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Optimizing Topical Delivery of Posaconazole through Design Expert-Designed Microsponge Formulation

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Abstract

This study aimed to prepare Posaconazole (PCZ) microsponges and formulate them into a hydrogel. The microsponges were prepared by using Design Expert's Box Behnken Design with ethyl cellulose and polyvinyl alcohol. In this drug-polymer ratio, surfactant (PVA) and stirring speed were considered as inputs to check the entrapment and the yield as dependent variables. The so-formed microsponges were further made intoa transdermal gel. The compatibilityand physical characterization including drug release were assessed. The study revealed that variations in various parameters across different formulations, with the optimized formulation (PM-3) demonstrating desirable attributes such as smaller particle size, higher % yield, % entrapment, and superior PCZ discharge profile. SEM analysis confirmed the favorable spherical morphology and porous structure of PM-3 microsponges, indicating their suitability for PCZ loading, discharge, and interaction with the skin environment. In vitroPCZrelease studies demonstrated the effectiveness of PM-3 in providing rapid initial discharge followed by sustained discharge over 12 h, suggesting potential for optimized PCZ delivery and therapeutic outcomes. Furthermore, viscosity studies indicated that PM-3 gel exhibited higher viscosity compared to conventional gel, potentially enhancing adherence to the skin surface and improving PCZ absorption. The superior antimicrobial activity of PMG-3, as evidenced by a larger zone of inhibition in the disk diffusion assay, further underscores its potential for effective control of microbial growth. This study successfully developed PCZ microsponges converted into a hydrogel. These findings suggest the promising utility of PCZ-loaded microsponge hydrogels in treating fungal infections.

Keywords:Antimicrobial, Box Behnken Design, Gel, Microsponges, Posaconazole.

Introduction

Topical treatment with Posaconazole (PCZ) represents a promising approach to addressing fungal infections of the skin and nails. PCZ, a triazole antifungal agent, exhibits broad-spectrum activity against various fungal pathogens, including dermatophytes, yeasts, and molds. Its

effectiveness stems from its ability to inhibit fungal cytochrome P450-dependent enzyme lanosterol 14-alpha-demethylase, thereby disrupting ergosterol synthesis, a crucial component of fungal cell membranes(Singh Malik *et al.*, 2016). Topical formulations of PCZ offer several advantages, including targeted delivery to the site of infection, reduced systemic exposure, and potentially lower risk of adverse effects compared to oral formulations. Additionally, topical application enables direct contact with the affected area, enhancing PCZ concentration and efficacy while minimizing systemic absorption. Overall, topical treatment with PCZ holds considerable promise for effectively managing fungal skin and nail infections, offering patients a convenient and potentially safer therapeutic option.

In recent times, there has been a surge in developing novel PCZ carrier systems to enhance bioavailability, achieve sustained discharge, and maintain localized effects in various skin layers. These systems, such as nanoparticles, liposomes, microparticles, and microsponges, overcome conventional administration limitations by targeting specific skin regions, minimizing systemic exposure, and prolonging therapeutic drug levels(Ahad, Chintaginjala, *et al.*, 2021). By penetrating the skin barrier and delivering PCZs to deeper layers, they offer new possibilities for treating infections, inflammatory conditions, and neoplastic diseases. Leveraging nanotechnology and controlled discharge, these advancements promise more effective and personalized dermatological therapies.

The poor water solubility of PCZs poses a significant challenge in transdermal delivery systems (TDDS), complicating the formulation of conventional dosage forms. This limitation hampers the development of effective drug delivery systems (DDS), as hydrophobic drugs struggle to dissolve in the skin's aqueous environment, leading to inadequate absorption and therapeutic efficacy. Overcoming this obstacle requires innovative formulation strategies, such as the incorporation of solubilizers, surfactants, or nanocarriers, to enhance drug solubility and optimize TDDS. Addressing the issue of poor water solubility is crucial for unlocking the full potential of TDDS and improving patient outcomes(Hariyadi *et al.*, 2023).

Posaconazole (PCZ) is a potent antifungal medication widely utilized in the treatment of diverse skin fungal infections, including athlete's foot, jock itch, ringworm, and yeast infections. Belonging to the azole class of antifungals, PCZ inhibits the synthesis of ergosterol, a vital component of fungal cell membranes, thereby impeding fungal growth and replication. Typically formulated into topical creams, lotions, and solutions, PCZ allows for direct application to affected skin areas, facilitating rapid absorption and targeted treatment. It exhibits broad-spectrum antifungal activity against various dermatophytes, yeasts, and molds, effectively

eradicating pathogens such as *Trichophyton* spp., *Candida* spp., and *Epidermophyton* spp. PCZ's fungicidal or fungistatic properties, depending on concentration and duration of exposure, alleviate symptoms like itching, redness, and inflammation, promoting skin healing. Clinically proven to be well-tolerated and safe for topical use in adults and children, PCZ provides rapid relief from fungal infections while ensuring patient safety. Overall, PCZ stands as a frontline therapeutic option for superficial skin fungal infections, offering effective treatment and improved skin health(Yasir Siddique *et al.*, 2021).

Antifungal-loaded microsponges (MS) represent a promising approach for enhancing topical PCZ delivery in the treatment of fungal infections. These MSsOffer several advantages, including sustained discharge of the antifungal agent, increased PCZ stability, and improved penetration into the skin layers. By encapsulating the antifungal PCZ within MS, controlled discharge kinetics can be achieved, prolonging PCZ action and reducing dosing frequency. Additionally, the porous structure of microsponges enhances PCZ loading capacity and promotes better adhesion to the skin, facilitating targeted delivery to the site of infection. Overall, antifungal-loaded MSholds great potential for optimizing topical PCZ therapy and improving patient outcomes in the management of fungal skin infections.

Materials and methods

Posaconazole (PCZ), provided as a gift sample from Cipla Limited, Bangalore. Eudragit S100 and sodium carboxymethylcellulose from Fischer Scientifics. Additionally, the fungal strain of *Candida albicans* culture was obtained from the Indian Institute of Science, Bangalore. All materials, including those from other suppliers, were of analytical grade and utilized as procured for the experiments conducted in this study.

Compatibility studies

The purePCZ, as well as its blend with excipients, were subjected to Fourier-transform infrared (FTIR) spectroscopy analysis. Spectra were recorded over the wavenumber range of 4000 to 400 PM^{-1} using an FTIR spectrophotometer.

Preparation of Microsphonges

The quasi-emulsion solvent diffusion technique was employed to fabricate PCZ microsponges. (PM). The internal phase comprised ethyl cellulose/Eudragit RS100 polymers and triethyl citrate (1% w/v) as a plasticizer. This internal phase was dissolved in 5 ml of a mixture of

dichloromethane (DPM) and ethanol (1:1). The external phase, consisting of polyvinyl alcohol (PVA) (surfactant), was completely dissolved in water. The internal phase was then added dropwise to the external phase under continuous stirring with a magnetic stirrerfor 60 min. The resulting product was filtered, washed with distilled water three times, and left to dry overnight in a calcium chloride desiccator. Preliminary trial batches were conducted to select the appropriate polymer, with Eudragit RS 100 being chosen as MSs were not formed with Eudragit RL 100. The MSs obtained were fluffy, prompting further studies to increase the polymer ratio using both ethyl cellulose and Eudragit RS 100 (Table 1)(Bhatia & Saini, 2018).

Table 1: Composition 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-Posaconazolemicrosponges; D:P- drug and polymer ratio; PVA-Poly vinyl Alcohol; rpm-rotations per minute

Evaluation of microsponges

Visual Inspection

The batches PM-1 to PM-17 were visually inspected for parameters such as color, consistency, homogeneity, and appearance in powdered form(Othman *et al.*, 2017).

% Yield

The % yield of PM-1 to PM-17 were determined by calculating the practical weight of microsponges obtained relative to the theoretical weight (polymer + PCZ)(Annepogu *et al.*, 2020).

Entrapment Efficiency

The entrapment efficiency (EE) (%) of batches PM-1 to PM-17 were calculated by measuring the absorbance of the sample in phosphate buffer saline pH 7.4. The %EE was determined by e.q.1(Fouziya *et al.*, 2022).

$$\%EE = \frac{Total\ amount\ \ of\ CMZ - amount\ \ of\ free\ CMZ}{Total\ amount\ \ of\ CMZ} X100 \text{--- (1)}$$

Particle Size Analysis

The average particle size of PM in batches PM-1 to PM-17 were determined using a binocular microscope equipped with a stage micrometer. The particle size was analyzed by spreading powdered MS on a clean glass slide and measuring those using stage and eyepiece micrometers(Mundarinti & Ahad, 2023).

Optimization of PCZ by Box Behnken design

In this investigation, the interplay between PCZ-polymer ratio, surfactant concentration, and stirring speed on the % EE and % yield were examined utilizing a Box-Behnken design. A total of 17 experimental runs were orchestrated using Design-Expert software, which employed a nonlinear computer-generated 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 b_0 serving as the intercept(Shravani *et al.*).

Loading of PM into a Gel

Initially, a precisely weighed amount of Carbopol 934 was taken and soaked in water for 24 h to ensure complete swelling of the polymer. To this base, PMequivalent to 1% w/w were uniformly dispersed. PEG-400 was incorporated into the mixture as a penetration enhancer to enhance PCZ 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 PCZ-loaded plain gel, replacing the MS with the PMN itself(Table 2)(Yadav *et al.*, 2017).

Table 2: Composition of PM loaded gels

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

Evaluation of PM gels

Physical assets

A visual inspection of the prepared MS-loaded hydrogels was conducted to assess their color, homogeneity, and consistency. Gels 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(Bansode *et al.*, 2019).

Viscosity assets

The viscosity of the formulations was assessed utilizing a Brookfield viscometer equipped with a small sample adapter and spindle no. 64. The rotational speed was gradually increased from 10 to 100 rpm, and the viscosity readings were recorded in centipoise (cps)(Rafat & Singh, 2017).

pH

The pH of the PCZmicrosponge-loaded hydrogel (PMG) formulations was determined using a digital pH meter. To conduct the measurement, one gram of gel was dissolved in 100 ml of distilled water and allowed to stand for 2h. After this incubation period, pH measurements were taken using a digital pH meter. The pH measurement process was repeated in triplicate for each formulation, and the average values were calculated to ensure the accuracy and reliability of the results(Mahaparale *et al.*, 2018).

Uniformity in PCZ content

To determine the PCZ content of the prepared PMG, 1 g of the gel, containingPCZ equivalent to 100 mg, was extracted with 30 ml of ethanol. The volume was then made up to 100 ml with phosphate buffer saline 7.4, and the solution was filtered. The resulting solution was analyzed by measuring the absorbance at 260 nm using a UV spectrophotometer after appropriate dilutions (e.q.2).

$$\text{PCZ content (\%)} = \frac{\text{Amount of PCZ in Solution (mg)}}{\text{Amount of PCZ in formulation (mg)}} \text{X100} \text{--- (2)}$$

This method allowed for accurate determination of the PCZ content in the PMG, ensuring its quality and efficacy for the intended use.

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 (redness) and edema (swelling). This evaluation aimed to identify any potential adverse reactions that could render the formulation unsuitable for use on the skin(Swetha *et al.*, 2010).

Spreadability assets

Spreadability refers to the ability of a gel formulation to spread over a given area upon application to the skin, which is crucial for its therapeutic effectiveness. In this test, 1 g of the formulation was placed within a 1 cm diameter circle marked on a ground glass slide. This slide was then sandwiched between another slide of the same dimensions, and a weight of 500 g was placed on the upper slide for 5 min. The increase in diameter due to gel spreading was recorded, and spreadability was calculated by e.q.3.

$$spreadbility \frac{mass\ (g) X\ (distance\ \ travelled\ \ by\ the\ gel)}{time\ \ (sec)} \text{--- (3)}$$

***In vitro*PCZ permeation assets**

In *the in vitro*discharge study of PMG, a Franz diffusion cell setup was employed. The formulation was placed in the donor compartment, while phosphate buffer saline (PBS 7.4) was added to the receptor compartment. 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 over the cellophane membrane, ensuring contact with the receptor medium. The entire assembly was then placed on a thermostatically controlled magnetic stirrer to maintain a constant temperature of 37± 0.5°C, with continuous stirring. At predetermined time 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 *in vitro*PCZdischarge profiles of the PMG were compared with those of the PCZ-loaded plain gel. Following suitable dilutions, the absorbance of the samples was measured at 260 nm using a UV-visible spectrophotometer(Ahad, Chinthaginjala, *et al.*, 2021).

Antifungal assets

For antimicrobial assessment, the Kirby-Bauer disk diffusion agar plate method was employed. Agar plates were prepared by pouring sterilized agar medium into Petri dishes and allowing it to solidify. A microbial suspension of *Candida albicans* was then spread evenly over the agar surface using sterile cotton swabs and allowed to dry for 10 min. Discs impregnated with the formulated gel containing PMG were aseptically placed onto the inoculated agar plates and incubated for 2 days. The presence of clear zones of inhibition around the test sample discs indicated antimicrobial activity. All assays were conducted in triplicate for accuracy and reliability(Salah *et al.*, 2018).

Results and discussion

Compatibility results

The Fourier Transform Infrared (FTIR) spectra analysis is a crucial technique used to assess the compatibility between PCZ and excipients in pharmaceutical formulations. In the context of this study, the FTIR spectra revealed that the characteristic peaks and stretches associated with PCZ were retained even in the presence of various excipients used in the formulation. This observation indicates that the chemical structure of PCZ remained unaffected by the excipients, suggesting good compatibility between the PCZ and the formulation components. The preservation of characteristic peaks and stretches in the FTIR spectra is essential as it signifies that the functional groups and chemical bonds within the PCZ molecule remained intact. Any significant alteration or disappearance of these characteristic peaks could indicate potential chemical interactions or incompatibility between the PCZ and excipients, which may compromise the stability or efficacy of the formulation (Figures 1 and 2).

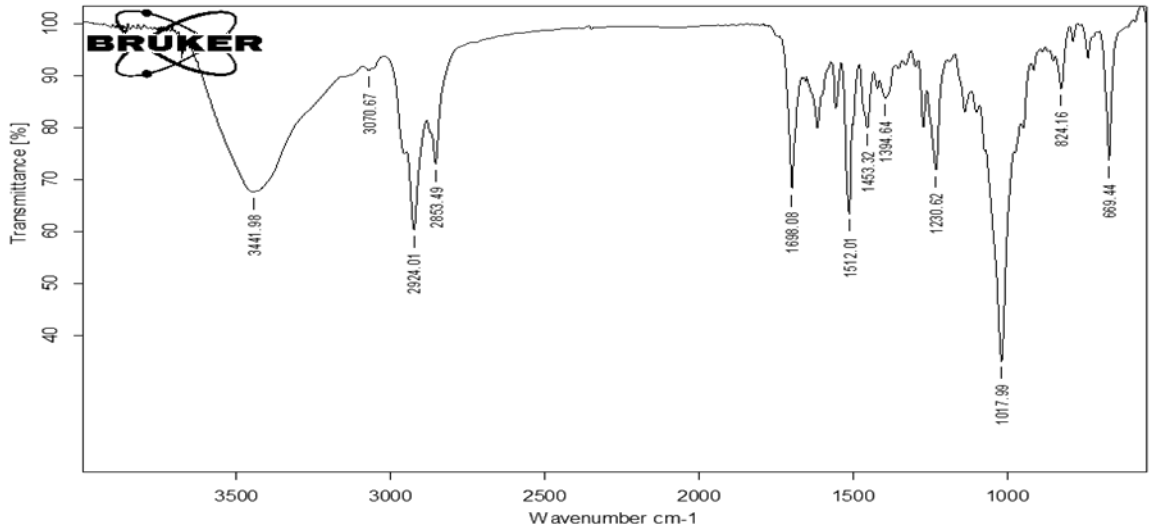

Fig.1. FTIR spectra of PCZ

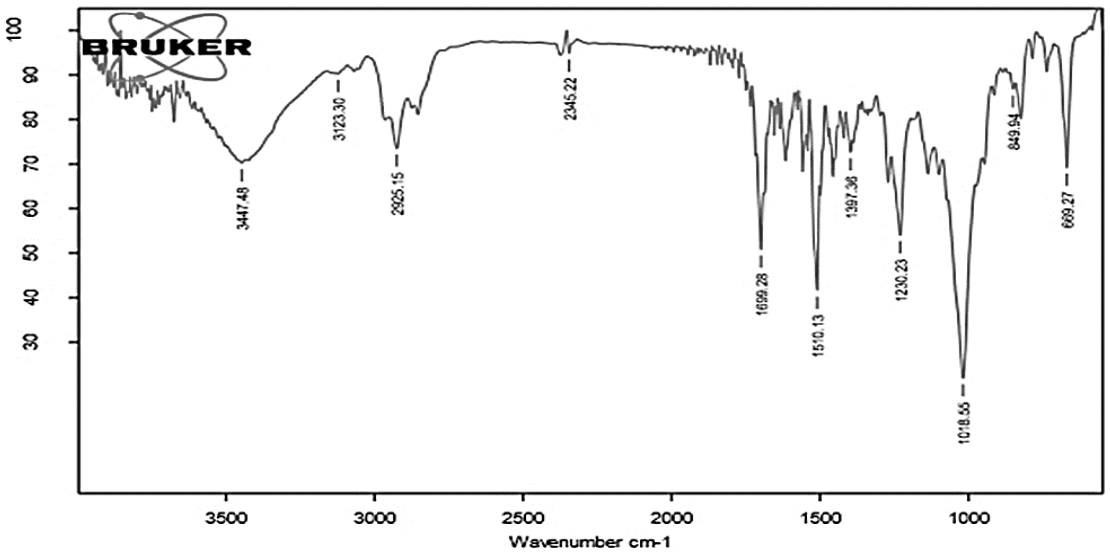

Fig.2. FTIR spectrum of PCZ with excipients

Results of physical assets

The particle size of the formulations ranged from 568.36±8.15 nm (PM-3) to 645.97±8.92 nm (PM-11), indicating variations in the size of the PMacross different formulations. This variation in particle size could impact the physical properties and performance of the formulations, such as their dispersibility, stability, and PCZdischarge kinetics.

The % yield of the formulations ranged from 80.10±3.26% (PM-11) to 98.5±1.52% (PM-3), reflecting differences in the efficiency of the PMpreparation process. Higher % yields indicate better recovery of the desired product, whereas lower yields may suggest losses during the manufacturing process or incomplete encapsulation of the PCZ within the MSs.

The PCZ's EE varied from 74.4±5.95% (PM-11) to 95.62±2.10% (PM-3), indicating the extent to which the PCZ was successfully encapsulated within the microsponge matrix. Higher EEindicates more effective PCZ loading and retention within the PMs, which is essential for achieving the desired therapeutic effect.

The pH values of the PMs ranged from 5.6±0.1 (PM-12) to 6.3±0.3 (PM-16), indicating a slightly acidic to neutral environment. This pH range is typically favorable for topical formulations as it is compatible with the physiological pH of the skin, minimizing the risk of irritation or adverse reactions upon application. The PCZ content varied among the formulations, ranging from 77.74±1.82% (PM-11) to 98.65±1.05% (PM-3), indicating differences in PCZ loading efficiency across the formulations. Higher PCZ content suggests better encapsulation and

retention of the active pharmaceutical ingredient within the PMmatrix. Spreadability values ranged from 8.06±0.06 to 9.88±0.18, reflecting the ability of the formulations to spread evenly over the skin surface upon application. Optimal spreadability is crucial for ensuring uniform PCZ distribution and enhanced therapeutic efficacy of topical formulations. Overall, the pH, PCZ content, and spreadability data provide valuable insights into the quality and performance characteristics of the PM, guiding further optimization and development efforts for topical drug delivery applications (Table 3).

Table 3. Physical assets of PCZ microsponges

Trial	Particle size (nm)	Yield (%)	EE (%)	pH	PCZ content (%)	Spreadability
PM-1	633.52±2.36	82.70±2.26	78.50±2.35	5.7±0.2	81.25±1.25	8.79±0.54
PM-2	638.97±5.52	81.20±2.26	76.80±2.61	5.8±0.3	79.82±2.25	8.95±0.66
PM-3	568.36±8.15	98.50±1.52	95.62±2.10	5.9±0.1	98.65±1.05	9.66±0.74
PM-4	572.58±7.16	97.40±0.62	94.52±5.62	6.2±0.2	97.12±1.30	9.88±0.18
PM-5	586.31±9.54	92.60±1.62	90.52±2.32	6.1±0.3	93.07±1.65	9.72±0.37
PM-6	592.30±9.82	91.70±0.62	89.25±2.54	5.8±0.1	92.26±2.52	9.65±0.14
PM-7	600.72±4.82	90.80±1.85	87.25±2.08	5.9±0.2	90.20±2.62	9.15±0.45
PM-8	601.25±6.35	89.90±4.15	87.10±3.96	6.2±0.4	91.18±3.65	8.57±0.65
PM-9	642.16±4.15	80.60±5.21	75.40±5.62	6.1±0.5	78.27±3.15	8.62±0.05
PM-10	578.58±5.92	96.80±2.98	93.60±4.85	5.8±0.3	96.98±1.02	9.64±0.25
PM-11	645.97±8.92	80.10±3.26	74.40±5.95	5.7±0.3	77.74±1.82	8.59±0.14
PM-12	582.66±7.18	95.10±0.52	92.36±6.85	5.6±0.1	95.66±2.08	9.64±0.05
PM-13	603.34±7.49	88.26±2.84	86.20±3.61	5.9±0.4	89.78±3.25	8.06±0.06
PM-14	606.88±9.82	87.80±3.65	85.40±7.15	5.8±0.2	88.21±1.05	8.97±0.15
PM-15	610.82±4.15	86.90±1.98	84.40±6.25	5.7±0.1	87.02±1.36	8.35±0.35
PM-16	612.91±5.62	85.40±0.85	83.60±4.62	6.3±0.3	86.65±2.64	8.27±0.23
PM-17	623.65±3.51	84.60±1.18	82.10±3.98	6.1±0.2	85.95±3.07	8.95±0.28

PM- Posaconazole microsponges; EE: entrapment efficiency; PCZ- Posaconazole

Effect of IV PCZ-polymer ration, surfactant, and stirring speed on %EE and % yield

The final equations in terms of coded factors provide valuable insights into the relationship between the independent variables (PCZ polymer ratio, surfactant concentration, and stirring speed) and the responses (EE and % yield). These equations allow for the prediction of response values based on specific factor levels, facilitating optimization of the formulation process.The equation for EE in terms of coded factors is as follows:

$$\%EE=+84.34-0.53X_1+8.87X_2-0.96X_3+0.15X_1X_2+0.28X_1X_3-0.06X_2X_3+3.31A^2-1.28X_2^2+0.89X_3^2$$

This equation indicates the impact of each factor (X_1, X_2, X_3) and their interactions (X_1X_2, X_1X_3, and X_2 X_3) on EE. The coefficients provide information on the magnitude and direction of the effect. For instance, positive coefficients suggest that increasing the level of the corresponding factor enhances EE, while negative coefficients suggest the opposite.Similarly, the equation for % yield in terms of coded factors is:

$$\% \text{ yield} = +86.59 - 0.55\ X_1 + 7.9\ X_2 - 0.725\ X_3 + 0.1\ X_1X_2 + 0.0\ X_1\ X_3 - 0.3\ X_2X_3 + 3.23\ X_1^2 + 0.129\ X_2^2 + 1.43\ X_3^2$$

This equation highlights the influence of each factor and their interactions on % yield. By analyzing the coefficients, one can discern the relative importance of each factor in controlling PCZdischarge from the formulation. Positive coefficients indicate factors that promote PCZdischarge, while negative coefficients suggest factors that hinder it.

The final equations regarding coded factors elucidate the relationship between the independent variables (PCZ-polymer ratio, surfactant concentration, and stirring speed) and the responses (EE and % yield). Moreover, the effects of these independent variables on % yield and %EE were visually represented using contour and 3D plots. Contour and 3D plots were generated to visualize the effects of PCZ polymer ratio, surfactant concentration, and stirring speed on both EE and % yield simultaneously. These graphical representations provide a comprehensive understanding of how changes in the independent variables impact the responses. Contour plots depict the relationship between two independent variables and the response while holding the third variable constant. By observing the contour lines, researchers can discern regions of optimal response values and identify the interaction effects between the variables(M. B. Patel *et al.*, 2021).

Three-dimensional plots offer a holistic view of the response surface, allowing researchers to visualize the complex interactions between all three independent variables and the response simultaneously. These plots provide insights into the combined effects of the variables and facilitate the identification of optimal formulation conditions. The contour and 3D plots effectively illustrate the influence of the PCZ-polymer ratio, surfactant concentration, and stirring speed on both EE and % yield. By analyzing these plots, researchers can identify the optimal formulation conditions that maximize both responses. Moreover, the contour and 3D

plots provide valuable guidance for PMs with desired characteristics, ultimately enhancing their efficacy and performance in therapeutic applications (Figure 3).

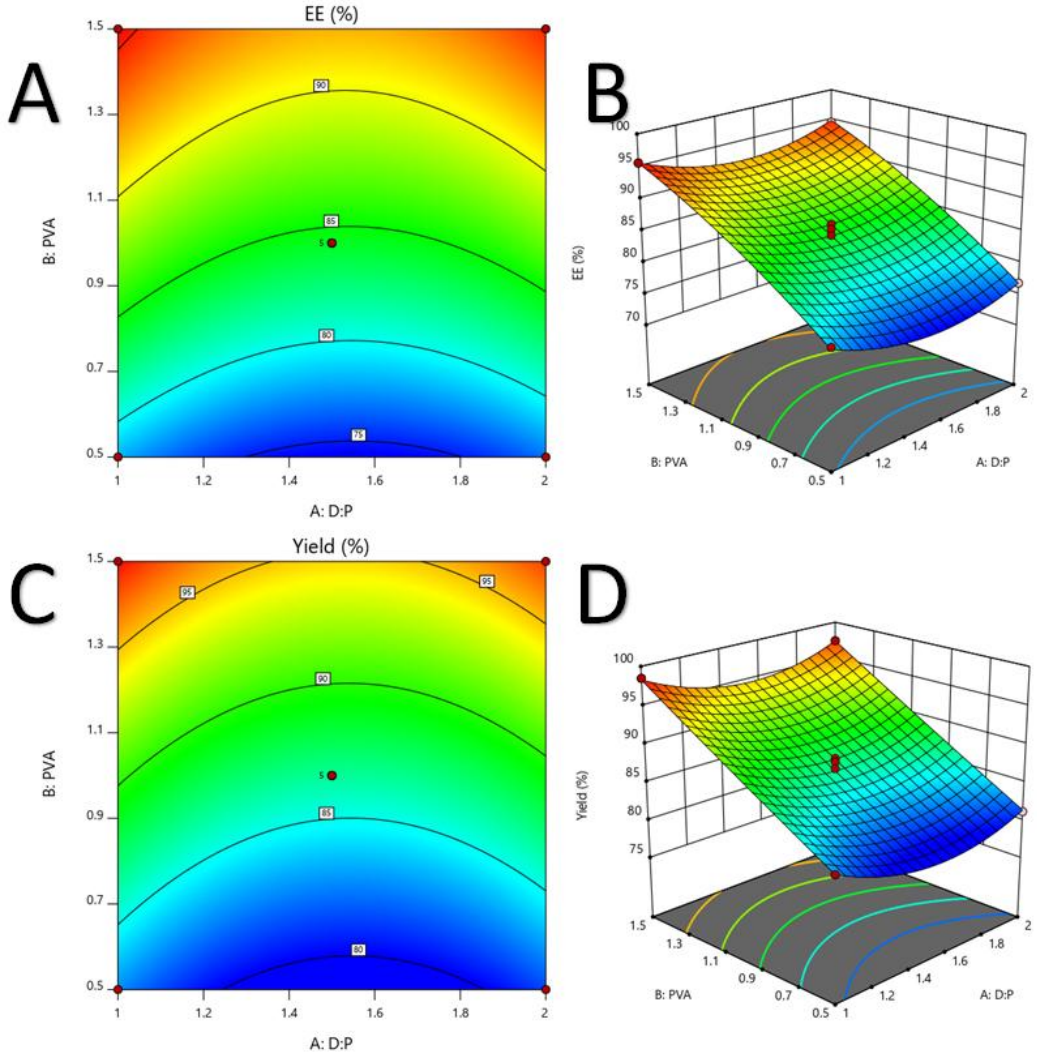

Fig.3. The effect of independent variables (PCZ-polymer ratio, surfactant concentration, and stirring speed) on % EE A) contour B) 3D and on % yield C) contour D) 3D plots

SEM assets

The SEM analysis of the optimized formulation (PM-3) revealed that the surface morphology of the PMwascharacterized by spherical shapes with uniform sizes. Additionally, the surface exhibited a porous structure, indicating the presence of pores or voids within the PMmatrix. These porous features could potentially enhance PCZ loading capacity, facilitate PCZdischarge, and promote interactions with the surrounding environment, which are advantageous for topical PCZ delivery applications(Sharma *et al.*, 2021). 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 drug delivery systems (Figure 4).

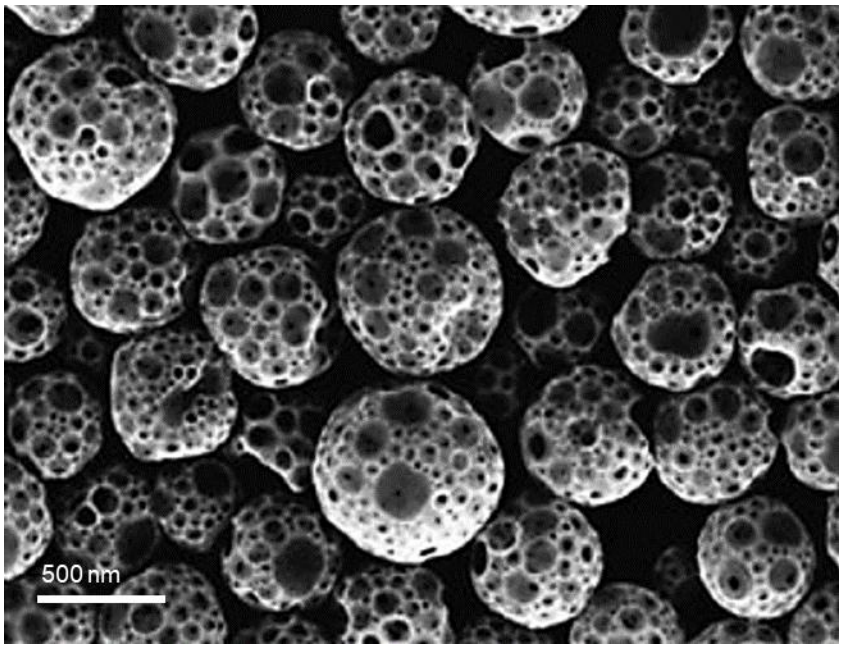

Fig.4. SEM image of PM-3

***In vitro*PCZdischarge results**

*In vitro*PCZdischarge studies demonstrated that the microsponge formulation (PM-3) exhibited superior PCZdischarge characteristics, displaying an initial rapid discharge followed by sustained dischargeover 12 h. This profile suggests that PM-3 effectively discharges the PCZ into the surrounding medium, offering an immediate therapeutic effect followed by prolonged PCZ availability for sustained action. Such controlled discharge behavior is desirable for topical formulations as it can optimize DDS, enhance therapeutic efficacy, and prolong the duration of action, thereby minimizing the frequency of application and improving patient compliance. The observed PCZdischarge profile of PM-3 highlights its potential as an effective DDS for achieving desired therapeutic outcomes in topical applications (Figure 5)(charan *et al.*, 2023).

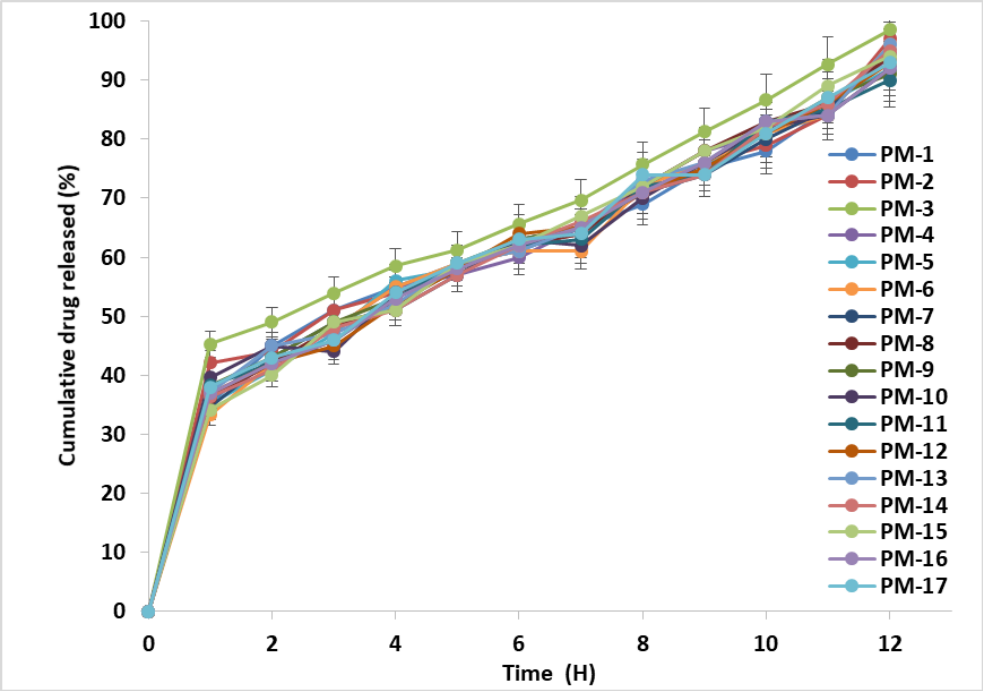

Fig.5. *In vitro*PCZdischargefrom the prepared PM

Viscosity results

Viscosity studies indicated that the PM gel formulation PM-3 exhibited superior viscosity compared to the conventional gel. This higher viscosity of PM-3 is advantageous as it can enhance the adherence of the gel to the skin surface, prolonging the contact time and facilitating better PCZ absorption. The increased viscosity of PM-3 may also contribute to improved spreadability and retention on the application site, ensuring efficient PCZ delivery and sustained therapeutic effect(Yadav *et al.*, 2017). Overall, the enhanced viscosity of PM-3 suggests its potential to provide better topical delivery and therapeutic efficacy compared to conventional gels (Figure 6).

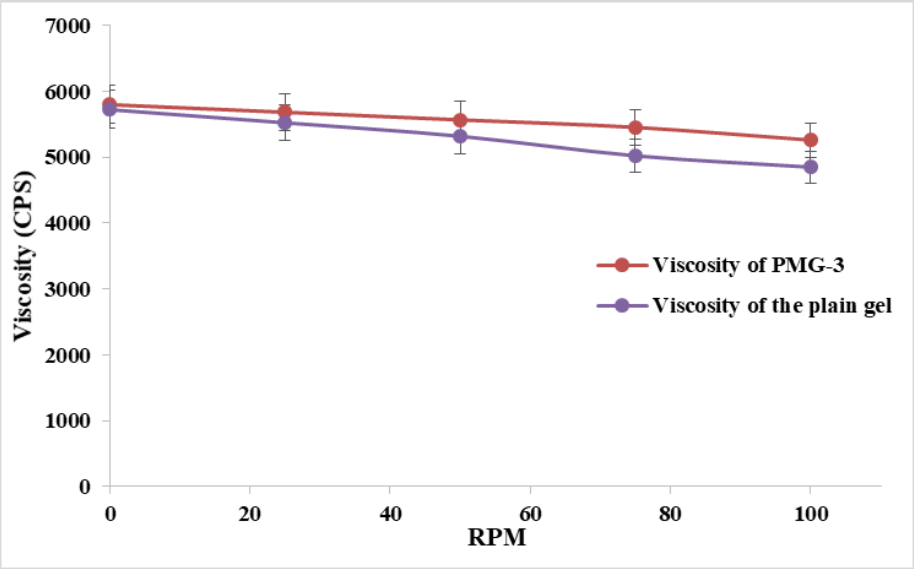

Fig.6. comparative viscosity of the optimizedPMG with normal gel

Antifungal results

In the antimicrobial assay, the optimized formulation (PMG-3) exhibited a distinct and improved zone of inhibition around the sample disc (Figure 6 B) in comparison to the plain gel (Figure 6 A). This clear and pronounced zone of inhibition indicates the enhanced antimicrobial activity of PMG-3 against the tested microorganism, likely attributed to the effective discharge of the active ingredient from the gel matrix. The larger zone of inhibition observed with PMG-3 suggests its potential for better control and prevention of microbial growth compared to the plain gel formulation(N. Patel *et al.*, 2016). This outcome highlights the importance of incorporating the PM into the gel matrix to augment its antimicrobial efficacy and therapeutic potential (Figure 7).

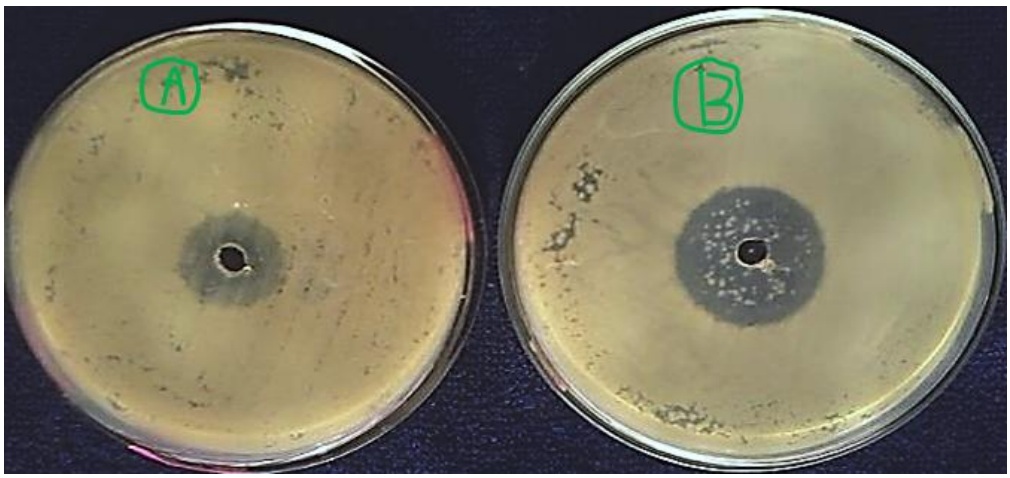

Fig.7. Zone of inhibition of A) plain gel B) PMG-3

Conclusion

This study was successful in developing Posaconazole (PCZ) microsponges and incorporating them into a hydrogel for topical application. Using Design Expert's Box Behnken Design, the micro sponges were efficiently prepared with ethyl cellulose and polyvinyl alcohol, considering drug-polymer ratio, surfactant (PVA) concentration, and stirring speed as key factors. The optimized formulation, PM-3, exhibited favorable characteristics such as smaller particle size, higher % yield, and superior PCZ discharge profile. SEM analysis confirmed the suitability of PM-3 microsponges for PCZ loading and interaction with the skin. In vitro PCZ release studies demonstrated rapid initial discharge followed by sustained release over 12 h, highlighting the potential for optimized PCZ delivery. Additionally, PM-3 gel showed higher viscosity compared to conventional gel, potentially improving PCZ absorption. The superior antimicrobial activity of PMG-3 further supports its potential for treating fungal infections. Overall, this study successfully developed PCZ-loaded microsponge hydrogels with promising applications in dermatology.

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