Journal of Pharmaceutical and Biological Research
ISSN: 2347-8330 | CODEN (USA): IJCPNH | Publisher: Pharma Research Library
Home Page: https://pharmaresearchlibrary.org/journals/index.php/jpbr
DOI: https://doi.org/10.30904/j.jpbr.2025.4886
J. Pharm. Bio. Res., 2025; 13(2): 81-89

Formulation Development and Characterization of Posaconazole Loaded Nanoparticle Gel

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A B S T R A C T

The present study focused on the development and evaluation of Posaconazole-loaded nanostructured lipid carriers (NLCs) as a novel topical delivery system to overcome limitations of conventional formulations such as poor solubility, low bioavailability, and inadequate tissue retention. Posaconazole, a broad-spectrum triazole antifungal, was incorporated into solid lipid nanoparticles (SLNs) and NLCs using the melt emulsification ultrasonication method, with optimization carried out through a full factorial design (3^3). Critical formulation factors including lipid-drug ratio, surfactant concentration, and sonication time significantly influenced particle size and entrapment efficiency. Optimized formulations demonstrated particle sizes ranging from 80–150 nm for NLCs with high entrapment efficiency (up to 85%) and stable zeta potential (–13 to –15 mV). Differential Scanning Calorimetry and Scanning Electron Microscopy confirmed successful drug encapsulation and uniform spherical nanoparticles, respectively. The optimized NLC was incorporated into an aloevera based carbopol gel, yielding a formulation with appropriate pH, viscosity, spreadability, and rheological characteristics suitable for topical application. *In vitro* permeation studies indicated enhanced skin retention of Posaconazole with minimal systemic penetration, while fluorescent microscopy confirmed deeper layer skin targeting. Antifungal evaluations demonstrated superior efficacy of the NLC gel against *Candida albicans* and *Aspergillus fumigatus*, with sustained activity, reduced fungal burden, and significant anti-inflammatory effects in *in vivo* models. Overall, the developed Posaconazole-loaded NLC gel showed promising potential as a safe and effective topical drug delivery system, combining enhanced local drug retention with improved therapeutic outcomes and reduced systemic side effects.

Keywords: *Candida albicans* and *Aspergillus fumigatus*, Differential Scanning Calorimetry, Scanning Electron Microscopy

A R T I C L E I N F O

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Article History
Received 20 July 2025
Revised 10 Aug 2025
Accepted 29 June 2025
Published 31 Oct 2025

Citation: Shirsath Prathmesh Durgadas, *et al.*Formulation Development and Characterization of Posaconazole Loaded Nanoparticle Gel. J. Pharm. Bio. Res., 2025, 13(2): 81-89.

Contents

1. Introduction

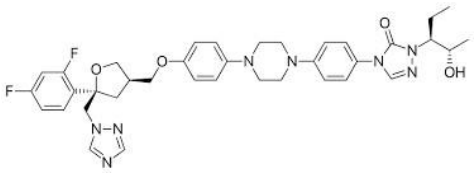


Fig.1: Posaconazole

Molecular Formula: $C_{37}H_{42}F_2N_8O_4$
Molecular Weight: 700.78 g/mol
IUPAC Name: 4-[4-[4-[4-[[[(3R,5R)-5-[(1H-1,2,4-triazol-1-yl)methyl]-5-(2,4-difluorobenzyl)tetrahydrofuran-3-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-1H-1,2,4-triazol-1-yl]benzonitrile
Chem Spider ID: 5293780

Density: ~1.36 g/cm³ (predicted)
Boiling Point: ~850.7 °C (predicted)
Melting Point: 170–172 °C
Flash Point: 9 °C
Refractive Index: Not readily available
Polar Surface Area: Not specified
LogP (Octanol/Water): Not explicitly listed, but it is lipophilic
Generic Name: Posaconazole
Brand Names: Noxafil, Posanol
Drug Category: Antifungal (Triazole class)
Indications: Treatment and prophylaxis of invasive fungal infections, especially in immunocompromised patients (e.g., Aspergillosis, Candidiasis, Mucormycosis)
Mechanism of Action:
Inhibits fungal lanosterol 14α-demethylase, disrupting ergosterol synthesis and compromising cell membrane integrity
Potency: Highly potent against Candida, Aspergillus, and Zygomycetes species, including strains resistant to other azoles
Tolerability: Generally well-tolerated; gastrointestinal upset is the most common side effect
Contraindications: Hypersensitivity to posaconazole or other azoles; caution with drugs that prolong QT interval
Adverse Effects: Nausea, vomiting, liver enzyme elevation, rash, QT prolongation
Availability: Oral suspension, delayed-release tablets, and intravenous formulations

2. Materials and Methods

Preliminary Evaluations of Posaconazole

Physiochemical Characterization of Posaconazole:

Melting point: It was measured by using digital auto melting point apparatus CE ISO 9001, Labtronics.

Differential Scanning Calorimeter (DCS)

The thermal properties of the Posaconazole (SC) was evaluated using DSC (EVO 131, SETARAM Instrumentations France). 5-7 mg of the drug was placed in aluminum crucibles, which were hermetically sealed/ crimped, while empty crucibles were employed as a reference. The sample was heated in the range of 30–250 °C at a heating and cooling rate of 10°C min−1 and 20°C min−1, respectively.

FTIR-ATR Model:

The spectrum of SC powder was recorded on ATR spectrometer (Alfa, Bruker, Berlin, Germany) over a frequency range of 4000-500 cm-1.

Solubility

Solubility of SC was determined in solvents such as ethanol, methanol, acetonitrile and water.

Spectrophotometric analysis of posaconazole:

Absorption spectra:

A solution of SC (1 mg/mL) was scanned on UV-3000 double-beam UV-Visible spectrophotometer (Labindia, India) over the wavelength range of 200-800 nm and wavelength corresponding to the peak maxima was noted.

Preparation of calibration plot of SC using UV spectrophotometer

(A) Standard solution

100 mg of SC was dissolved in 50 mL of methanol in a volumetric flask to prepare a stock solution of 2 mg/mL. The stock solution of SC was subsequently diluted with methanol to obtain a series of dilutions ranging from 0.4 to 2 mg/mL. The absorbance of these solutions was read on the spectrophotometer and standard curve was plotted between absorbance and drug concentration.

Analytical Method Development and Validation by UV visible spectrophotometer: The developed method was validated for specificity, precision, linearity and range, accuracy, robustness, limit of detection and limit of quantification.

UV spectroscopic method validation for determination:

The validation of an analytical method is the process of determining the suitability of a given methodology by laboratory studies that the method in question can meet the requirement for the intended use. Method validation is not simply a measure of performance of the total analytical system, (ICHQ2A, 1994). The process of analytical method validation ensures that the proposed analytical methodology is accurate, specific, reproducible, and rugged for its intended use.

Development of Nanoformulation by using High Pressure Homogenizer Technique: In this study, the stabilizer that was screened on the basis of the suspending effect on drug NPs using high-pressure homogenizer technique. Two-level multifactor, Plackett Burman design was used to evaluate the impact of formulation, and process variables like homogenization pressure, speed of high-speed homogenizer (primary Nanoformulation), time of homogenizer (primary Nanoformulation), number of cycles on responses like particle size, drug content and entrapment efficiency. After finding the critical process variables, the actual values of these process variables were optimized. Three-factor five-level full factorial, 5^3 Central composite design (CCD) of RSM are applied. They are the well-set approach for optimization of effects of factors, formulation variables and process parameters of HPH on dependent responses like particle size, entrapment efficiency and drug content of prepared Nanoformulation with a view to developing highly soluble and stable NPs. Developed NPs were dried using lyophilizer an optimized method for drying of NPs to stabilize the Nanoformulation. The resultant NPs were characterized for saturation solubility, drug content, entrapment efficiency, zeta potential, particle size, surface topographical studies, dissolution efficiency.

Design of Experiments

QTPP: To determine the QTPP, risk, regulatory, scientific and practical aspects are considered. The main goal of the study is the determination of the TPQP and target product profile. Control space can also be helped to determine the operability region. CPPs & CMAs were picked to achieve the target that is predefined in this study. The determined CPPs, CMAs and QTPP are disclosed in Table.

Risk analysis study by Plackett-Burman Design: Process variables that affect the CQAs of NPs formulation were screened by a group of experiments using Plackett–Burman (PB) screening design for the development of NPs using wet media milling. We can screen a large number of factors with a few runs by using the PB design. Another important

part of PB designs was the option of dummies, the component whose level does not change .The only main effects can be estimated by the PB design, as they are the resolution of three designs. From the large set of experimental factors, PB designs are typically used to identify a few but significant factors. Design-Expert (Version 11.0.5.0, Stat-Ease Inc., MN), involving eight independent variables, generated 12 experiment trials for Sertaconazle. Experiments were performed in a randomized order according to the run number that was arranged by the software. The response values were the mean measurements taken in three duplicates. ANOVA (Analysis of variance) was used to estimate the significance of main effects and interactions. Factors with a negligible effect on the response at a significance level of 95% were screened out. The remaining factors that affected the response were optimized further.

Plackett-Burman Design for Sertaconazle Nanoformulation
For estimation of process and formulation variables for Posaconazole Nanoformulation eight independent variables were screened viz., speed of high-speed homogenizer (primary Nanoformulation) (X1), time of homogenizer (primary Nanoformulation) (X2), homogenization pressure (X3), number of cycles (X4), concentration of SL (X5), concentration of SLS (X6), concentration of tween 80 (X7) and concentration of PC (X8). The response variables selected were particle size (Y1), drug content (Y2) and entrapment efficiency (Y3) based on trials drawn during preliminary batches.

Optimization by Central Composite Design

A response surface method, CCD using a five-level full factorial study was performed to explore the optimum levels of the variables after following Plackett–Burman screening design and identifying critical formulation and process variables. This methodology consists of two groups of design points, which include two-level factorial design points (-1 & +1), axial or star points (- α & + α) along with center points (0). Therefore, three selected independent variables that have the highest percentage contribution were selected from PB design and further studied at five different levels coded as -α, -1, 0, +1, and + α using CCD. The value for α (1.6817) was calculated to fulfill the design rotatability. Dependent or response variables selected are particle size (Y1), drug content (Y2) & entrapment efficiency (Y3). The CCD matrix was designed by using Design Expert® software (Version 11.0.5.0, Stat-Ease Inc., MN), with 20 runs, including one replication of fractional points, one axial point and six replicated center points. According to the obtained CCD matrix, the 20 Nanoformulation formulations were prepared and evaluated for responses to proceed with model fitting (Pandya, 2011). For the current optimization study, different RSM computations were performed and polynomial equations that contain quadratic and interaction terms were produced for all the dependent factors using the MLRA approach. Along with this, output files obtained from DOE, two-dimensional contour plots were also constructed.

Process analytical technology (PAT) – Particle size analysis, entrapment efficiency, and drug content

Size analysis of fabricated NPs particles were measured using Zetasizer 300 HAS (Malvern Instruments, Malvern, UK), while entrapment efficiency and drug content was determined using UV-visible spectrophotometer at 260 nm wavelength, respectively which was used for PAT for particle size, entrapment efficiency and drug content analysis.

Formulation of Nanoformulation

NPs were fabricated using high-pressure homogenizer (HPH) (Panda PLUS 2000, GEA Niro Soavi, Germany). To prevent blocking of the homogenizer valve, the coarse powder of drugs was first eventually dispersed in an aqueous stabilizer solution using digital homogenizer (UltraTurrax T25, Jahnke & Kunkel, Staufen, Germany) at 8000 rpm for one hour to form primary Nanoformulation. The primary Nanoformulation was further processed through an HPH with three homogenization cycles at 250, 700, and 1200 bars, followed by maximum cycles at 1500 bars. By varying the no. of cycles of homogenization and keeping process temperature constant at 25 ºC different particle sizes of NPs were obtained.

Lyophilization of NPs

Liquid NPs formulations were processed for lyophilization using a lab freeze dryer. After HPH, NPs were dispersed with an optimized concentration of cryoprotectant in a glass vial which is semi stoppered with slotted rubber closures and was pre-freeze at -30 ºC for 12 h. The primary drying was performed at -53 °C for 24 h and the pressure maintained was 0.016 mBar. The secondary drying was performed at 10 °C for 8 h followed by 25 °C for 4 h by increasing the temperature at a rate of 0.5 °C/min. Finally, the temperature of the cold trap was maintained at -53 °C during the entire process. Resultant powder of NPs was used future for subsequent evaluation studies.

Characterization of NPs

Residual moisture

Using Karl Fischer (Vigo – Matic M.D.) titrator moisture content of dried products was evaluated. To the titration vessel accurately 20 mL of anhydrous methanol was transferred & titrated to the end-point. A sample of 10µl of accurately measured water was used to systemize the Karl Fischer reagent. Then accurately weighed samples were suspended in anhydrous methanol and electromagnetic endpoint titration was carried.

Light microscopy

The contents of the lyophilized product were analyzed for a preliminary study of glass morphology using light microscopy. The samples (mg) were kept on a glass slide under a drop of cedarwood oil and are covered using coverslip. The oil prevents moisture absorption as well as improves the resolution power of the objective lens. Dried samples are seen under ×40 magnification, and using Hyper HAD color video camera the photomicrographs were captured using Motic images (National Optical and Scientific Inc., San Antonio, Texas).

Particle Size Analysis

The size of optimized NPs particles was measured using Zetasizer 300 HAS (Malvern Instruments, Malvern, UK). Prior to size determination, lyophilized Nanoformulation was redispersed in distilled water. Data obtained were mean

average values of three independent samples that are prepared under the same formulation conditions.

Surface Topographical Studies

SEM: The SEM was used to study the morphology of the surface of Sertaconazle NPs which examines sphericity, discreteness and surface properties of NPs. SEM studies were done using SEM (JEOL JSM-6360, Japan) at 20 kV accelerating voltage and high vacuum. Before analysis, lyophilized NPs were first placed on two-sided carbon tape and then, sputtered with gold-palladium alloy up to 3-5 nm of the thickness.

Crystallographic Investigation

X-ray Crystallography (XRD)

The XRPD spectra of pure drug, physical mixture (PM) and optimized NPs were obtained using an X-ray diffractometer (Philips analytical XRD, PW 3710) with Cu-Kα radiation (1.54 Å), at 40 kV, 40 mA by passing through a nickel filter. The samples were analyzed in the 2θ angle range of 5 to 80º. The range and the chart speed were 5×10^3 CPS and 10 mm/º2θ, respectively.

Differential Scanning Calorimetry (DSC)

The thermal behavior of pure drug, physical mixture (PM) with excipients and optimized

NPs were studied using a Perkin Elmer 4000e module controlled by PYRIS Version-11.1.0.0488 (PerkinElmer, Inc., USA.). For each analysis, before heating under nitrogen purging (20 mL/min), the samples of 1 mg were kept in sealed aluminum pans, and scanned at a scanning rate of 10ºC/min, for a temperature range of 30ºC to 350ºC.

Particle Charge (Zeta-potential)

Zeta potential analysis was performed to determine the stability of developed NPs. Charges that are present on the surface of drug-loaded droplets were measured using Zetasizer 300 HSA (Malvern Instruments, UK). Analysis time was kept for the 60 sec and average ZP, charge, and mobility of optimized formulation batches of NPs were determined. After diluting samples with bidistilled water adjusted to a conductivity of 0.0850 mS/cm at room temperature, the zeta potential was measured.

FTIR

FTIR of a drug, physical mixture (PM) and optimized lyophilized NPs were analyzed using the FTIR spectrophotometer (Agilent CARY 630 FTIR) to study the compatibility between drug and stabilizers. Every specimen was analyzed by keeping them on ATR diamond crystal by pro software of Agilent resolutions. Every spectrum of samples was collected from an average of 21 single scans at 4 cm^{-1} resolution in the absorption area of 800–4000 cm^{-1}.

Saturation Solubility Studies

Saturation solubility was done with the addition of a surplus amount of pure drug and optimized lyophilized NPs in ten ml of distilled water. Then, samples were agitated using orbital shaker (Remi instruments limited, Mumbai) for 48 h at 25ºC, then centrifuged to remove the solid content as a residue and the amount of drug present in the supernatant layer was analyzed spectrophotometrically using a UV-visible spectrophotometer at 260 nm.

Total Drug Content:

An aliquot of Nanoformulation (0.5 ml) was dried by evaporation. Further, in methanol residue was dissolved followed by filtration using 0.45 µm filter paper. The samples were then analyzed using a UV-visible spectrophotometer (Shimadzu-1700, Japan) at λ max of 260 nm. The total drug content (TDC) and percentage TDC were calculated from equations 3 and 4.

TDC = (Vol. total/ Vol. aliquot) x drug in aliquot x 100

% TDC = TDC / TAD x 100

Where vol. total/vol. an aliquot is the total volume of Nanoformulation to the taken aliquot volume and the total amount of drug i.e. TAD is the drug used for the fabrication of Nanoformulation.

Entrapment efficiency: Entrapment efficiency (% EE) was determined by ultracentrifugation of 2 mL of sample for 30 min at 10,000 rpm at 4°C using cold centrifuge (Remi CM 12 Plus, Mumbai). The supernatant was used to determine free drug content (FDC). Obtained sediment was washed with a 0.1 N NaOH solution to determine surface adsorbed drugs (SAD). Using UV-visible spectrophotometer all drug solutions were quantified in triplicates at 260 nm. PC is the percentage of drug entrapped in nanoparticles and can be calculated using given formula,

% EE = TDC – (FDC + SAD)/TAD x 100.

***In- Vitro* Drug Release**

Dissolution studies on pure drug powder and their optimized NPs were performed using USP type-II apparatus. Weighed quantities of samples were transferred into the dissolution apparatus (Electro lab TDT-08 L, India) containing 900 mL of SGF with pH 1.2, simulated intestinal fluid with pH 6.8 and pH 7.4, respectively as a medium. The shaft speed was set to 50 rpm at medium temperature 37±0.5 ºC. Samples (5 mL each) were withdrawn at 10, 20, 30, 40, 50 and 60 min of time points and the fresh buffer was added for sink condition maintenance. The samples were collected and filtered using the Whatman filter paper (0.25 µm, Whatman Inc., USA) and inspected using a UV spectrophotometer at 260 nm. The release profile of NPs was correlated with the pure drug.

Formulation, Characterization and Evaluation of Nano Gel

Preparation of nano gel

Preparation of aloevera extract

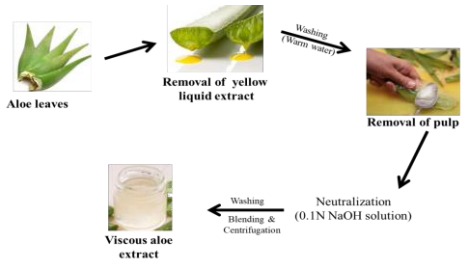


Fig.2: Preparation of aloe-vera extract

Aloe leaves were cut from the plant and placed vertically to remove the toxic yellow liquid from the leaves .This was followed by washing of leaves with warm water and removal of pulp from the aloe leaves. The pulp was treated with 0.1N sodium hydroxide solution to neutralize

the acidic aloe. The pulp was then washed with warm distilled water followed by its blending and centrifugation to remove any fibrous/leafy remnants from the viscous pulp. Sediments were then subjected to multiple extractions employing water till complete extraction of mucilage was achieved. The supernatant and the extracted pulp were combined, filtered and stored in refrigerator till further use.

Preparation of aloe-carbopol gel

To the viscous aloe extract (50 mL), 1% w/w carbopol 974 (gelling agent) was added and allowed to hydrate for 24h. Later, 0.2% w/w methyl paraben (preservative) was added and blend was mixed till a homogenous viscous solution was formed. This was followed by filtration (muslin cloth) and centrifugation to remove any undissolved substance (if present). The supernatant was then treated with triethanolamine (TEA) under stirring till a pH 5.5-6.0 was achieved. This neutralization process results in the formation of gel, which was later stored in refrigerated conditions for future use.

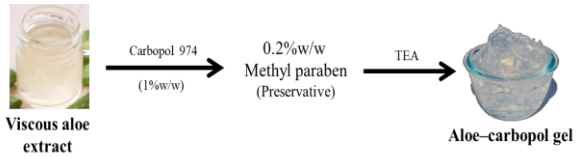


Fig.3: Preparation of aloe-carbopol gel

Preparation of nano loaded gel

Optimized nano preparation (blank/with drug) was added to aloe-carbopol gel and stirred to obtain the homogenous gel (creamy gel; emulgel) with drug concentration equivalent to 20 mg/g of gel. The gel was then subjected to sonication and centrifugation at 5000 rpm for 10 min to remove the entrapped air bubble and stored in refrigerator till further use.

Fig.4: Preparation of nano gel

Physiochemical characterization of nano gel:

(A) Transparency, smoothness and relative density

The nano gel (5 mL) was taken in test tube and evaluated for its transparency by visual examination. Gel was rubbed between the fingers and observed for its smoothness, homogeneity or roughness. The relative density of the gel was determined by comparing weight of formulation (1 mL) with equivalent weights of distilled water using relative density (RD) bottle.

(B) pH: 10 g of the sample (blank/drug loaded nano gel) was placed in a beaker and the surface pH of formulation was measured in triplicate using Elico pH meter.

(C) Moisture content: 2 g of aloevera based carbopol gel, blank and Posaconazole loaded nano-gel and marketed preparation Onabet, 2% w/w) were accurately weighed and kept in desiccator containing anhydrous calcium chloride. Formulations were weighed at regular interval of 12h for 3 days or till a constant weight was achieved and the percentage moisture loss was calculated using the formula:

$$\%\ Moisture\ loss = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

(D) Rheology

The rheological behavior of blank and drug loaded nano gel was determined using MCR-52 dynamic Rheometer (Anton Paar, Germany) employing parallel plate geometry PP50 (50 mm diameter) with a measuring gap of 1.0 mm. Water loss during the experiment was prevented by covering the shearing geometry with low viscosity liquid paraffin. Temperature of sample was maintained at 25°C throughout the study. The flow characteristic of the gel was determined by comparing dynamic viscosity of the system with the shear rate (0-100s-^{1}) at controlled shear stress and varying shear stress (0-100 Pa) at controlled shear rate under isothermal conditions.

(E) Spreadability

250 mg of nano-formulation (aloe-carbopol gel, blank/ AZA loaded nano gel and marketed preparation) was placed on the glass slab using 1 mL syringe and noted for its diameter. Another glass plate of similar dimensions was placed over it and was loaded with the weight of 500 g for 5 min to press the sample to uniform thickness The increase in diameter (in cm) of the preparation was taken as a comparative value for spreadability (Bachhav and Patravale, 2010).

(F) Drug content uniformity: Three nano gel samples (equivalent to 5mg of drug) from varied locations of dispensing container were taken and dissolved in HPLC grade methanol.The suspension was slightly heated over the water bath to allow complete extraction of drug followed by its centrifugation at 7000 rpm for 10 min. The supernatant was appropriately diluted with mobile phase, filtered and analyzed spectrophotometrically at 260 nm.

3. Results and Discussion

Prelimnary Evaluations of Posaconazole Physiochemical Characterization of SC Melting point: The melting point of Posaconazole was found to be 166°C which was similar to the earlier reports indicating the purity of sample.

Differential scanning calorimetry (DSC)

DSC, a confirmatory test for the drug purity, was used to measure the thermal attributes of the drug. The DSC thermogram of pure sample revealed a single sharp melting endotherm, having a peak temperature of 109.19°C with an onset and end-set temperature of 93.7°C and 161.4°C, respectively. The results were found to be in concordance with the findings of an endothermic peak for SC at 161.4°C.

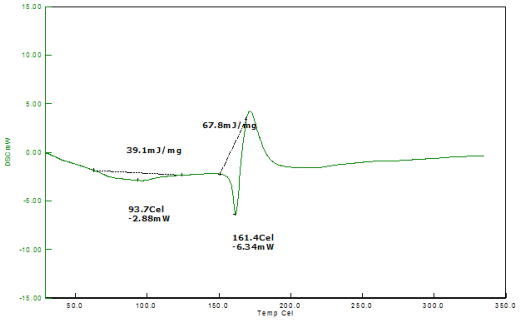


Fig.5

FT-IR: The characteristic peaks of SC. The ATR spectra depicted the absorption peak at 3461.53cm-^{1}indicating the presence of C-H group. The characteristics C=O and C-O peaks corresponding to COOH group were observed at 1485.30cm-^{1},1436.9cm-^{1} respectively. The results obtained were similar to the prior reported data.

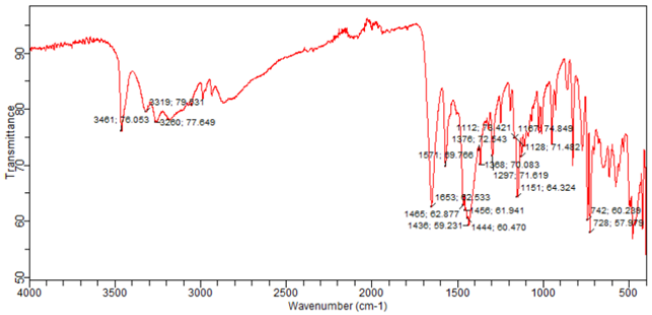
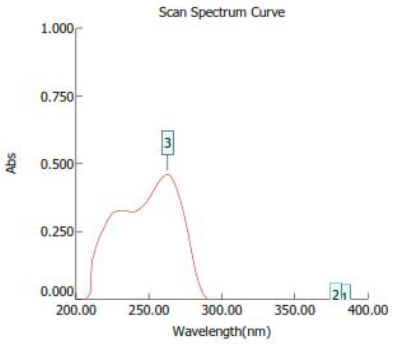


Fig.6

UV Spectroscopy: Absorbance spectrum of a 10 µg/mL solution of SC in methanol was obtained from 400-200 nm. As seen in the Fig, λ max was found to be at 260 nm and the absorbance was 0.460.



1	Peak	384.00	-0.109
2	Peak	378.00	-0.103
3	Peak	260.00	0.460

Fig.7: UV-visible spectrum of Posaconazole

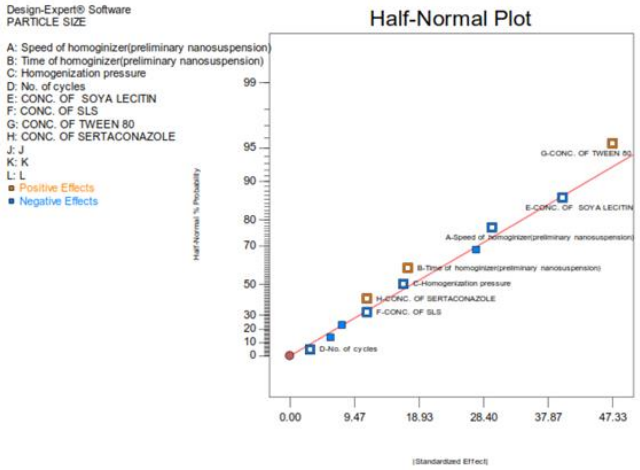


Fig.8: The Half-Normal Plot Displaying the Significant Process and Formulation Variables on Particle Size (Y1)

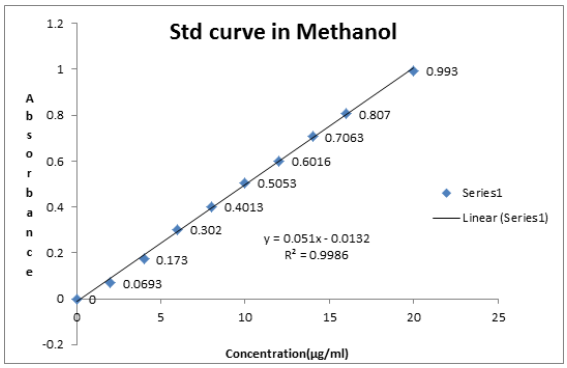


Fig.9: Standard curve of SC in methanol

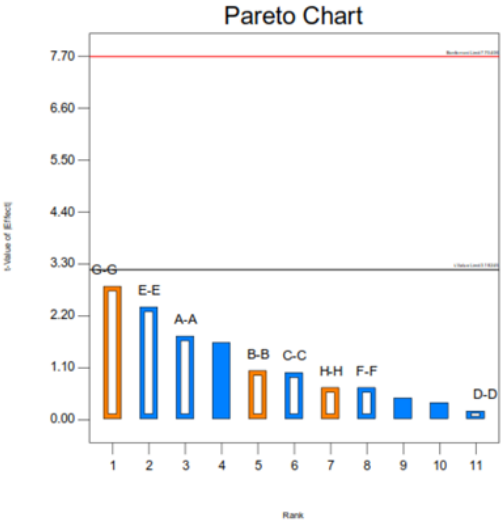


Fig.10: Pareto Charts Displaying Significant Process and Formulation Variables on Particle Size (Y1)

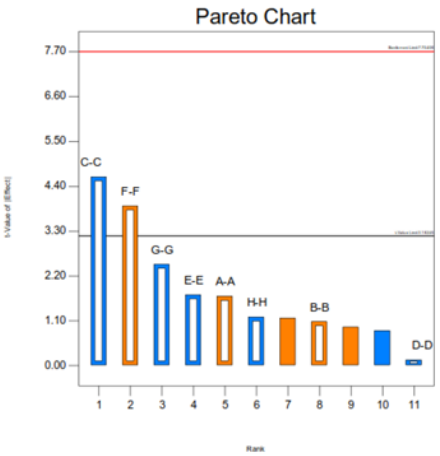


Fig.11: Pareto Charts Displaying Significant Process and Formulation Variables on Drug Content (Y2)

For the Drug content (Y2), the most contributed and significant factors were the tween 80 concentration (X7), the concentration of SLS (X6), and homogenization pressure (X3), respectively. The R^2 value was 0.9450 indicate a significant fit for the model being tested. From ANOVA the p-value for main effects obtained was 0.0767, which was not statistically significant; hence, by using CCD most significant factors were further

evaluated. Drug content has a chief part in a therapeutic activity at a given dose of SC in NPs.

Drug content = +91.91+0.67 * A+0.42 *B-1.82*C - 0.06 * D - 0.69 * E +1.54 * F - 0.98 *G - 0.47 *H

Polynomial Equation represents that, drug content (Y2) was decreased with increased SL concentration, speed of homogenizer in primary NPs stage with a decreased time of homogenizer. It also decreases with increased tween 80 concentrations, SC and SLS. Followed by increasing pressure and no of cycles of homogenizer, respectively. From all the process variables, the percentage contribution of the tween 80 concentration (11.33 %), the concentration of SL (28.16 %) and homogenizer pressure (39.35 %) influence drug content, respectively. Thus, 98.76 % of drug content in HPCNPs could be achieved by performing the experiment using 0.15 % (v/v) of Tween 80, 15 mg of SL with homogenization pressure of 25000 Bars. For the Entrapment efficiency (Y3), the most contributed and significant factors were the concentration of soya lecithin (X5), time of homogenizer(X2), homogenization pressure (X3), respectively. The R^2 value was 0.8189 indicating a significant fit for the model being tested. From ANOVA the p-value for main effects obtained was 0.3613, which was not statistically significant; hence, by using CCD most significant factors were further evaluated. Entrapment efficiency has a chief part in entrapment of SC in stabilizer vesicles to stabilize NPs. Following polynomial equation can describe Y3,

Entrapment efficiency = +88.30417+0.49583*A-1.83583* B1.68750*C+0.12750*D- 0.83417*E-0.62417*F-0.62750* G+0.63083*H

Polynomial equation represents that, entrapment efficiency (Y3) was decreased with an increasing concentration of SC, followed by increasing the time of homogenizer and the number of cycles of homogenization. It also decreases with increased concentration of tween 80, soya lecithin and SLS, followed by the increasing pressure of homogenizer and speed of homogenizer, respectively. From all the process variables, the percentage contribution of concentration of soya lecithin (6.82 %), time of homogenizer (33.02 %) and homogenizer pressure (27.90%) influences entrapment efficiency, respectively. Thus, to achieve 92.46 % of entrapment efficiency in SCNPs, experiments can be performed by using 30 min of homogenization, 15 mg of soya lecithin with homogenization pressure of 5000 Bars.

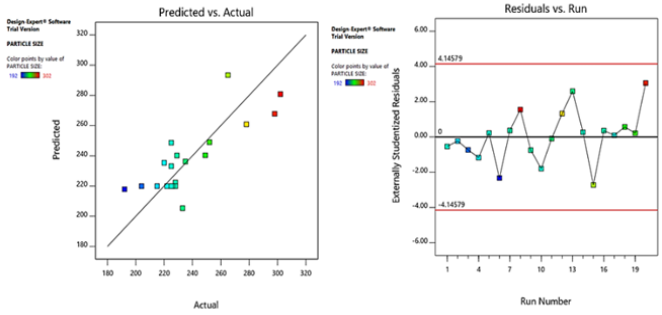


Fig.12: Residual and Predicted Plots Displaying the Impact of Independent Factors on Y1 Response (Particle Size)

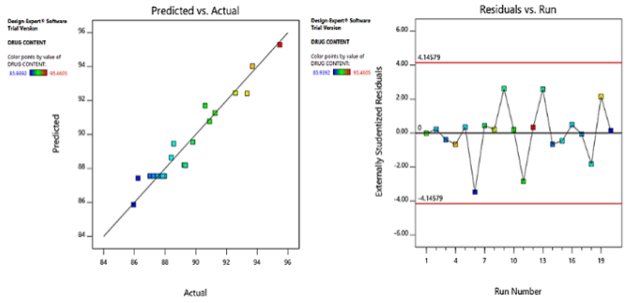


Fig.13: Residual and Predicted Plots Displaying the Impact of Independent Factors on Y2 Response (Drug Content).

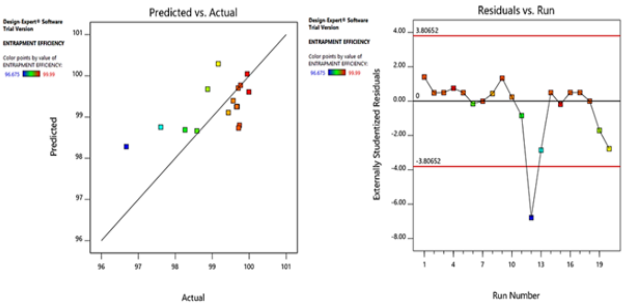


Fig.14: Residual and Predicted Plots Displaying the Impact of Independent Factors on Y3 Response (Entrapment Efficiency).

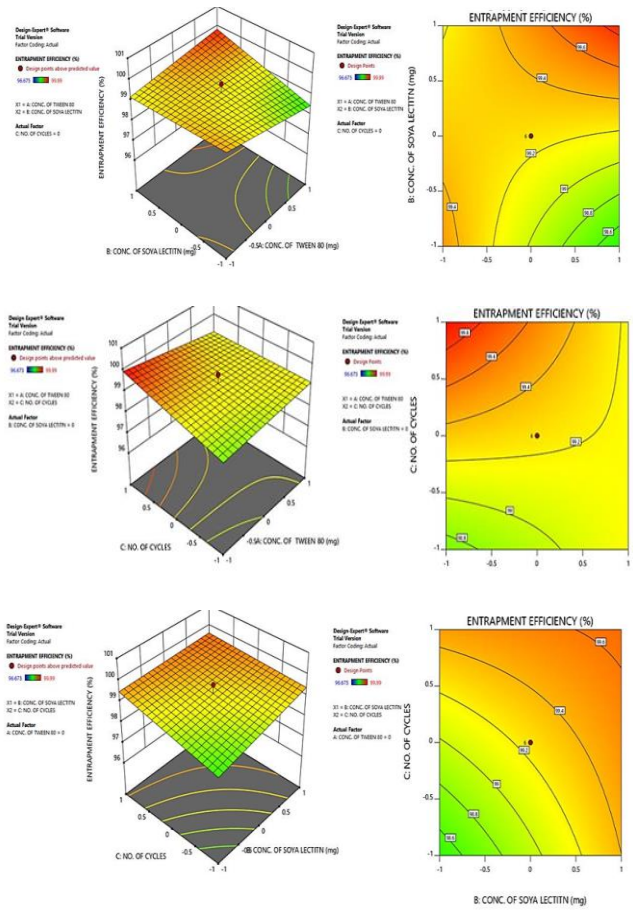


Fig.15: 3D Surface Response Plot and Contour Plot Displaying the Impact of Independent Factors on Y3 Response (Entrapment Efficiency).

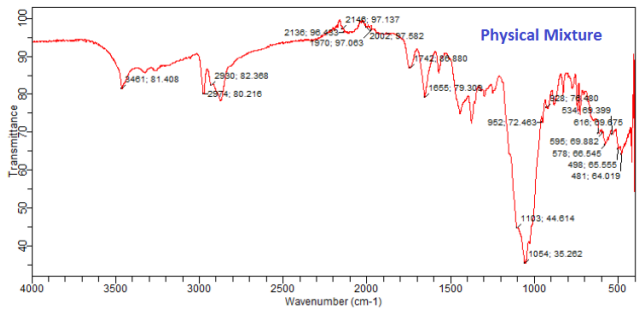


Fig.16: FTIR Physical Mixture

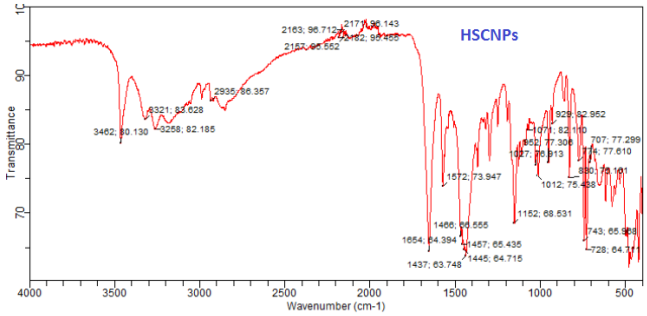


Fig.17: FTIR HSCNPs

Table.1: Residual values of CQAs of optimized formulations

Response parameters	CMAs/CPPs			CQA		
	Conc. of Soya lecithin (mg)	No. of cycles	Conc. Of Tween 80 (%v/v)	Particle size (nm)	Drug content (%)	Entrapment efficiency (%)
Software-predicted results	15	31	0.15	246.036	89.411	99.36
Actual obtained results	15	28	0.20	220±0.37	92.23±0.45	99.52±0.52
Residual values (%)	-	-	-	-1.15	0.223	0.240

*CMAs= critical material attributes; CPPs =critical processing parameters; CQAs= critical quality attributes

Table.2: Results of optimized batches obtained from an overlay plot of Design expert software

Optimized batch	Independent variables			Dependent variables					
				Observed value			Predicted value		
	A	B	C	Y1	Y2	Y3	Y1	Y2	Y3
HSCNP1	0.151	15	28	219±0.25	93.52±0.55	99.01±0.45	220.148	87.139	98.79
HSCNP2	0.141	15	29	235±0.31	91.41±0.54	98.68±0.48	236.492	91.076	98.77
HSCNP3	0.159	15	31	250±0.37	92.23±0.45	98.52±0.52	252.009	93.770	98.79

A = Tween 80 concentration (mL); B= Concentration of soya lecithin (mg); C = no. of cycles; Y1= Particle size (nm); Y2 = Drug Content (%); Y3 = Entrapment efficiency (%)

4. Conclusion

Posaconazole (PCZ), a broad-spectrum triazole antifungal, though primarily indicated for invasive fungal infections, has recently gained attention for its potential applicability in topical drug delivery systems due to its potent antifungal, anti-inflammatory, and immunomodulatory properties. Despite its therapeutic promise, Posaconazole is limited by poor aqueous solubility, low bioavailability, and challenges in crossing biological barriers such as the stratum corneum. Conventional formulations (oral suspensions, tablets, topical creams) often fail to provide effective site-specific drug delivery and are associated with variable absorption, poor tissue retention, and systemic side effects. These drawbacks necessitate the development of novel delivery platforms to achieve targeted, sustained, and safe topical delivery of Posaconazole. Nano-based delivery systems such as liposomes, niosomes, ethosomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) have emerged as viable strategies to overcome solubility and penetration issues. SLNs and NLCs, in particular, are attractive due to their biocompatibility, stability, higher drug-loading capacity, and ability to provide controlled release with minimal systemic permeation. Incorporating Posaconazole into such nanocarriers could potentially enhance its therapeutic effectiveness by improving skin retention, tissue targeting,

and antifungal efficacy while reducing drug-related adverse effects. The present investigation was designed to formulate and evaluate Posaconazole-loaded lipid nanocarriers (SLNs/NLCs) for topical application. The aim was to enhance skin penetration and retention, improve controlled release, and reduce side effects associated with conventional Posaconazole formulations. Physicochemical and antimicrobial evaluations of Posaconazole were carried out using in-vitro approaches. Differential Scanning Calorimetry (DSC) confirmed the crystalline purity of Posaconazole, with melting transitions in agreement with reported literature. Antifungal susceptibility testing (agar diffusion, MIC analysis) revealed Posaconazole to exhibit superior inhibitory activity against Candida albicans and Aspergillus fumigatus compared to standard antifungals (fluconazole, itraconazole), with MIC values in the low microgram per milliliter range, affirming its high potency. Various formulation methods (solvent injection, melt emulsification, low-temperature solidification, and ultrasonication) were screened to prepare SLNs/NLCs using optimized solid and liquid lipids along with surfactants selected on the basis of Posaconazole solubility. Among these, melt emulsification followed by ultrasonication was found to be the most suitable technique. Preliminary trials identified critical formulation variables such as lipid-to-drug ratio, surfactant concentration, and sonication time as influential factors affecting particle size and entrapment efficiency. To statistically optimize these parameters, a full factorial design (3^3) was employed using particle size (PS) and entrapment efficiency (EE) as response variables. The quadratic model was found to best fit the experimental data, as confirmed by ANOVA, low PRESS values, and close agreement between predicted and adjusted R^2. Particle sizes for SLNs ranged from 200–500 nm, and for NLCs 80–150 nm, while entrapment efficiencies varied between 50–85%, indicating strong drug incorporation into the lipid matrices. The optimized formulations exhibited zeta potential values around –13 to –15mV, confirming their stability. DSC analysis demonstrated reduced enthalpy and disappearance of Posaconazole endotherms in drug-loaded carriers, suggesting successful encapsulation. Scanning Electron Microscopy (SEM) revealed uniformly distributed spherical nanoparticles in the nanometric range. The optimized NLC formulation was further incorporated into an aloevera-based carbopol gel to enhance applicability. The formulated nanogel was evaluated for pH, viscosity, spreadability, homogeneity, occlusivity, all of which indicated suitability for topical application. Rheological studies confirmed shear-thinning behavior, ensuring ease of spread on skin. In-vitro permeation and retention studies demonstrated significantly higher skin retention of Posaconazole-loaded NLCs compared to drug suspensions and marketed formulations, with minimal transdermal penetration (<10%), indicating localized drug action. Fluorescent microscopy using rhodamine 6G confirmed deeper skin layer targeting by NLCs. In-vitro and in-vivo antifungal studies showed that the Posaconazole NLC gel effectively reduced fungal burden, suppressed inflammatory responses, and restored normal skin physiology in infected models. Overall, the findings highlight Posaconazole-loaded NLC gels as a promising topical drug delivery platform with improved skin penetration, higher drug retention, and better safety, potentially revolutionizing the topical management of fungal infections.

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