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Research Article

Empowering Topical Delivery: Box Behnken Design Optimization of Posaconazole Microsponge Hydrogel for Improved Management of Fungal Infections

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ABSTRACT

This study aimed to prepare posaconazole (PNZ) microsponges and formulate them into a hydrogel, optimizing the drug-polymer ratio and other parameters using Design Expert's box behnken design. The microsponges were synthesized considering drug-polymer ratio, surfactant (PVA), and stirring speed as inputs, while the vesicular size and PNZ content were evaluated as dependent variables. The prepared microsponges were then merged into a hydrogel. Compatibility studies between the PNZ and excipients were conducted, and the physicochemical parameters of the microsponges and hydrogels were assessed. Statistical analysis was performed to evaluate variance among the factors. Microsponges formulated with a polymer ratio of 1:1 with PNZ (PM-3) exhibited favorable attributes, including smaller vesicular size, drug content, high %yield, %entrapment, and superior PNZ discharge profile. Scanning electron microscopy confirmed the round morphology and spongy assembly of PM-3 microsponges, indicating their suitability for PNZ loading and release. *In-vitro* release studies demonstrated rapid initial discharge followed by sustained release over 12 hours for PM-3, indicating potential for optimized PNZ delivery. Viscosity studies revealed a higher viscosity of PM-3 gel compared to conventional gel, potentially enhancing adherence to the skin surface and PNZ absorption. Additionally, PM-3 exhibited superior antifungal activity in disk diffusion assays, indicating effective control of fungal growth. This study successfully developed PNZ-loaded microsponge hydrogels, showing promising utility in treating fungal infections. PM-3 formulation demonstrated superior characteristics, suggesting its potential for optimized PNZ delivery and therapeutic outcomes.

INTRODUCTION

Skin applications for antifungal treatment involve the topical administration of medications to target fungal ailments affecting the skin. These ailments include athlete's foot and ringworm to more pathetic forms such as fungal nail infections and candidiasis. Topical antifungal treatments are preferred for superficial fungal ailments due to their localized action, minimal systemic side effects, and ease of application. Commonly used topical antifungal agents include azoles, allylamines, polyenes, and ciclopirox. These medications work by inhibiting fungal cell membrane synthesis or disrupting fungal cell wall integrity, thereby suppressing fungal growth and proliferation.[1] Skin applications for antifungal treatment involve applying the medication right to the affected area, typically in the form of semi-solid applications, sprays, or dusts. The choice of formulation based on the type and severity of the fungal ailments, the location of the affected area, and patient preferences.[2] To ensure effective treatment, proper application techniques should be followed, including cleaning and parching the infected part prior to smearing the medication, applying a thin layer of the drug to shelter the infected part and nearby

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skin, and gently rubbing the medication into the skin until it is absorbed. It is essential to adhere to the prescribed treatment regimen, including the frequency and duration of application, to achieve optimal therapeutic outcomes. Skin applications for antifungal treatment can effectively alleviate symptoms such as itching, redness, scaling, and discomfort associated with fungal ailments. However, in cases of severe or persistent infections, oral antifungal medications or combination therapy may be necessary for comprehensive treatment.[3]

Posaconazole (PNZ) has shown efficacy in the management of various fungal ailments, including those affecting the skin. As a triazole antifungal agent, PNZ works by suppressing the production of ergosterol, resulting in disturbing fungal development and propagation. In dermatology, PNZ has been used to treat a range of superficial fungal ailments of the skin, including dermatophytosis (such as ringworm), candidiasis, pityriasis versicolor, and various forms of cutaneous mycoses. These infections can present with symptoms such as itching, redness, scaling, and discomfort and may occur in areas such as the feet (e.g., athlete's foot), groin (e.g., jock itch), scalp, nails, and other parts of the body. PNZ exists as oral tablets, suspensions, and topical formulations such as creams, lotions, or gels.[4] Topical PNZ formulations are applied straight to the affected area of the skin, providing localized treatment and minimizing systemic side effects. These formulations offer convenience and ease of application, making them suitable for use in both outpatient and inpatient settings. Clinical studies and case reports have demonstrated the efficacy of topical PNZ in the controlling of various fungal skin infections. Its broad-spectrum antifungal activity, along with its favorable safety profile, makes it a valuable option for treating superficial fungal ailments, especially in cases where other topical antifungal agents have proven ineffective or when systemic therapy is not indicated. When using topical PNZ for skin infections, it is important to follow the prescribed treatment regimen, including the frequency and duration of application, as absorbed by a healthcare professional. Patients should also be advised to clean and dry the affected area before applying the medication and to continue treatment until the infection resolves completely, even if symptoms improve earlier.[5]

Microsponges (MS) are innovative drug delivery systems (DDS) characterized by their porous structure and ability to encapsulate a drug within their matrix. These microscopic particles, typically with 5 to 300 µm size, are composed of biocompatible polymers such as ethyl cellulose, methylcellulose, or polyvinyl alcohol. The unique structure of MS allows them to absorb and retain large quantities of liquids or substances, making them ideal candidates for controlled discharge and targeted delivery of drugs. They can be loaded with both hydrophilic and hydrophobic drugs, offering versatility in formulation design. Additionally, MS can protect sensitive APIs from degradation and improve their stability, extending shelf life and enhancing therapeutic efficacy. MS are fabricated using various techniques, including emulsion polymerization, solvent evaporation, spray drying, and precipitation. These methods allow for precise control over particle size, porosity, and drug loading capacity. Surface modification techniques such as coating or cross-linking can further tailor the properties of MS to meet specific formulation requirements. One of the key advantages of MS-based DDS is their ability to provide sustained release of drugs over an extended period. By controlling the rate of drug release, MS can minimize dosing frequency, reduce side effects, and improve patient compliance. This sustained release profile is achieved through diffusion of the drug from the interior of the MS matrix to the surrounding environment. MS finds applications in various pharmaceutical and cosmetic formulations, including topical creams, lotions, gels, and foams. In dermatology, they are particularly useful for delivering drugs to the skin for the treatment of acne, psoriasis, eczema, fungal ailments, and other dermatological conditions. Their porous structure enables efficient drug penetration into the skin layers, enhancing therapeutic outcomes.[6]

The objective of this investigation was to fabricate MS containing PNZ and subsequently incorporate them into a hydrogel formulation. To achieve this goal, the study focused on optimizing crucial factors such as the drug-polymer ratio and various other parameters utilizing Design Expert's Box Behnken Design methodology. The aim was to meticulously tailor the PNZ-polymer composition and other key variables to attain MS with desirable characteristics for effective DDS.

Materials And Methods

Materials

Cipla Limited, Bangalore generously provided posaconazole (PNZ). Eudragit S100, ethyl cellulose, triethyl citrate, triethanolamine, and carbopol 934 P were procured from Fischer Scientific. The fungal strain *of Candida albicans* culture was sourced from the Indian Institute of Science, Bangalore. All materials, including those acquired from other suppliers, were of analytical grade and used without further modification for the experiments conducted in this study.

Compatibility Studies

Both the pure PNZ and its formulations containing various excipients were subjected to fourier-transform infrared (FTIR) spectroscopy analysis to investigate their molecular structure and interactions. This analytical technique allowed for the examination of functional groups present in the compounds by recording spectra across a range of wavenumbers from 4000 to 400 cm^{-1} with an FTIR spectrophotometer. By analyzing the resulting spectra, any changes or shifts in absorption peaks could deliver visions into the composition, bonding, and connections

of PNZ and the excipients. This characterization step is crucial for understanding the compatibility of PNZ with the selected excipients and for assessing the feasibility of the formulation for further development.

Preparation of Microsphonges

The quasi-emulsion solvent diffusion technique made the MS PNZ (PM). The internal phase, comprising ethyl cellulose/Eudragit RS100 polymers and triethyl citrate (1% w/v) as a plasticizer, was dissolved in a mixture of dichloromethane (DCM) and ethanol (1:1). Meanwhile, the external phase, containing polyvinyl alcohol (PVA) as a surfactant, was dissolved in water. The internal phase was then included slowly to the external phase under continuous rousing for 60 minutes with a magnetic stirrer. The resulting product was filtered, rinsed with distilled water thrice, and dried overnight in a calcium chloride desiccator. Preliminary trials were conducted to determine the suitable polymer, with Eudragit RS 100 being selected due to the inability to form MS with Eudragit RL 100. The obtained MS exhibited a fluffy appearance, prompting further investigations to optimize the polymer ratio using ethyl cellulose and Eudragit RS 100 (Table 1).[7]

Evaluation of MS

Visual inspection

Batches PM-1 to PM-17 were subjected to thorough visual inspection to evaluate several key parameters. This assessment included examining the color, consistency, homogeneity, and overall appearance of the formulations in their powdered state. Each batch was carefully observed to ensure uniformity in color, texture, and distribution of components throughout the formulation. Any deviations or inconsistencies in these visual characteristics were noted and documented for further analysis and consideration during the formulation optimization.[8]

Table 1: Formulations of PM

Trial	*Ingredients*			
	D:P	*PVA (mg)*	*rpm*	*Triethyl citrate (% w/v)*
PM-1	1:1	0.5	1000	1
PM-2	1:2	0.5	1000	1
PM-3	1:1	1.5	1000	1
PM-4	1:2	1.5	1000	1
PM-5	1:1	1.0	800	1
PM-6	1:2	1.0	800	1
PM-7	1:1	1.0	1200	1
PM-8	1:2	1.0	1200	1
PM-9	1:2	0.5	800	1
PM-10	1:1.5	1.5	800	1
PM-11	1:1.5	0.5	1200	1
PM-12	1:1.5	1.5	1200	1
PM-13	1:1.5	1.0	1000	1
PM-14	1:1.5	1.0	1000	1
PM-15	1:1.5	1.0	1000	1
PM-16	1:1.5	1.0	1000	1
PM-17	1:1.5	1.0	1000	1

PM- Posaconazole MS; D:P- drug and polymer ratio; PVA-Poly vinyl Alcohol; rpm-rotations per minute

%Yield

The percentage yield of batches PM-1 to PM-17 was judged by calculating the practical weight of the MS obtained relative to the theoretical weight, which includes both the polymer and PNZ. This calculation provides insight into the efficiency of the MS fabrication process, indicating the proportion of the intended product that was successfully obtained.[9]

PNZ entrapment

The entrapment efficiency (EE) (%) of batches PM-1 to PM-17 was calculated by measuring the absorbance of the samples in PBS (pH 7.4). The %EE was determined using equation 1, which allows for the quantification of the amount of PNZ effectively trapped within the MS relative to the total amount of PNZ present in the formulation. This calculation provides appreciated visions into the effectiveness of the MS in encapsulating the desired PNZ component.[10]

$$\%EE = \frac{Total\ amount\ of\ PNZ - amount\ of\ free\ PNZ}{Total\ amount\ of\ PNZ} X100 \quad ...(1)$$

Vesicular size analysis

The mean vesicular size of the MS in batches PM-1 to PM-17 was determined using a binocular microscope equipped with a stage micrometer. The VS analysis involved spreading powdered MS on a clean glass slide and measuring them using both stage and eyepiece micrometers. This method gives the idea about the size distribution of the MS, aiding in understanding their physical characteristics and potential applications.[11,12]

Optimization of PNZ by box behnken design

In this investigation, the interplay between the PNZ-polymer ratio, surfactant concentration, and stirring speed on the VS and DC was examined utilizing a Box-Behnken design. A total of 17 experiments were orchestrated using Design-Expert software, which employed a quadratic model equation. This equation was formulated to account for the responses (Y) associated with each combination of factor levels, encompassing linear coefficients (b_1, b_2, b_3), interaction coefficients (b_{12}, b_{13}, b_{23}), and quadratic coefficients (b_{11}, b_{22}, b_{33}), with b0 serving as the intercept. The design enabled a comprehensive exploration of the effects of these factors on outputs, providing valuable insights into the optimization process.[13]

In a Box-Behnken Design, the full model equation describes the association between the response variable (Y) and the

inputs (X_1, X_2, ..., X_k), including all possible effects such as linear, quadratic, and interaction terms. The reduced model equation, however, is a simplified version that excludes non-significant terms, typically determined through statistical analysis like *p-value*s or lack-of-fit tests.

Loading of PM into a gel

Carbopol 934 was taken and placed in water for 24 hours to ensure complete swelling of the polymer. To this base, PNZ MS equivalent to 1% w/w were uniformly dispersed. PEG-400 was incorporated into the mixture as a penetration enhancer to enhance PNZ delivery through the skin. Additionally, methylparaben and propylparaben were added as preservatives to ensure the stability of the formulation. Triethanolamine was then added dropwise with gentle stirring using a homogenizer to adjust the pH of the gel. The same procedure was followed to prepare the PNZ-loaded plain gel (Table 2).[14]

Evaluation of PM Gels

Physical assets

The MS-loaded hydrogels were visually inspected to assess their color, homogeneity, and consistency. Hydrogels are expected to exhibit a pleasant appearance, indicating uniform dispersion of the MS and appropriate consistency. This visual examination helps ensure that the hydrogels meet aesthetic standards and are suitable for further evaluation and potential use in topical applications.[15,16]

Table 2: Formulae of PM-loaded gels

Trial	*Ingredients*					
	PM (%) (Drug: Polymer)	*Carbopol 934 P*	*PEG-400*	*MP*	*PP*	*Triethanolamine*
PMG-1	1:1	1	1	0.02	0.01	q.s
PMG-2	1:2	1	1	0.02	0.01	q.s
PMG-3	1:1	1	1	0.02	0.01	q.s
PMG-4	1:2	1	1	0.02	0.01	q.s
PMG-5	1:1	1	1	0.02	0.01	q.s
PMG-6	1:2	1	1	0.02	0.01	q.s
PMG-7	1:1	1	1	0.02	0.01	q.s
PMG-8	1:2	1	1	0.02	0.01	q.s
PMG-9	1:2	1	1	0.02	0.01	q.s
PMG-10	1:1.5	1	1	0.02	0.01	q.s
PMG-11	1:1.5	1	1	0.02	0.01	q.s
PMG-12	1:1.5	1	1	0.02	0.01	q.s
PMG-13	1:1.5	1	1	0.02	0.01	q.s
PMG-14	1:1.5	1	1	0.02	0.01	q.s
PMG-15	1:1.5	1	1	0.02	0.01	q.s
PMG-16	1:1.5	1	1	0.02	0.01	q.s
PMG-17	1:1.5	1	1	0.02	0.01	q.s

PMG- Posaconazole microsponge gel; PEG- Polyethylene glycol; MP-Methyl paraben; PP-Propyl paraben; q.s-quantity sufficient

Viscosity assets

Measuring viscosity with a Brookfield viscometer using a small sample adapter and spindle no. 64, involved gradually increasing rotational speed from 10 to 100 rpm and recording viscosity readings in centipoise. This assessment offers valuable insights into the flow characteristics of the formulations, which are critical for their suitability as topical gels.[17,18]

pH

The pH of the PNZ microsponge-loaded hydrogel (PMG) was judged with a digital pH meter. About 1 g of gel was solubilized in 100 mL of distilled water and allowed to stand for 2 hours. After incubation, pH measurements were taken using the digital pH meter. This process was repeated in triplicate for each formulation, and the average values were calculated to safeguard the accuracy and reliability of the results. pH measurements offer crucial insights into the potential irritation and compatibility of the formulations with the skin.[17,19]

Uniformity in PNZ content

To ascertain the PNZ content in the prepared PMG, a process was followed wherein 1 g of the gel, containing PNZ equivalent to 100 mg, underwent extraction with 30 mL of ethanol. Subsequently, the volume was brought to 100 mL with phosphate buffer saline (pH 7.4), followed by filtration of the solution. The resultant solution underwent analysis by measuring absorbance at 260 nm using a UV spectrophotometer after necessary dilutions. This method allows for accurate quantification of PNZ content in the gel formulation, ensuring its quality and consistency (e.q.2).[20]

$$\text{PNZ content (\%)} = \frac{\text{Amount of PNZ in Solution (mg)}}{\text{Amount of PNZ in formulation (mg)}} \text{X100} \quad \ldots(2)$$

Skin irritation observations

A skin irritation test was conducted on human volunteers to assess the safety of the final PMG for topical use. Approximately, 1 g of the formulated gel was applied to a sensitive area of the skin, such as the wrist portion of the hand. The application site was then monitored for signs of irritation, including erythema and edema. This evaluation aimed to identify any potential adverse reactions that could render the formulation unsuitable for use on the skin. Such tests are essential to ensure the safety and tolerability of topical formulations before their widespread application in clinical settings or consumer use.[21,22]

Spreadability assets

Spreadability, a vital factor determining the therapeutic efficacy of gel formulations, denotes their capacity to cover a defined area upon application to the skin. In this assessment, 1 g of the formulation was deposited within a 1-cm diameter circle delineated on a ground glass slide. Subsequently, this slide was sandwiched between

another of identical dimensions, and a weight of 500 g was applied to the upper slide for a duration of 5 minutes. The expansion in diameter due to gel spreading was noted, and spreadability was computed using a designated formula. This test provides valuable insights into the practical application and coverage of the gel on the skin surface, aiding in the optimization of formulation characteristics for enhanced therapeutic outcomes (e.q.3).[23]

$$spreadbility = \frac{mass\ (g) X\ (distance\ travelled\ by\ the\ gel)}{time\ (sec)} \quad ...(3)$$

In-vitro PNZ permeation assets

For the *in-vitro* discharge study of PMG, a setup utilizing Franz diffusion cells was utilized. The formulation was placed in the donor compartment, while phosphate buffer saline (PBS 7.4) served as the receptor medium in the adjacent compartment. To facilitate PNZ diffusion, a cellophane membrane, previously soaked in PBS 7.4, was positioned between the donor and receptor compartments. Subsequently, 1 g of the formulation was evenly spread onto the cellophane membrane, ensuring complete contact with the receptor medium. The assembly was then positioned on a thermostatically controlled magnetic stirrer, maintaining a constant temperature of 37 ± 0.5°C with continuous stirring. At predetermined intervals, 1-mL samples were withdrawn from the receptor compartment and replaced with an equal volume of PBS 7.4 to maintain sink conditions. The discharge profiles of PNZ from PMG were compared with those of PNZ-loaded plain gel. After appropriate dilution, sample absorbance was measured at 260 nm using a UV-visible spectrophotometer, facilitating the evaluation of PNZ release kinetics and formulation efficacy.[24,25]

Antifungal assets

For antifungal evaluation, the Kirby-Bauer disk diffusion agar plate method was employed. Firstly, agar plates were prepared by pouring sterilized agar medium into Petri dishes and left to solidify. Next, the fungal suspension of *Candida albicans* was evenly spread across the agar surface using sterile cotton swabs and allowed to dry for 10 minutes. Following this, discs impregnated with the formulated gel containing PMG were carefully placed onto the inoculated agar plates in an aseptic manner. The plates were then incubated for two days under appropriate conditions. The presence of clear zones of inhibition surrounding the test sample discs indicated antifungal activity against *C. albicans*. To ensure accuracy and reliability, all assays were conducted in triplicate.[21,26]

Results And Discussion

Compatibility Results

The FTIR analysis played a pivotal role in elucidating the interaction between PNZ and the various excipients used in the formulation process. Remarkably, the FTIR spectra revealed that the characteristic peaks and stretches associated with PNZ remained consistent even in the presence of the diverse excipients employed. This consistent profile indicates that PNZ maintained its molecular structure without significant alteration, suggesting favorable compatibility with the formulation components. The preservation of PNZ's characteristic peaks in the FTIR spectra is indicative of the integrity of its functional groups and chemical bonds. This integrity is paramount for maintaining the stability and efficacy of PNZ within the formulation. The absence of any significant alterations or disappearances of these peaks signifies minimal chemical interactions or incompatibilities between PNZ and the excipients. Such interactions could potentially compromise the quality and performance of the formulation, leading to undesirable effects. The observed compatibility between PNZ and the excipients utilized in the formulation process underscores the suitability of the formulation for pharmaceutical applications. This compatibility ensures that PNZ retains its therapeutic efficacy and stability within the formulation, enhancing its potential for successful pharmaceutical development and clinical use. Overall, the FTIR analysis provides valuable insights into the compatibility of PNZ with the formulation components, thereby contributing to the optimization and development of effective pharmaceutical formulations for clinical applications (Fig. 1).

Preliminary screening

The preliminary trials aimed to identify the most suitable polymer for the formation of microspheres (MS). Among the polymers evaluated, Eudragit RS 100 emerged as the optimal choice, while Eudragit RL 100 exhibited limitations in forming microspheres. This selection was likely attributed to the specific physicochemical properties of Eudragit RS 100, such as its solubility characteristics and film-forming ability, which facilitated the formation of microspheres.

The microspheres obtained with Eudragit RS 100 displayed a fluffy appearance, indicating the presence of void spaces within the matrix. This observation suggested the need for further optimization to enhance the density and uniformity of the microspheres. The

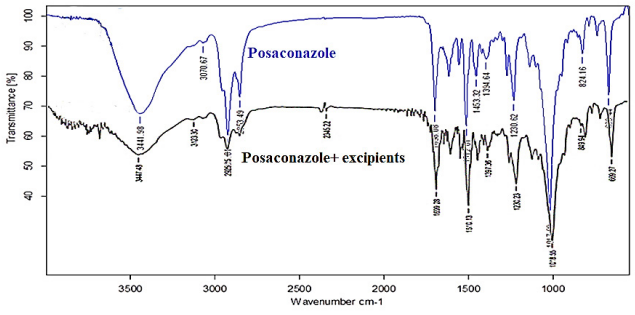

Fig. 1: FTIR spectra of PNZ pure and with excipients

utilization of additional polymers, such as ethyl cellulose, in combination with Eudragit RS 100 presents a promising approach to address this issue.

Results of Physical Assets

The VS distribution of the formulations exhibited a range from 568.36 ± 2.34 nm (PM-3) to 645.97 ± 5.35 nm (PM-11), indicating variability in the size of the PNZ-loaded MS (PMs) across different formulations. Such variations can influence key physical attributes and performance aspects of the formulations, including dispersibility, stability, and the kinetics of PNZ discharge.

The %yield of the formulations ranged from 80.10 ± 2.55% (PM-11) to 98.5 ± 2.44% (PM-3), indicating differences in the efficiency of the PM preparation process. Higher %yields imply better recovery of the desired product, whereas lower yields may suggest losses during manufacturing or incomplete encapsulation of PNZ within the MS matrix.[27]

PNZ's %EE varied from 74.4 ± 1.94% (PM-11) to 95.62 ± 1.72% (PM-3), indicating the extent to which PNZ was successfully encapsulated within the MS matrix. Higher EE values indicate more effective loading and retention of PNZ within the PMs, essential for achieving the desired therapeutic effect.[28]

The pH values of the PMs ranged from 5.6 ± 0.3 (PM-12) to 6.3 ± 0.4 (PM-16), indicating a slightly acidic to neutral environment. This pH range is typically favorable for topical formulations as it aligns with the physiological pH of the skin, minimizing the risk of irritation or adverse reactions upon application.[29]

PNZ content varied among the formulations, ranging from 77.74 ± 2.33% (PM-11) to 98.65 ± 2.49% (PM-3), indicating differences in PNZ loading efficiency across the formulations. Higher PNZ content suggests better encapsulation and retention of the active pharmaceutical ingredient within the MS matrix.[30]

Spreadability values ranged from 8.06 ± 0.14 to 9.88 ± 0.07, reflecting the formulations' ability to spread evenly over the skin surface upon application. Optimal spreadability is crucial for ensuring uniform distribution of PNZ and enhancing therapeutic efficacy in topical formulations (Fig. 2).

Selection of Dependent Variables

In the formulation of microsponge gels, the dependent variables vesicular size (VS) and drug content (DC) are typically selected based on the desired characteristics of the final product, such as its intended application, release profile, and stability. Design Expert software is utilized to optimize these variables by employing statistical experimental design methodologies, such as the design of experiments (DoE).

Firstly, the formulation goals and constraints are defined, including the target vesicular size range and desired drug content per unit volume of the gel. These parameters

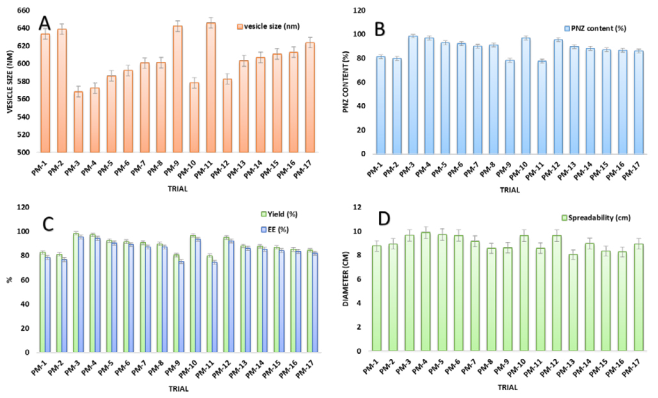

Fig. 2: A) Vesicle size B) PNZ content C) Yield and entrapment efficiency D) spreadability of the prepared microsponges

are essential for achieving optimal drug delivery characteristics, such as controlled release and enhanced efficacy.

Design Expert software then facilitates the creation of a design matrix, where various formulations are systematically generated by varying the independent variables, such as the type and concentration of polymers, surfactants, cross-linkers, and other excipients.

The collected data are then analyzed using statistical tools within the Design Expert software to identify the significant factors affecting vesicular size and drug content, as well as their interactions.

Through iterative experimentation and analysis guided by Design Expert software, the optimal formulation with the desired vesicular size and drug content can be determined. This approach enables the development of microsponge gels with tailored properties for specific pharmaceutical applications, such as topical drug delivery or controlled-release formulations.

Deciding on the Levels of the Variables in Box Behnken's Design

The following approaches were used in the selection of the input variables range.

Range of practical values

The levels are often selected to cover the range of practical values for each independent variable. This ensures that the experimental design encompasses the relevant operating conditions for the system being studied. For example, if the independent variable is concentration, the levels might represent low, medium, and high concentrations based on the expected range for the formulation.

Prior knowledge or literature data

Existing knowledge or data from literature reviews, previous experiments, or pilot studies can inform the selection of levels. This information helps in determining the plausible range of values that are likely to influence the response variables. It may also guide the selection of

levels that are known to produce significant effects on the responses.

Resolution and precision

The levels are chosen to achieve a balance between resolution (the ability to detect significant effects) and precision (the reliability and accuracy of the estimated response surface). Typically, three levels are used for each factor in a Box-Behnken design, allowing for the estimation of linear, quadratic, and two-factor interaction effects while minimizing the number of experimental runs.

Equidistant levels

In a Box-Behnken design, the levels of each independent variable are usually spaced equidistantly between the low and high values to maintain uniformity and facilitate statistical analysis. This ensures that the design points are evenly distributed within the experimental space, improving the efficiency of the design.

Statistical considerations

The selection of levels may also be influenced by statistical considerations, such as the desire to minimize correlation between factors or to avoid extreme values that could lead to instability or non-linearity in the model.

By considering the above all the levels of the independent variables were selected.

Effect of Independent Variables on the Responses

The final equations derived in terms of coded factors offer valuable insights into the intricate relationship between the independent variables, namely the PNZ polymer ratio, surfactant concentration, and stirring speed, and the corresponding responses, including vesicular size (VS) and drug content (DC). These equations serve as powerful predictive tools, enabling researchers to forecast response values based on specific factor levels, thereby facilitating the optimization process of the formulation.

Each equation delineates the influence of individual factors (X_1, X_2, X_3) and their interactions (X_1X_2, X_1X_3, and X_2X_3) on both VS and DC. The coefficients within these equations provide crucial information regarding the magnitude and direction of the effects. Positive coefficients indicate that increasing the level of the corresponding factor enhances the response, while negative coefficients signify the opposite trend. The equations can be expressed as follows.

$$VS=+611.52+2.02A-32.30B+3.91C-0.3075AB-1.37AC+0.0675BC-12.68A^2+4.52B^2-3.69C^2$$

$$DC=+87.52-0.3487A+8.92B-0.725C-0.025AB+0.4475AC-0.1975\ BC+3.10A^2-1.41B^2+1.05C^2$$

Furthermore, these equations shed light on the relative importance of each factor and their interactions in wcontrolling PNZ discharge from the formulation. By analyzing the coefficients, researchers can discern which factors play a dominant role in influencing DC. Positive coefficients suggest factors that facilitate DC.[31]

ANOVA for the Quadratic Model to the Response

ANOVA examining the quadratic model concerning the responses variable is as per Table 3.

The model F-value of 26.67 demonstrates the statistical significance of the model. With only a 0.01% chance of such a high F-value occurring randomly, it suggests that the model effectively accounts for the variation in the data. The *p-values* below 0.05 for model terms indicate their significance, with terms B and A^2 being significant in this case. Conversely, values above 0.1 suggest that model terms are not significant. When many model terms are insignificant (excluding those essential for hierarchy), simplifying the model may improve its performance. Regarding the lack of fit, the F-value of 0.19 indicates its lack of significance compared to pure error. With an 89.83% chance of observing such a lack of fit F-value due to noise, it suggests that the model adequately fits the data. A non-significant lack of fit is preferable, indicating that the model captures data variability effectively without requiring further adjustments.

The model F-value of 47.84 highlights the substantial impact of the model on the data, with only a 0.01% chance of such a result occurring randomly. Model terms with *p-value*s below 0.05, such as B and A^2, are considered significant, while those exceeding 0.1 are not. Simplifying the model by removing non-significant terms, except for those necessary for hierarchy, may improve its performance. Regarding lack of fit, the F-value of 0.33 indicates its insignificance compared to pure error. An 80.54% likelihood of observing this lack of fit F-value due to noise suggests that the model adequately fits the data. A non-significant lack of fit implies effective capture of data variability without further adjustments.

Visual representations of the equations through contour and 3D plots offer a comprehensive understanding of how changes in the independent variables affect both VS and DC simultaneously. Contour plots illustrate the relationship between two independent variables and the response while holding the third variable constant. By examining contour lines, researchers can identify regions of optimal response values and elucidate the interaction effects between variables. On the other hand, three-dimensional plots provide a holistic view of the response surface, enabling researchers to visualize the complex interactions between all three independent variables and the responses concurrently. These plots offer insights into the combined effects of the variables and aid in identifying optimal formulation conditions.[32]

Overall, the contour and 3D plots effectively illustrate the influence of the PNZ-polymer ratio, surfactant concentration, and stirring speed on both VS and DC. By leveraging these graphical representations, researchers can identify optimal formulation conditions that maximize both responses, thereby enhancing the efficacy and performance of PNZ-loaded MS in therapeutic applications.

Table 3: ANOVA analyzing the quadratic model of the response variable

ANOVA for the quadratic model to the response-1 (VS)						
Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	*9324.04*	*9*	*1036.00*	*26.67*	*0.0001*	*significant*
A-D:P	32.76	1	32.76	0.8436	0.3889	
B-PVA	8348.90	1	8348.90	214.96	< 0.0001	
C-rpm	122.07	1	122.07	3.14	0.1195	
AB	0.3782	1	0.3782	0.0097	0.9242	
AC	7.45	1	7.45	0.1919	0.6745	
BC	0.0182	1	0.0182	0.0005	0.9833	
A^2	676.98	1	676.98	17.43	0.0042	
B^2	85.93	1	85.93	2.21	0.1805	
C^2	57.49	1	57.49	1.48	0.2632	
ANOVA for the quadratic model to the response-2 (DC)						
Model	694.82	9	77.20	47.84	< 0.0001	significant
A-D:P	0.9730	1	0.9730	0.6029	0.4629	
B-PVA	636.00	1	636.00	394.09	< 0.0001	
C-rpm	4.20	1	4.20	2.61	0.1505	
AB	0.0025	1	0.0025	0.0015	0.9697	
AC	0.8010	1	0.8010	0.4963	0.5039	
BC	0.1560	1	0.1560	0.0967	0.7649	
A^2	40.50	1	40.50	25.10	0.0015	
B^2	8.41	1	8.41	5.21	0.0564	
C^2	4.68	1	4.68	2.90	0.1325	

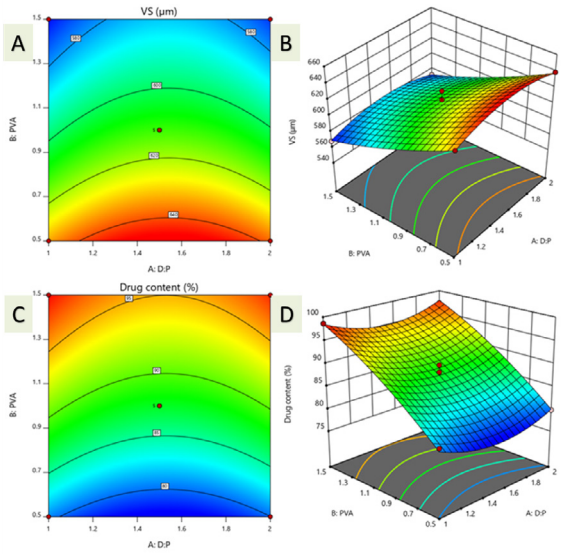

Fig. 3: The effect of independent variables (PNZ-polymer ratio, surfactant concentration, and stirring speed) on %VS A) contour B) 3D and on DC C) contour D) 3D plots

Moreover, these plots provide valuable guidance for tailoring MS with desired characteristics, ultimately optimizing their therapeutic effectiveness (Fig. 3).

In QbD experiments, the *p-value* is pivotal for assessing the significance of various factors on product quality attributes. It determines whether observed differences are statistically significant or due to chance, aiding in the identification of critical process parameters (CPPs) and prioritizing factors affecting product quality. By analyzing *p-values*, researchers select critical parameters for control, optimize conditions, and understand factor interactions, which are crucial for developing robust pharmaceutical products.

Optimizations

The optimized conditions for achieving a vesicular size of 623.78 µm with a DC of 83.56% were determined to be a PNZ-polymer ratio of 1:1.04, PVA concentration of 0.66, and stirring speed of 1041.65. These parameters were identified through systematic optimization using Design Expert software. The selection of these specific conditions highlights the intricate balance required among the variables to achieve the desired outcomes in MS. The drug-polymer ratio plays a crucial role in determining the PNZ content within the MS, with higher ratios typically leading to increased PNZ loading. However, an excessive amount of polymer relative to the PNZ can result in larger vesicular sizes, potentially impacting the formulation's

efficacy and performance. Similarly, the concentration of PVA, as a surfactant, significantly influences the formation and stability of the MS. A higher concentration of PVA can promote the formation of smaller vesicles by reducing surface tension and facilitating the emulsification process. Conversely, excessively high concentrations may lead to instability or aggregation of the MS. The stirring speed during the formulation process also plays a critical role in controlling the size and distribution of the microspheres. Higher stirring speeds typically result in smaller vesicle sizes by promoting better dispersion and homogenization of the components. However, excessively high speeds can lead to shear forces that may affect the integrity of the MS. The optimization of these parameters to achieve the desired VS and DC underscores the importance of systematic experimental design in formulation development.[33] By identifying the optimal conditions, researchers can ensure the reproducibility and consistency of the formulation process, ultimately leading to improved product quality and performance (Fig. 4).

SEM Assets

The SEM analysis of the optimized formulation (PM-3) revealed that spherical shapes with uniform sizes characterized the surface morphology of the PM. Additionally, the surface exhibited a porous structure, indicating the presence of pores or voids within the PM matrix. These porous features could potentially enhance PNZ loading capacity, facilitate PNZ discharge, and promote interactions with the surrounding environment, which are advantageous for topical PNZ delivery applications. Overall, the SEM images provide valuable insights into the structural characteristics of the PM, confirming its suitability for further investigation and potential application in topical DDS.

In-vitro PNZ Discharge Results

The *in-vitro* PNZ discharge studies revealed that the microsponge formulation PM-3 displayed notable discharge characteristics, presenting an initial rapid release followed by sustained discharge over 12 hours. This discharge profile indicates the effective and controlled release of PNZ from PM-3, offering immediate therapeutic benefits followed by a sustained and prolonged release for extended action. The observed discharge pattern of PM-3 is advantageous for topical applications as it ensures optimized DDS, enhances therapeutic efficacy, and prolongs the duration of action. By providing an initial burst release, PM-3 can promptly address the immediate treatment needs, while the sustained release ensures a continuous supply of PNZ, thereby minimizing the frequency of application and improving patient compliance. The superior discharge behavior of PM-3 underscores its potential as an effective DDS for achieving desired therapeutic outcomes in topical applications. This controlled release profile aligns with the requirements of topical formulations, offering a promising approach for controlling various dermatological conditions, including fungal ailments (Fig. 5).

Evaluation of the MS hydrogel

Viscosity results

The viscosity analysis revealed that the PM gel formulation PM-3 demonstrated significantly higher viscosity when compared to the conventional gel. This elevated viscosity attribute of PM-3 holds several advantages for its application in topical PNZ delivery. Firstly, the increased viscosity of PM-3 facilitates better adherence of the gel to the skin surface. This improved adhesion prolongs the contact time between the gel and the skin, thereby enhancing the absorption of PNZ into the skin layers. Moreover, the higher viscosity of PM-3 contributes to its improved spreadability over the skin surface. This enhanced spreadability ensures more uniform coverage of the affected area, promoting efficient delivery of PNZ to the target site. Additionally, the elevated viscosity of PM-3 aids in the retention of the gel on the application site. This prolonged retention period ensures a sustained release of PNZ over time, thereby prolonging the therapeutic effect and reducing the frequency of application (Fig. 6).

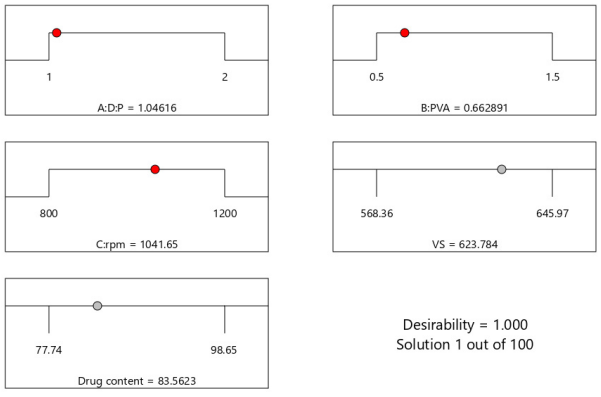

Fig. 4: Optimization chart for getting smaller vesicular size with better %drug content of the vesicles

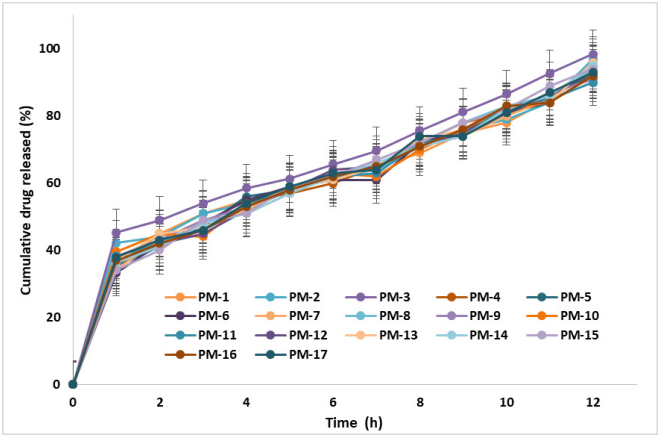

Fig. 5: *In-vitro* PNZ discharge from the prepared PM

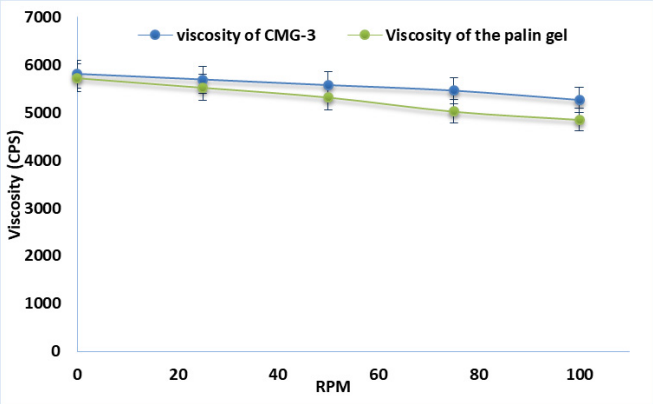

Fig. 6: Comparative viscosity of the optimized PMG with normal gel

Fig. 7: Zone of inhibition of A) PNZ hydrogel B) optimized PMG-3

Antifungal results

In the antifungal assay, the optimized formulation (PMG-3) demonstrated a significant enhancement in antifungal activity compared to the plain gel (Fig. 6 A). Specifically, PMG-3 exhibited a distinct and expanded zone of inhibition around the sample disc (Fig. 6 B), indicating its improved efficacy against the tested fungi. The pronounced zone of inhibition observed with PMG-3 suggests its enhanced ability to inhibit the growth and proliferation of microorganisms. This enhanced antifungal activity can be attributed to the effective discharge of the active ingredient, PNZ, from the gel matrix of PMG-3. The optimized formulation likely facilitates the sustained release of PNZ, ensuring a prolonged exposure of the microorganism to the PNZg, thereby enhancing its efficacy in controlling microbial growth. The larger zone of inhibition observed with PMG-3 underscores its potential for more effective control and prevention of microbial infections compared to the plain gel formulation. This outcome highlights the significance of incorporating the PNZ-loaded MS into the gel matrix to augment the antifungal efficacy and therapeutic potential of the formulation (Fig. 7).

Conclusion

The preliminary evaluation revealed that Eudragit RS 100 proved optimal for microsphere formation, while Eudragit RL 100 was unsuitable. The fluffy appearance of Eudragit RS 100 microspheres led to the exploration of polymer ratio optimization with ethyl cellulose. Moving forward, the focus will be on refining these parameters to enhance drug loading, control release kinetics, and achieve uniformity, advancing microsphere formulations for controlled drug delivery systems.

This study successfully developed posaconazole (PNZ) MS and integrated it into a hydrogel for topical administration. Employing a Design Expert, Box Behnken Design, the MS was efficiently fabricated using ethyl cellulose and polyvinyl alcohol, with the drug-polymer ratio, surfactant (PVA) concentration, and stirring speed optimized as key factors. The optimized formulation, PM-3, exhibited desirable characteristics, including a smaller vesicle size, higher %yield, and superior PNZ discharge profile. SEM analysis confirmed the suitability of PM-3 MS for PNZ loading and interaction with the skin. *In-vitro* PNZ release studies showcased a rapid initial discharge followed by sustained release over 12 hours, suggesting the potential for optimized PNZ delivery. Additionally, PM-3 hydrogel demonstrated higher viscosity compared to conventional gel, potentially enhancing PNZ absorption. The superior antifungal activity of PMG-3 further reinforces its potential for treating fungal infections. In summary, this study successfully developed PNZ-loaded microsponge hydrogels with promising applications in dermatology.

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