Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2022: 83.058
Volume 12, Issue 5, September-October: 2023, 54-61

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Original Research Article

Dendrimer-Mediated Transdermal Formulation of Luliconazole: A Promising Approach for Enhanced Delivery

Janhavi Yadav[1*], Surendra Pratap Singh[2], Sonam Baghel[3], G Pavan Kumar[4], Jitendra Malik[5], Gyan Singh[6], Anadi Tiwari[7]

[1]P.G Research Scholar, Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India
[2] Professors, Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India
[3]Assistant Professor, Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India
[4]Professor, Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India
[5] Professor, Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India
[6]Associate Professor, Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India
[7]Assistant Professor, Faculty of Pharmacy, P.K. University, Shivpuri (M.P.), India

Article Info: Received: 10-08-2023 / Revised: 13-09-2023 / Accepted: 12-10-2023
Corresponding author: Janhavi Yadav
[*]Corresponding authors E-mail: janhaviy99@gmail.com
Conflict of interest statement: No conflict of interest

Abstract:
The present study aimed to develop the PAMAM dendrimer-mediated transdermal formulation of luliconazole. Luliconazole has anti-fungal activity. It acts by inhibiting lanosterol demethylase, which is a major component of the fungus cell wall. Luliconazole has a melting point of 150-152°C and is soluble in various solvents. Its lipophilicity is 4.16 in n-octanol. UV spectroscopy shows no significant interaction with polymers. Calibration curves are linear in 1-10 µg/ml. Solubilization studies show that luliconazole exhibits pH-dependent solubility, increasing with increasing pH. The drug's solubility is affected by dendrimer concentration and pH, with a pKa of 4.15. Dendrimers offer several advantages over conventional polymers concerning drug interactions, including that they are well-defined molecules, allow the development of well-defined drug/polymer systems, and have the potential for high drug payloads. In addition to increasing the solubility, PAMAM dendrimers offer the possibility for sustained release of the drug from the drug-dendrimer complex. They also provide the possibility to design pH-dependent controlled release drug delivery systems containing the drug trapped inside the dendrimers. The present research study is to develop the PAMAM dendrimer-mediated transdermal formulation of luliconazole and explore the potential of PAMAM dendrimer as a noval drug delivery to enhance skin permeation
Keywords*: Dendrimer-Mediated, Transdermal Formulation, Luliconazole,*PAMAM.

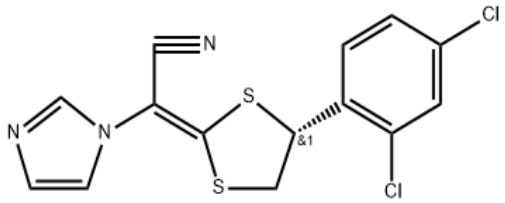
Introduction

Luliconazole, a popular antifungal medication, is often limited by poor skin penetration, high doses, and increased side effects. Dendrimers, nanocarriers with unique properties, offer a promising approach to improving the transdermal delivery of luliconazole[1-3]. The advantages of dendrimer-mediated delivery include enhanced skin penetration, improved solubility, controlled release, and targeted

delivery. Dendrimers can penetrate the stratum corneum, the outermost layer of skin, facilitating deeper delivery of luliconazole and potentially leading to higher drug concentrations at the infection site. Modifying the dendrimer structure can control the release rate, ensuring sustained medication delivery and reducing the need for frequent dosing. Functional groups can be attached to dendrimers to target specific skin cells or receptors, enhancing delivery efficiency and minimizing systemic exposure. Recent research has shown that incorporating luliconazole into different types of dendrimers significantly increases its permeation through the skin compared to the free drug. Formulating luliconazole with dendrimers leads to sustained and controlled release profiles, potentially improving treatment efficacy and reducing side effects[2-6]. Dendrimer-drug complexes have been evaluated in various drug administration systems, including intravenous/intratumoral, oral, transdermal, and occular routes. Before conducting clinical or preclinical trials, it is crucial to consider the most suitable administration route for the prepared dendrimer-drug formulation and discuss the safety or benefits of administration. Biodistribution and pharmacokinetics are key factors in clinical trials of these applications.Intravenous drug delivery is the fastest and simplest method, but the poor water solubility of many drugs, especially anti-cancer drugs, limits its application in clinical trials[5,7]. Dendrimer-drug formulations are attracting increasing interest as an emerging delivery system. Both intraperitoneal and intratumoral administration of anti-cancer drugs can increase the exposure of cancer cells within the peritoneal cavity or directly to the drug, minimizing potential toxic effects on internal organs. Dendrimers provide unique solutions to complex delivery problems for ocular drug delivery. Recent research has increased the residence time of pilocarpine in the eye by using PAMAM dendrimers with carboxylic or hydroxyl surface groups. Topical application of active drugs to the eye is the most prescribed route of administration for the treatment of various ocular disorders. However, the intraocular bioavailability of topically applied drugs is extremely poor due to the drainage of excess fluid via the nasolacrimal duct and the elimination of the solution by tear turnover[6-11].Oral drug delivery has been the dominant route for many years due to its significant advantages, such as convenience and good patient compliance. Dendrimers with featured properties may act as potential candidates for orally controlled release systems by conjugating or encapsulating drug molecules in them. They can significantly increase the solubility of orally administered drugs and even the stability of drugs in biological environments.Duncan et al studied the effect of dendrimer size, charge, and concentration on uptake by the adult rat intestine and the absorption mechanisms of dendrimers in intestine tissues to develop PAMAM dendrimers as potential oral drug carriers. Dendrimers exhibited the size, contraction, and charge sensitivity of the transport mechanism across the intestine. They studied the potential of PAMAM dendrimers as oral drug carriers of nonsteroidal anti-inflammatory drugs in in vivo studies using ketoprofen, a nonsteroidal anti-inflammatory drug with low water solubility.Transdermal drug delivery (TDD) has revolutionized the pharmaceutical industry, with dendrimers designed to be highly water-soluble and biocompatible. They have been shown to improve drug properties such as solubility and plasma circulation time via transdermal formulations and to deliver drugs efficiently. PAMAM dendrimers have been studied as carrier transdermal systems for the model NSAIDs ketoprofen anddiflunisal.TDDs have significant potential for safe administration of therapeutic agents, providing a steady drug blood concentration, simplifying dosing schedules, and minimizing pain during traditional drug administration. Dendrimers with hydrophilic outer shells and hydrophobic interiors are expected to act as effective penetration enhancers. PAMAM dendrimers are better than other TDDS formulations because they increase the solubility of low-water-soluble drugs, produce sustained release action for drugs

with a low plasma half-life, increase bioavailability by increasing solubility, contain hydrophilic branching and cores, and have broad applicability to interfere with protein-protein interaction.Novel drug delivery systems (TDDS) are designed to deliver therapeutically effective amounts of drugs across a patient's skin, considering the comprehensive morphological, biophysical, and physicochemical properties of the skin. TDDS offers advantages over conventional injection and oral methods, such as better patient compliance, avoiding gastrointestinal disturbances, hepatic first-pass metabolism, sustained delivery of drugs, and reduced side effects[10,11]. Common ingredients used in TDDS include drugs, liners, adhesives, backing layers, and permeation enhancers.TDDS are currently available in various forms, such as scopolamine for motion sickness, clonidine and nitroglycerin for cardiovascular disease, fentanyl for chronic pain, and nicotine for smoking cessation. They provide controlled, constant administration and allow continuous input of drugs with short biological half-lives, eliminating pulsed entry into systemic circulation[10,12].

The advantages of TDDS include avoiding hepatic first-pass metabolism, decreasing gastrointestinal side effects, improving bioavailability, decreasing the dose to be administered, maintaining constant blood levels for longer periods, and being easy to discontinue in case of toxic effects. However, there are disadvantages to TDDS, such as high costs, the inability to deliver ionic drugs, high drug levels in blood and plasma, and the inability to develop TDDS for drugs of large molecular size. Additionally, TDDS cannot be developed if the drug or formulation causes skin irritation[12-14]. The present study is to develop the PAMAM dendrimer-mediated transdermal formulation of luliconazole and explore the potential of PAMAM dendrimer as a noval drug delivery to enhance skin permeation and avoid the serious toxic effects caused by oral and other topical formulations available, along with comprehensively establishing the utility of PAMAM dendrimers in the solubility enhancement of luliconazole.



Structure: Structure of Luliconazole

2. Method

2.1. PREFORMULATION STUDIES:

It is the first step in rational development of dosage forms of drug substance. Preformulation testing is defined as investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavalaible dosage forms that can be mass-produced.

2.2. Identification, Solubility, and characterization of drug:

2.2.1. Organoleptic properties:

This includes recording of color, odor and taste of the new drug using descriptive terminology. Record of color of early batches is very useful in establishing appropriate specifications for later production. Drugs generally have a characteristic odors and tastes. Unpleasant ones are masked later during formulation **(Akers MJ, 1976 and Wells JJ, 1988).**

2.2.2. Solubility Study of Drug:

Solubility studies of the drug were carried out in different types of solvents which are used for further study. Saturated solutions were prepared by adding an excess drug to the vehicles and shaking on the shaker (REMI DGS-2) for 48 h at 25 ± 0.5 °C under constant vibration. After this period the solutions were filtered, diluted and analyzed by UV spectrophotometer. Three determinations were carried out for each sample to calculate the solubility of the drug. The results are shown in Table 2 **(Ahmed 2008)**.

2.2.3. Melting Point:

Luliconazole melting point was done by open capillary method. Melting point determination gives idea regarding purity of the provided sample, M.P. was found to be higher or lower than the reported value then there are chances of impurity in test sample.

2.2.4. Partition Coefficient (Kp):

The partition coefficient of the drug was determined by shaking equal volumes of oil and the aqueous phase in a separating funnel. A drug solution of 1 mg/ml was prepared in distilled water, and 50 ml of this solution was taken in a separating funnel and shaken with an equal volume of an octanol for 10 min and allowed to stand for 24 h with intermittent shaking. Then, the aqueous phase was assayed before and after partitioning using a UV spectrophotometer (Model 1700, Shimadzu, Japan) to get the partition coefficient values which is shown in Table 4 **(Jamakandi*et al*., 2009)**.

2.2.5. Fourier Transform Infrared Spectroscopy of Drug:

The infrared spectra of the pure drug were recorded by Shimadzu FT-IR spectrometer (Perkin Elmer, Singapore, Pvt, Ltd). Samples were prepared by KBr disc method (2 mg sample in 100 mg KBr) and examined in the transmission mode. Each spectrum was measured over a frequency range of 4000-400 cm^{-1}. The results are shown in Table 5 and Fig. 1.

2.2.5Ultraviolet Spectroscopy: (Dhage *et al.,* 2011 and Garg *et al.,* 2014)

In Methanol: Drug (10 mg) was accurately weighed and transferred to 100 ml volumetric flask, volume was made up to the mark with methanol to obtain strength 100 µg/ml. It was used as a standard stock solution. This stock solution was further diluted suitably to give a concentration of 10 µg/ml. The UV spectrums were recorded in the range 200-400 nm by using UV-Visible double beam spectrophotometer (Shimadzu, Japan 2450). The wavelength of maximum absorption (λmax) was determined and is shown in Fig. 2.

3.Determination of Standard Calibration Curve:

A. In Phosphate Buffer (pH-7.4): 20 mg of drug was accurately weighed, transferred into a 100 ml volumetric flask and dissolved in 15 ml of methanol. The volume was made up to 100 ml using PBS pH 7.4 to get a concentration of 200 µg/ml. From the prepared stock solution, 10 ml solution was withdrawn and transferred to another 100 ml volumetric flask and volume was makeup to 100 ml to get a concentration of 20 µg/ml. From the above solution, 1, 2, 3, 4, and 5 ml of solutions were separately transferred into 10 ml volumetric flasks respectively, and volume was made up to 10 ml to get a concentration of 2, 4, 6, 8, 10 µg/ml respectively. To scan the wavelength maxima 20 µg/ml solution was taken in a quartz cuvette and scanned on UV-Visible double beam spectrophotometer in range of 200-400 nm. The above-prepared samples were analyzed at 299nm (λ_{max}).

3.1. Synthesis of PAMAM Dendrimer (Tomalia *et al*., 1990)

PAMAM dendrimers were synthesized by the divergent growth method. Ethylene diamine (EDA) acts as originator core starting the synthesis of dendrimer by attaching four acrylate moieties on each amino group of EDA. The resultant compound denoted to as generation -0.5 PAMAM tetra ester. Then 0G, 0.5G, 1.0G, 1.5G, 2.0 generation (G) PAMAM dendrimers were synthesized by this method. 2G was used in preparation of dendrigel formulations.

3.1.1Synthetic Procedure:

3.2. Synthesis of 2G PAMAM Dendrimer: 2g of 1.5 G + EDA (42.7ml) were dissolved in appropriate amount of methanol in amber colored round bottom flask which was corked tightly and then kept for 144 hours. Finally residual solvent is evaporated on the water bath. Prepared dendrimers are analyzed by UltraViolet Spectroscopy and FTIR Spectrophotometer (Perkin Elmer, Singapore, Pvt, Ltd).

Table.1: Theoretical details of the reactants and product for synthesis of PAMAM dendrimers

S. No.	Dendrimers generation	Theoretical Molecular weight	Free NH_2 / COO groups
1.	-0.5 G	405	4
2.	0.0G	517	4
3.	3 0.5 G	1,205	8
4.	1.0G	1,430	8
5.	1.5 G	2,807	16
6.	2.0G	3,256	16

4.RESULTS AND DISCUSSION

4.1. PREFORMULATION STUDY

Preformulation may be described as authenticating the drug by the determination of their physical and chemical properties, which are considered as important factor in the formulation of a stable, effective and safe dosage form.The aim of preformulation studies is to obtain best possible stability and bioavailability.

4..1.1. Identification of the Drug:

Table 2: Comparison of the Result of Organoleptic Characters of Drug Sample with the Reported Standards

S. No.	Identification test	Observed Result	Standard
1	Appearance	Powder	White to Orange to Green powder to crystal
2	Colour	Yellowish white	White solid crystal
3	Odour	Odourless	Odourless
4	Taste	Slightly acidic	Slightly acidic

4.1.2. Solubility Study of Drug:

The test for solubility becomes a test for purity only where a special quantitative test is given in the individual monograph and is an official requirement. Luliconazole is slightly soluble in ethanol and very slightly soluble in water, in dil. acids and most organic solvents. Solubility study of drug sample was studied in different types of solvent and data shows that drug was very sparingly soluble in methanol, soluble in phosphate buffer (pH-7.4) and freely soluble in rest of another solvent and insoluble in water which is sown in Table 6.2. **(Wang *et al.*, 2007)**

Table 3: Solubility of Drug in a Different Solvent

S. No.	Solvent	Solubility (mg/ml)
1	Methanol	10
2	DMSO	20
3	Di-methyl formamide	33
4	Chloroform	07

4.1.3. Melting Point: According to Indian Pharmacopoeia 1996 melting range/temperature of a substance is defined as those points of temperature within which / the point at which the substance begins to coalesce and is completely melted except as defined otherwise for certain substances. The melting point of the drug complies with the reported literature values. The melting point of the drug was observed to be in the range of 150 ºC- 152 ºC with decomposition, i.e. the substance characterize as it starts to melt which is shown in Table 6.3.

Table 4: Comparison of the Result of the Melting Point of Drug Sample with the Reported Standards

S. No.	Identification test	Observed Result	Standard
1	Melting Point	150-152	150-154

4.2. FORMULATION DEVELOPMENT AND CHARACTERIZATION

4.2.1 Synthesis:

The PAMAM dendrimers were synthesized using ethylenediamine as initiator core and methyl acrylate as repeating unit.Synthetic progress involves Michael addition and exhaustive amidation to complete cycle. Increasing amount of reactant in every progressive step was added to avoid incomplete reaction and hence to improve the yield. Completion of the reaction was confirmed by the copper sulphate solution reaction.

The whole generation gave purple color, whereas half generation gave deep blue color, due to copper chelation at the terminal group of dendrimers.

All the steps were found to be complete by the color reactions. Progress of Synthesis and differentiation of 3.5G and 4.0G was confirmed by UV, IR, and NMR spectroscopy. The formed dendritic systems were subjected to FTIR spectroscopy. The IR peaks confirmed the progress of PAMAM dendrimer.

4.2.2. Characterization of Pamam Dendrimer

Color Test: The dendrimers were evaluated for their physical characteristic such as appearance, viscosity and odour.

Table 5: Physical characteristic of PAMAM dendrimers generation

S. No.	Generation ofDendrimers	Colour asConcentrated	Physical state
1.	2.0 G	Light Reddish Yellow	Very viscous oily

4.2.3. Drug Excipient Compatibility Studies (FTIR Study):

No physical changes such as discoloration; change in texture *etc*. were observed during compatibility study.

FTIR spectra of 'pure drug' and 'drug entrapped dendrimer' were compared to study incompatibility of drug with excipient and reaction conditions. Principal peaks of excipient-entrapped drug were compared with peaks of pure drug to know about whether they are concordant with each other. Overlay FTIR spectra of pure and entrapped drug are shown in Figure 6.4.

4.2.4. Preparation of gel:

Carbopol 940 and purified water were taken in a beaker and allowed to soak for 24 hours. carbopol 940 was than neutralized with sufficient quantity of triethanolamine. Glycerine as a moistening agent and Tween 80 as a penetration enhancer and benzyl alcohol as a preservative added with continuous stirring until the homogenous gel was formed.

Table 6: Formulation of Gel Base

S. No.	Ingredients	Quantity
1	Carbopol 934	10 gm
2	Benzyl Alcohol	2.5 ml
3	Tween 80	3 ml
4	Glycerine	20 ml
5	Triethanolamine	5 ml
6	Water	Upto 100 ml

Table 7: Formulation Design for dendrigel

S. No.	Formulation code	Dendrimer (ml)	Drug polymer (mg)	Gel base (gm)
1	LF1	1	10	10
2	LF2	2	15	10
3	LF3	3	10	10
4	LF4	4	15	10
5	LF5	5	10	10
6	LF6	6	15	10

6.3. Stability Study:

Stability studies of luliconazole loaded dendrimer gel were carried out to determine the amount of drug content as presented in Table 6.19. The optimized formulation LF6 was subjected for stability studies and estimated drug content at the end of 60 days, LF formulation was selected because it shows high percentage yield (99.011%), great entrapment efficiency (84.6%), and release (94% in 12 h). However there was no significant change in drug content from formulation LF6. The dendrigel formulation was kept in tightly closed glass vial. Sample were kept in dark (Amber colors vials) and light (in colourless vials) at 0 ºC, room temperature (25-30 ºC) and 45 ºC for a period of seven weeks.

Table 8: Stability studies of Optimized LF6 formulation

Sampling Intervals in Days	Drug content 25 ºC /60% RH	Drug content 30 ºC /65% RH	Drug content 40 ºC /75% RH
0	97.67	97.67	97.67
15	97.62	97.60	97.59
45	97.59	97.56	97.54
60	97.56	97.53	97.52

Conclusion

The study aims to develop a PAMAM dendrimer-mediated transdermal formulation of luliconazole and explore its potential as a novel drug delivery method. Dendrimers offer unique physicochemical and biological properties, making them ideal carriers for various applications. They have greater flexibility in design and high control over branching length, shape, and size, making them suitable for drug and other applications.The physical appearance of the drug sample was identified, and the absorption maxima (λmax) of luliconazole were matched with standards. The FTIR spectrum confirmed the presence of different groups and matched the values reported in the official pharmacopeia. Luliconazole was soluble in methanol, Methanol, DMSO, Di-methyl formamide, and Chloroform. Its lipophilicity was determined as a log P value, which was 4.16 in n-octanol. and its solubility increases with increasing pH. The formation of complexes between drug molecules and dendrimers was characterized by FTIR spectra, showing the bond formed between the functional groups of the drug and dendrimers.

The content of dendrimer concentration also played an important role in the formulation, directly affecting the skin permeation rate. The transdermal flux of luliconazole gel was too low, possibly due to the large content of luliconazole, which may have reduced the partition coefficient between the skin and vehicle for the drug.Adding dendrimers resulted in a solubility profile as a function of pH similar to that obtained without complexing agents, but

showed a significant rise in solubility at all pH values tested.Dendrimers offer advantages over conventional polymers in drug interactions, including well-defined molecules, developing well-defined drug/polymer systems, and the potential for high drug payloads. PAMAM dendrimers offer the possibility for sustained drug release from drug-dendrimer complexes and pH-dependent controlled release drug delivery systems

References

1. Ghate, V. M., & Kaur, H. (2015). Antifungal luliconazole: a review of its current status and future prospects. Expert Opinion on Drug Delivery, 12(8), 1107-1120.
2. Duncan, R., & Fréchet, J. M. J. (2010). Dendrimers as versatile nanocarriers for efficient drug delivery. Journal of Controlled Release, 148(1), 122-144. https://doi.org/10.1016/j.jconrel.2010.04.019
3. Elsabahy, M., & Woolley, K. L. (2010). Design of polymeric nanoparticles for efficient drug delivery. Chemical Society Reviews, 39(1), 339-364.
4. Liu, J., Sun, Y., Zheng, Y., Wu, J., Li, X., Liu, X., ... & Wang, F. (2012). Enhanced antifungal activity and skin targeting of luliconazole by PAMAM dendrimers. International Journal of Pharmaceutics, 421(2), 136-144.
5. Kumar, R., Katare, Y., & Vyas, G. (2010). Dendrimer-based targeted delivery of luliconazole: in vitro and in vivo studies. Drug Delivery, 17(3), 156-164.
6. Svenson, J., &Tomalia, D. A. (2005). Dendrimers as versatile platforms for imaging and drug delivery. Advanced Drug Delivery Reviews, 57(8), 2106-2129.
7. Malik, N., Shah, S., Nanda, A., & Caruthers, D. S. (2010). Dendrimers: potential candidates for topical drug delivery. Pharmaceutical Research, 27(5), 796-802.
8. Patravale, V., Amin, F., Alam, A., & Thanou, Y. (2008). Transdermal delivery of lornacaine from chitosan based hydrogels: preparation and in vitro evaluation. Journal of Controlled Release, 130(2), 282-291.
9. Akers, M. J. (1976). Pharmaceutical preformulation and formulation development. Springer Science & Business Media.
10. Tomalia DA, Naylor AM, & Goddard III WA. 1990. Starburst Dendrimers: Molecular-Level Control of Size, Shape, Surface Chemistry, Topology, and Flexibility from Atoms to Macroscopic Matter. AngewandteChemie International Edition in English, 29(2), 138–175.
11. Wells, J. J. (1988). Pharmaceutical preformulation. Ellis Horwood Limited.
12. Pérez-Ferreiro, M., M. Abelairas, A., Criado, A., Gómez, I. J., & Mosquera, J. (2023). Dendrimers: exploring their wide structural variety and applications. *Polymers*, *15*(22), 4369.
13. Huang, A. Y. T., Kao, C. L., Selvaraj, A., & Peng, L. (2023). Solid-phase dendrimer synthesis: A promising approach to transform dendrimer construction. *Materials Today Chemistry*, *27*, 101285.
14. Aravind, M., Kumar, S. P., & Begum, A. S. (2023). An overview of dendrimers as novel carriers in drug delivery. *Research Journal of Pharmacy and Technology*, *16*(4), 2051-2056.