International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 6, 2024

Original Article

ADVANCING TOPICAL POSACONAZOLE DELIVERY: BOX BEHNKEN DESIGN MICROSPONGE HYDROGEL OPTIMIZATION AND EXTENSIVE *IN VIVO* INVESTIGATION

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Received: 18 Apr 2024, Revised and Accepted: 24 Aug 2024

ABSTRACT

Objective: The primary aim of this study was to develop a novel hydrogel formulation containing Posaconazole (PCZ) encapsulated within microsponges. Furthermore, the study aimed to assess the permeation properties of this formulation *in vivo* using a mouse model.

Methods: To achieve this aim, a series of seventeen trials were conducted using the Box Behnken Design methodology. These trials were designed to optimize the production of PCZ Microsponges (PCZ MS), which were subsequently incorporated into a hydrogel matrix. Skin permeation studies were then performed to evaluate the ability of the PCZ microsponge-based hydrogel to deliver the drug across the skin barrier. These studies involved comparison with a standard hydrogel formulation lacking microsponges.

Results: This study assessed the efficacy of microsponge gel formulation PM-3 for drug entrapment, yield, and sustained release compared to a conventional gel. PM-3 displayed the highest entrapment efficiency of 98.5% and a yield of 95.62%, indicating a direct correlation with the 1:1 drug-polymer ratio. Moreover, PM-3 exhibited sustained drug release over 12 h, releasing 83.82% of PCZ compared to 65.31% with the normal gel, suggesting its potential for prolonged therapeutic action. These findings underscore the promise of microsponge-based hydrogels, like PM-3, in enhancing therapeutic outcomes through sustained drug release, warranting further exploration for clinical applications.

Conclusion: The findings of this study highlight the promising potential of microsponge-based hydrogels as effective carriers for localized drug delivery, particularly in the context of treating skin fungal infections.

Keywords: Drug permeation, Microsponge hydrogel, Posaconazole, Skin fungal infections, Topical

DOI: https://dx.doi.org/10.22159/ijap.2024v16i6.51167 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Topical treatment with Posaconazole (PCZ) for skin conditions has gained attention due to its antifungal properties and potential efficacy in managing various dermatological conditions. PCZ, a triazole antifungal agent, is commonly used orally for systemic fungal infections. However, its topical application has shown promise in treating localized fungal infections of the skin, such as dermatophytosis (ringworm), *candidiasis*, and *pityriasis versicolor* [1]. PCZ's broad-spectrum antifungal activity makes it suitable for addressing infections caused by a wide range of fungal pathogens [2, 3].

The strategy involves the use of nanotechnology-based delivery systems, such as nanoemulsions, nanosuspensions, or lipid nanoparticles, which can enhance the solubility, stability, and permeability of PCZ through the skin. These nanocarriers can encapsulate the drug and improve its permeation by promoting its penetration into the deeper layers of the skin [4-6].

Microsponge (MS) based hydrogels for topical application offer a promising approach for enhancing drug permeation across the skin barrier, thereby improving the efficacy of topical drug delivery. MS are porous, cross-linked polymer particles with a high capacity for absorbing and entrapping drug molecules. When incorporated into hydrogel formulations, provides a controlled discharge mechanism, drug discharge, and prolonged drug delivery to the target site [7-9].

This study aims to develop a novel hydrogel formulation incorporating Microsponges (MS) containing PCZ and assess its *in vivo* skin permeation for potential topical delivery of PCZ. A comparative evaluation with conventional hydrogels is conducted to determine their efficacy in treating skin fungal infections.

MATERIALS AND METHODS

Posaconazole was gifted from Cipla Limited, Bangalore. Eudragit S100 and sodium carboxymethylcellulose, triethyl citrate, dichloromethane, and polyvinyl alcohol from Fischer Scientific. All materials, including those from other suppliers, were of analytical grade and utilized as procured for the experiments conducted in this study.

Preparation of microsponges

The quasi-emulsion solvent diffusion technique was employed to fabricate PCZMS (PM). In this method, the internal phase consisted of a mixture of ethyl cellulose and Eudragit RS100 polymers, with triethyl citrate (1% w/v) as a plasticizer. This internal phase was dissolved in 5 ml of dichloromethane and ethanol (1:1). The external phase, containing Polyvinyl Alcohol (PVA) as a surfactant, was completely dissolved in water. The internal phase was then added dropwise to the external phase under continuous stirring with a magnetic stirrer for 60 min. The resulting product was filtered, washed with distilled water three times to remove any residual solvent, and left to dry overnight in a calcium chloride desiccator to obtain the MS [10].

Experimental design

A box Behnken Design was employed for the optimization procedure, with the ratio of PCZ: polymer (X_1), PVA (X_2), and stirring rate (X_3) selected as the three prime independent variables (table 1). % entrapment efficiency (EE) (Y_1) and % yield (Y_2) were measured as the dependent variable. Design-Expert®-13 Software was utilized for the generation and evaluation of the statistical experimental design, enabling systematic exploration and optimization of the formulation parameters (table 1) [11-13].

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

% Yield

The % yield of PM-1 to PM-17 was judged by calculating the practical weight of MS obtained relative to the theoretical weight, which includes both the polymer and PCZ. This calculation was performed using e. q.1 [14, 15].

$$\% \text{ yield} = \frac{\text{practical weight of microsphonges}}{\text{weight of drug and polymers took}} \text{X100 --- (1)}$$

Entrapment efficiency

The EE of batches PM-1 to PM-17 was calculated based on the absorbance of the sample in phosphate buffer saline pH 7.4. The % EE was judged using e. q.2 [16, 17].

$$\%\text{EE} = \frac{\text{Total amount of PCZ} - \text{amount of free PCZ}}{\text{Total amount of PCZ}} \text{X100 --- (2)}$$

Loading of PM into a gel

In the formulation process, a precisely weighed amount of Carbopol 934 was initially taken and soaked in water for 24 h to ensure complete swelling of the polymer. Following this, PM equivalent to 1% w/w was uniformly dispersed into the swollen Carbopol 934 base. Additionally, PEG-400 was incorporated into the mixture as a penetration enhancer to enhance the delivery of PCZ through the skin. To ensure stability, methylparaben and propylparaben were added as preservatives. Triethanolamine was then added dropwise with gentle stirring using a homogenizer to adjust the pH of the gel to the desired level. The same procedure was followed to prepare the PCZ-loaded plain gel. This formulation process allows for the development of a gel formulation containing PCZ-loaded MS, facilitating enhanced PCZ delivery through the skin with the aid of penetration enhancers and ensuring stability through the inclusion of preservatives (table 2) [18, 19].

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 hydrogel; PEG-Polyethylene glycol; MP-Methyl paraben; PP-Propyl paraben; q. s-quantity sufficient

***In vitro* PCZ permeation assets**

In the *in vitro* discharge study of PMG (Posaconazole microsponge hydrogel), a Franz diffusion cell setup was utilized. The formulation was placed in the donor compartment, while phosphate buffer saline (PBS 7.4) was added to the receptor compartment. A cellophane membrane, pre-soaked in PBS 7.4, was interposed between the donor and receptor compartments. Subsequently, 1 g of the PMG formulation was uniformly spread over the cellophane membrane to ensure consistent contact with the receptor medium. The entire assembly was then positioned on a thermostatically controlled magnetic stirrer to maintain a constant temperature of 37±0.5 °C, with continuous stirring [20]. 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 and ensure accurate measurement of PCZ discharge. The *in vitro* discharge profiles of PCZ from PMG were compared with those of the PCZ-loaded plain gel formulation. Following suitable dilutions, the absorbance of the samples was measured at 260 nm using a UV-visible spectrophotometer. This comparative analysis allowed for the evaluation of the discharge kinetics and efficacy of PMG in delivering PCZ compared to the plain gel formulation [21].

In vivo studies

Mice grouping and treatment

The study utilized 8–11 w old, healthy mice of both sexes. Before experimentation, approval was obtained from the Institutional Animal Ethics Committee (IAEC) at CES, Kurnool (Approval No: 1305/Po/Re/S/09/CCSEA). For the study, a topical dose of 5% gel was applied daily to the shaved dorsal skin or region of the mice for 7 consecutive days (Control group-Vaseline treatment; Negative control group-hydrogel base, Test group-PMG-3 optimized gel, and normal hydrogel with PCZ). This dosing regimen allowed for the evaluation of the topical formulation's effects on the skin over a week-long period and provided insights into its potential efficacy and safety for dermatological applications [22].

Skin irritation study

Gel samples were applied to the shaved skin, and the appearance, as well as any signs of edema and/or erythema, were evaluated at specific time points of day 1 to 7 after application. This systematic assessment allowed for the monitoring of immediate and delayed skin reactions following the application of the gel samples. Any observed changes in skin appearance, such as redness or swelling, were documented at each time point to assess the potential irritant or sensitizing effects of the gel formulations. This evaluation protocol ensured comprehensive characterization of the skin response to the applied treatments and provided valuable insights into the safety profile of the gel samples [23].

Ex vivo permeation studies

Healthy mice were sacrificed to collect their skins, which were then utilized for *ex vivo* permeation studies. Topical gels containing pure PCZ and aPM-based gel (PMG-3) were prepared and permeated through the excised dorsal skin of the mice. The procedure closely resembled that of *in vitro* PCZ diffusion studies, ensuring consistency and comparability in the experimental setup and methodology. This approach allowed for the assessment of the permeation characteristics of the gel formulations through the mouse skin *ex vivo*, providing valuable insights into their potential for transdermal PCZ delivery [24].

Guesstimate of PCZ reserved in the skin layers

After completion of the experiments, the skin was removed from the diffusion apparatus. To estimate the undiffused PCZ, the surface of the skin specimen was washed ten times with 1 ml of distilled water, and the PCZ content in the washings was judged spectrophotometrically. Similarly, the diffused PCZ in the receptor compartment was estimated using a similar method. For the estimation of the PCZ retained inside the skin, the epidermis and dermis layers were effectively separated using the classical heat method. The skin specimen was placed in a sealed bag and submerged in a water bath maintained at 52 °C for 30 sec; then, the dermis and epidermis were separated by peeling and placed in 10 ml of methanol. The samples were vortexed for 5 min and then centrifuged at 8000 rpm for 15 min. The supernatants were filtered, and the filtered supernatants of the dermis and epidermis tissue suspensions were further extracted with methanol and filtered again. Finally, the filtrate was serially diluted and analyzed using UV spectrophotometry to determine the PCZ content in the dermis and epidermis layers. This comprehensive method allowed for the quantification of the PCZ retained within the different layers of the skin specimen, providing insights into the distribution and penetration of the PCZ in the skin [25].

RESULTS AND DISCUSSION

Results of % yield and PCZ entrapment

The evaluation of EE revealed notable variations among the formulations, with formulation PM-3 exhibiting the highest EE at 98.5%, while PM-11 displayed the lowest at 80.1%. Conversely, % yield demonstrated a similar trend, with PM-3 yielding the highest at 95.62% and PM-11 yielding the least at 74.4%. These findings suggest a correlation between the drug-polymer ratio and the resultant MS characteristics. Specifically, formulations with a 1:1 ratio of drug to polymer, such as PM-3, yielded MS with optimal EE and yield. This indicates the importance of carefully selecting the drug-polymer ratio to achieve desirable MS assets. Such studies were also done by Khattab and Nattouf in 2021 [26]. The observed variations underscore the significance of formulation optimization in MS development, as it directly impacts the final product's performance and characteristics (fig. 1).

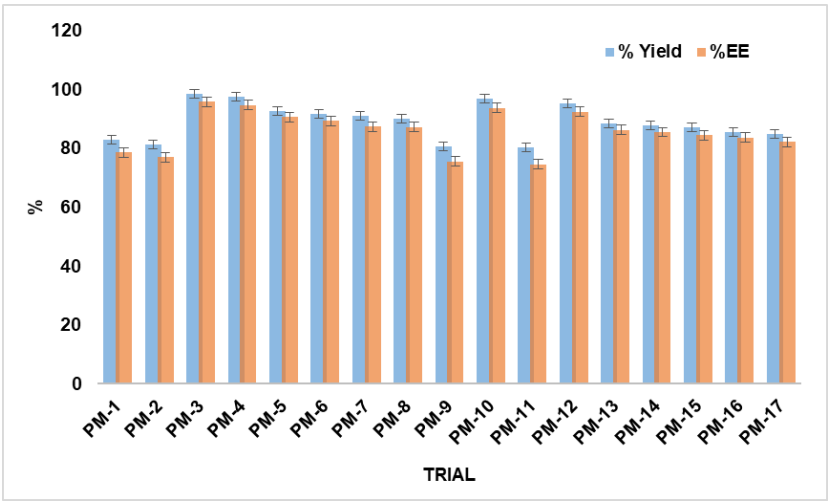

Fig. 1: % yield and % entrapment for the prepared microsponges (data given in mean ±SD; n=3)

In vitro PCZ discharge results

The results of the *in vitro* PCZ discharge studies revealed notable differences among the various formulations tested. Specifically, the MS formulation PM-3 exhibited superior PCZ discharge characteristics compared to other formulations. PM-3 demonstrated an initial rapid discharge of PCZ, followed by extended discharge over 12 h. This discharge profile indicates that PM-3 effectively

discharges PCZ into the surrounding medium, providing an immediate therapeutic effect followed by prolonged availability of PCZ for prolonged action. Patel *et al.*, 2017 did such studies on nicorandil microsponges and achieved release up to 12h [27].

The controlled discharge behavior observed with PM-3 is highly desirable for topical formulations. By optimizing DDS, PM-3 can enhance therapeutic efficacy and prolong the duration of action, thereby minimizing the frequency of application and improving patient compliance. The extended discharge of PCZ from PM-3 offers the potential to maintain therapeutic levels of the PCZ over an extended period, which is crucial for the effective treatment of fungal infections. Overall, the observed PCZ discharge profile of PM-3 highlights its potential as an effective DDS for achieving desired therapeutic outcomes in topical applications. Further studies, including *in vivo* efficacy and safety evaluations, are warranted to fully elucidate the clinical relevance and potential of PM-3 as a topical formulation for the treatment of fungal infections (fig. 2).

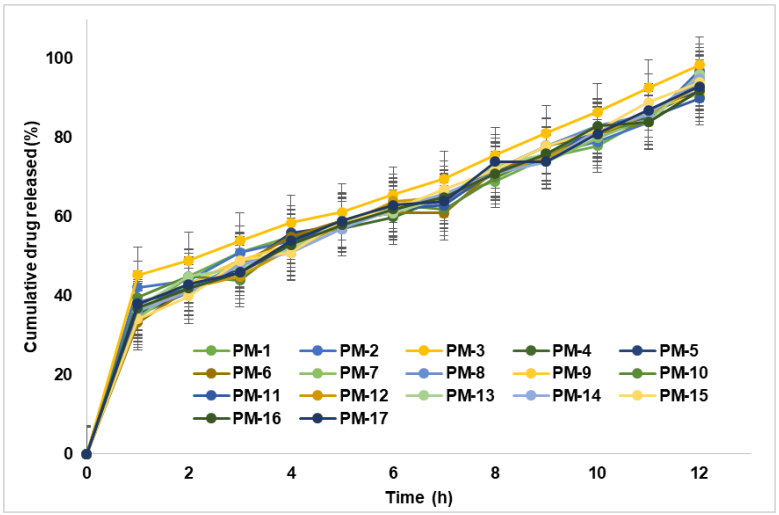

Fig. 2: *In vitro* PCZ discharge from the prepared PM (data given in mean ±SD; n=3)

Skin irritation study

Following the application of the optimized formulation onto the skin, the appearance of edema and/or erythema was evaluated daily for 7 d. Throughout the assessment period, no signs of irritation, including edema or erythema, were observed, indicating the formulation's excellent skin tolerance. This absence of adverse reactions suggests that the optimized formulation is well-tolerated and non-irritating, further supporting its potential for safe and effective use in topical applications Tripathi *et al.,* 2019 studied skin irritation studies using MS gels and found the gels were free from irritation [28].

Ex vivo permeation studies

The PCZ discharge profiles obtained for PCZ hydrogel and the optimized PMG-3 formulation were compared. The marketed PCZ hydrogel exhibited PCZ discharge for 8 h, reaching approximately 65.31% of PCZ, with no further discharge observed thereafter. In contrast, the PMG-3 formulation showed extended discharge till the end of 12 h and reached a maximum of 83.82%. Both formulations followed the Higuchi kinetic model for PCZ discharge, as evidenced by high regression coefficients of 0.8768 for the PCZ hydrogel and 0.8097 for the optimized PMG-3 formulation. Ivanova *et al.*, 2019, performed such studies using diltiazam MS gels [29]. These findings suggest that the PMG-3 formulation offers prolonged PCZ discharge compared to the normal hydrogel (fig. 3).

Guesstimate of PCZ reserved in the skin layers

The results suggest that the MS formulation (PMG-3) aids in localizing the PCZ within the skin, exerting a local effect on fungal infections. The reduced deposition of the PCZ in the dermis layer indicates that it did not penetrate the bloodstream through dermal blood vessels. Kumar *et al.,* 2017, skin retention of silver sulfadiazine from the MS gel [30]. Similarly, the normal hydrogel formulation also showed minimal PCZ penetration into the dermis layer, enhancing the localized effect and mitigating the risk of systemic side effects (table 3).

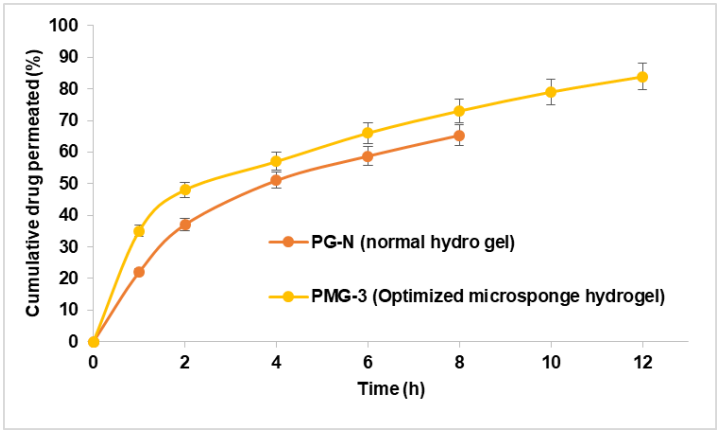

Fig. 3: Comparison of skin permeation of optimized microsponge hydrogel (PMG-3) with normal hydrogel (PG-N) (data given in mean ±SD; n=3)

Table 3: Reserved PXM in the skin layers

Trial	Epidermis	Dermis	Diffused drug
Optimized PMG-3	58.62±0.66	29.63±0.84	11.75±0.26
Marketed	86.39±0.75	7.26±0.47	6.35±0.25

Value in mean±SD; n=3

CONCLUSION

Posaconazole (PCZ) MSs were successfully prepared and characterized. Results indicated that MS prepared with a 1:1 ratio of PCZ to polymer exhibited excellent entrapment and yield. These MS were then incorporated into a topical hydrogel, which demonstrated superior therapeutic effects in treating fungal infections compared to normal hydrogels. Sustained drug discharge was achieved for up to 12 h with the microsponge-based hydrogel, highlighting its potential for prolonged drug delivery. *In vivo* and *ex vivo* permeation studies further confirmed the enhanced efficacy of the microsponge-based hydrogel in alleviating psoriasis. Consequently, the prepared microsponge-based hydrogels present a promising carrier for the effective local treatment of skin fungal infections.

ABBREVIATIONS

PCZ – Posaconazole; MS – Microsponge; EE-Entrapment Efficiency; PM-Posaconazole Microsponge; PMG-Posaconazole Microsponge Gel; PVA-Polyvinyl Alcohol; PEG-Polyethylene Glycol; MP-Methyl Paraben; PP-Propyl Paraben; rpm-Rotations per Minute; PBS-Phosphate Buffer Saline; IAEC-Institutional Animal Ethics Committee; CCSEA-Committee for Control and Supervision of Experiments on Animals

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Rekha Rani came up with the conception, acquisition, analysis, and interpretation of the data for the work; Ravi Prakash arranged the data, and Devanna N proofed the data. All authors read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

All authors declare no conflict of interest.

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