoparticles.

Korsmeyer-Peppas equation showed characteristic Fickian drug release mechanism against the non-Fickian mechanism of the nanoparticles (Table 6).

Spreadability and Extrudability Test

The spreadability as well as Extrudability are the essential parameters of semisolid dosage form which are dependent on viscosity and affects the patient compliance. Optimized nano-gel showed satisfactory results for the both parameters. Spreadability was fond to be 7.4±0.5 cm with excellent extrudability (+++).[17]

Antifungal study

The therapeutic benefit of the prepared nano-gel was confirmed by the zone of inhibition method. Drug, marketed formulation, and nano-gel showed 18 mm, 15 mm, and 20 mm inhibition zones, respectively, as shown in Figure 10. These results indicate that the antifungal activity shown by prepared Luliconazole nanogel is greater than that of normally marketed Luliconazole. This is due to enhanced permeation and solubility by nanoparticles. Similar results were observed by Kumar M *et al.* 2019.[18]

Skin irritation test

After confirming the antifungal activity of prepared nano-gel, skin irritation study was carried out on Albino Wister rats to confirm the safety of prepared formulation for proposed use. Formulation was applied on the shaved skin of rats and animals were observed for any sign of skin irritation for 60 min. No redness, edema and erythema were observed (Figure 11). Results clearly indicate the suitability and safety of prepared formulation in management of topical fungal infections.

CONCLUSION

In proposed research, we prepared luliconazole nanoparticles by PLGA which were further dispersed in the gel of Carbopol 934 and HPMC K4M. At early stage, nanoparticles were optimized by using 3^2 factorial design and further lyophilized. Optimized nanoparticles were further loaded in gel of Carbopol and HPMC which was again optimized by using 3^2 factorials designs to set suitable concentration of polymer. Optimized formulation showed satisfactory extrudability and spreadability. A comparative antifungal study between drug, standard and optimized nano-gel showed significant zone inhibition than drug and marketed formulation. No, any sign of skin irritation was observed on Albino Wister rats. Based on available evidences, there is scope to consider the prepared nano-gel as the suitable substituent to current fungal treatment.

ACKNOWLEDGEMENT

Authors express their sincere thanks to management and Principal METs Institute of Pharmacy, BKC: Adgaon, Nashik for providing necessary facilities to carry out this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: Analysis of Variance; **CDR:** Cumulative drug release; **DOE:** Design of experiments; **DSC:** Differential Scanning Colorimetry; **HPH:** High-Pressure Homogenizer; **MIC:** Minimum Inhibitory Concentration; **NPs:** Nanoparticles; **P80:** Polysorbate 80/Polyoxyethylene Sorbitan Monooleate; **RH:** Relative Humidity; **SEM:** Scanning Electron Microscopy; **XRD:** X-ray diffraction.

SUMMARY

Large population of the globe is suffering from the fungal infection. Fungal infections are preferably treated by the topical application of antifungal drugs. Conventional topical dosage form needs to be applied frequently and in high dose due to poor penetrability. Current research involves the formulation of 'luliconazole nano-gel'. Drug nanoparticles were prepared by using the PLGA 50:50 polymer and further dispersed in gel obtained from Carbopol 934 and HPMC K4M. Two-stage optimization was carried out viz. fabrication of nanoparticles and development of nano-gel by using 3^2 factorials designs. During a zone of inhibition test, prepared formulation showed better antifungal action than standard therapy. No signs of skin irritation were observed during the skin irritation test.

REFERENCES

1. Kainz K, Bauer MA, Madeo F, Carmona-Gutierrez D. Fungal infections in humans: the silent crisis. Microb Cell. 2020;7(6):143-5. doi: 10.15698/mic2020.06.718, PMID 32548176.
2. Ameen M. Epidemiology of superficial fungal infections. Clin Dermatol. 2010;28(2):197-201. doi: 10.1016/j.clindermatol.2009.12.005, PMID 20347663.
3. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses. 2008; 51;Suppl 4: 2-15. doi: 10.1111/j.1439-0507.2008.01606.x , PMID 18783559.
4. Garg A, Sharma GS, Goyal AK, Ghosh G, Si SC, Rath G. Recent advances in topical carriers of anti-fungal agents. Heliyon. 2020;6(8):e04663. doi: 10.1016/j.heliyon.2020.e04663, PMID 32904164.
5. Chapla VM, Zeraik ML, Leptokarydis IH, Silva GH, Bolzani VS, Young MC, *et al.* Antifungal compounds produced by Colletotrichum gloeosporioides, an endophytic fungus from *Michelia champaca*. Molecules. 2014;19(11):19243-52. doi: 10.3390/molecules191119243, PMID 25421415.
6. Naik A, Kalia YN, Guy RH. Transdermal drug delivery: overcoming the skin's barrier function. Pharm Sci Technol Today. 2000;3(9):318-26. doi: 10.1016/s1461-5347(00)00295-9, PMID 10996573.
7. Dismukes WE. Introduction to antifungal drugs. Clin Infect Dis. 2000;30(4):653-7. doi: 10.1086/313748, PMID 10770726.
8. Laddha UD, Kshirsagar SJ, Sayyad LS, Ahmed MT, Gaikwad SS, Udavant PB, *et al.* Development of surface modified nanoparticles of curcumin for topical treatment of diabetic retinopathy: *in vitro, ex vivo* and *in vivo* investigation. J Drug Deliv Sci Technol. 2022;76:103835. doi: 10.1016/j.jddst.2022.103835.
9. Laddha UD, Kshirsagar SJ. Formulation of PPAR-gamma agonist as surface modified PLGA nanoparticles for non-invasive treatment of diabetic retinopathy: *in vitro* and *in vivo* evidences. Heliyon. 2020;6(8):e04589. doi: 10.1016/j.heliyon.2020.e04589, PMID 32832706.
10. Laddha UD, Kshirsagar SJ. Formulation of nanoparticles loaded *in situ* gel for treatment of dry eye disease: *in vitro, ex vivo* and *in vivo* evidences. J Drug Deliv Sci Technol. 2021;61:102112. doi: 10.1016/j.jddst.2020.102112.
11. Kumar S, Singh S, Senapati S, Singh AP, Ray B, Maiti P. Controlled drug release through regulated biodegradation of poly (lactic acid) using inorganic salts. Int J Biol Macromol. 2017;104(A):487-97. doi: 10.1016/j.ijbiomac.2017.06.033, PMID 28624369.
12. Joshi VY, Yashaswini G, Acharya AB, Bheemachari HV, Annegowda HV, Niraula B. Formulation and evaluation of semisolid dosage forms of an anti-inflammatory drug. 3 Biotech. 2019;9(7):248. doi: 10.1007/s13205-019-1769-6, PMID 31218172.
13. Swain S, Barik SK, Behera T, Nayak SK, Sahoo SK, Mishra SS, *et al.* Green synthesis of gold nanoparticles using root and leaf extracts of *Vetiveria zizanioides* and *Cannabis sativa* and its antifungal activities. BioNanoScience. 2016;6(3):205-13. doi: 10.1007/s12668-016-0208-y.
14. Rout Y, Behera S, Ojha AK, Nayak PL. Green synthesis of silver nanoparticles using *Ocimum sanctum* (Tulashi) and study of their antibacterial and antifungal activities. J Microbiol Antimicrob. 2012;4(6):103-9. doi: 10.5897/JMA11.060.
15. Mandala SM, Thornton RA, Milligan J, Rosenbach M, Garcia-Calvo M, Bull HG, *et al.* Rustmicin, a potent antifungal agent, inhibits sphingolipid synthesis at inositol phosphoceramide synthase. J Biol Chem. 1998;273(24):14942-9. doi: 10.1074/jbc.273.24.14942, PMID 9614099.
16. Acute dermal irritation/corrosion: Test No. 404. OECD guidelines for the testing of chemicals, Section 4. Paris: OECD Publishing; 2015.
17. Mahmood A, Rapalli VK, Waghule T, Gorantla S, Singhvi G. Luliconazole loaded lyotropic liquid crystalline nanoparticles for topical delivery: QbD driven optimization, *in vitro* characterization and dermatokinetic assessment. Chem Phys Lipids. 2021;234:105028. doi: 10.1016/j.chemphyslip.2020.105028, PMID 33309940.
18. Kumar M, Shanthi N, Mahato AK, Soni S, Rajnikanth PS. Preparation of luliconazole nanocrystals loaded hydrogel for improvement of dissolution and antifungal activity. Heliyon. 2019;5(5):e01688. doi: 10.1016/j.heliyon.2019.e01688, PMID 31193099.

Cite this article: Chhajed SS, Laddha UD, Sonanwane SS, Pingale M, Thorat P, Kshirsagar SJ, *et al*. Formulation, Development and Characterization of PLGA-Luliconazole Nanoparticles Loaded Gel System for Topical Fungal Treatment. Indian J of Pharmaceutical Education and Research. 2024;58(1s):s187-s195.