

RESEARCH ARTICLE

Preparation of Posaconazole Nanosponges for Improved Topical Delivery System

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ABSTRACT

The objective of the current research was to develop the posaconazole (PCZ) loaded NS into the carbopol 934 polymeric gel for prolonged drug release and improved topical delivery; seven different nanosponge formulations of PCZ were formulated using the emulsion solvent diffusion method using various amounts of polymer (ethylcellulose, EC). The aqueous and dispersed phases were prepared using polyvinyl alcohol (PVA) and dichloromethane. The prepared nanosponges (NS) were studied for particle size, structural appearance, and *in vitro* drug release. Furthermore, the selected formula was formulated as hydrogel and was evaluated for physical characteristics, drug content, and *in-vitro* drug release. Morphological studies revealed irregular shapes, rough and porous surfaces of nanosponges. The particle sizes were in the range of 201.6 ± 29.9 to 4904.7 ± 540.4 nm. *In-vitro* release studies revealed the sustained release pattern of the drug-loaded nanosponges. The lyophilized PCZ-NS formula had a 12-fold increase in saturation solubility over PCZ pure powder. Fourier transform infrared spectroscopy (FTIR) of the selected formula showed no significant shifts in the positions of wavenumbers compared to that of pure drug. This indicates there is no interaction between drug and excipients used. PCZ NS loaded hydrogel significantly improved the dissolution rate, which was significantly higher ($p < 0.05$) than that of pure PCZ hydrogel.

Keywords: Antifungal, Drug Delivery, Formulation, Nanosponges, Posaconazole, Topical gel Transdermal.

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INTRODUCTION

Fungal infections consider an important public health problem because of the prolonged treatment required for the disease, and frequent recurrence of infection, Moreover they are generally considered difficult to manage. In addition, the therapeutic efficacy of oral antifungals is limited due to unfavorable physicochemical properties and their toxicity effects. Dermatophytosis, caused by *Trichophyton rubrum*, is the most common cutaneous fungal infection worldwide, which represents the cause of between 80% and 90% of all chronic and recurrent infections.¹ Thus, a novel drug delivery system was developed to prevent such limitations. Nanosponge (NS) is a porous nanocarrier system that can entrap a drug of choice and improve its availability on the dermal layer, rate of permeation, and retention time. In the current research, a novel azole antifungal drug, posaconazole (PCZ), having a wide spectrum of activity and potency against dermatophytes, is used as a model drug. It has low aqueous solubility and less dermal availability.² Nanosponges enhance the solubility of poorly water-soluble drugs, prolong the release, and improve the bioavailability of the drug by modifying

the pharmacokinetic parameters of active constituents.³ Nanosponge offers controlled drug delivery for topical use, is an emerging technology for topical drug delivery, and offers entrapment of ingredients. Therefore it is believed to contribute towards reduced side effects, improved stability, increased elegance, and enhanced formulation flexibility.⁴ The objective of the present study was to develop the PCZ loaded NS into the carbopol 934 polymeric gel for prolonged drug release. The formulated dosage form enhances the drug entry deeper into the skin layer, which helps to eliminate the fungal infections of the dermal region so that recurrent infection will be terminated (Figure 1).

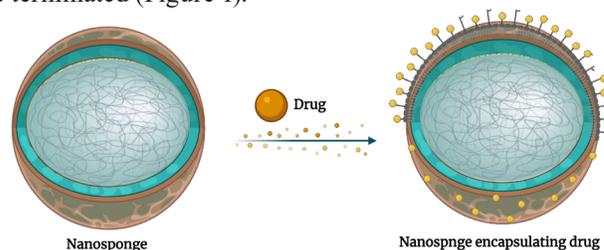


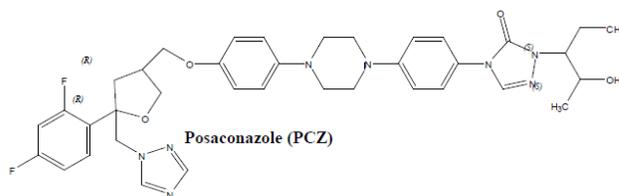
Figure 1: Nanosponges encapsulating drug [Created with BioRender.com]

Table 1: Composition of PCZ NS

Formula	Nanosponge composition					
	PCZ (g)	EC(g)	PVA %	Stirring Speed (rpm)	DCM (mL)	DW (mL)
F1	0.6	0.6	0.5	1000	20	100
F2	0.6	0.3	0.5	1000	20	100
F3	0.6	0.2	0.5	1000	20	100
F4	0.3	0.3	0.5	1000	20	100
F5	0.6	0.3	1	1000	20	100
F6	0.6	0.3	0.5	1500	20	100
F7	0.6	0.3	0.5	800	20	100

Table 2: Composition of PCZ NS loaded-hydrogel

Ingredients w/w %	F1-Pure PCZ	F2-PCZ NS
Posaconazole (gm)	0.3	NS eq. to 0.3 gm
Carbopol 934 (gm)	0.3 %	0.3 %
Triethanolamine	Few drops	Few drops
Ethanol 95 % (v/v)	20	20
Propylparaben (gm)	0.05 mg	0.05 mg
Deionized water q.s	100	100

**Figure 2:** Chemical structure of Posaconazole.⁵

PCZ is a novel oral antifungal triazole with potent and broad-spectrum activity against opportunistic, endemic, and dermatophytes fungi. Furthermore, PCZ also possesses activity against zygomycetes both *in vitro* and *in vivo*, distinguishing it from all available azoles.⁷ It has very low aqueous solubility limiting its dermal availability and acting as a barrier for topical delivery. It can be classified as a BCS II compound.⁵ According to chemical structure, PCZ is nominated as 4-[4-[4-[4-[[[(3R,5R)-5-(2,4-difluorophenyl) tetrahydro-5-(1H-1,2,4-triazol-1-ylmethyl)-3 furanyl] methoxy] phenyl]-1-piperazinyl] phenyl]-2-[(1S,2S)-1-ethyl-2-hydroxy propyl]-2,4-dihydro-3H-1,2,4-triazol-3-one; molecular formula of $C_{37}H_{42}F_2N_8O_4$; MW of 700.8 and melting point of 170°C to 172°C.⁶

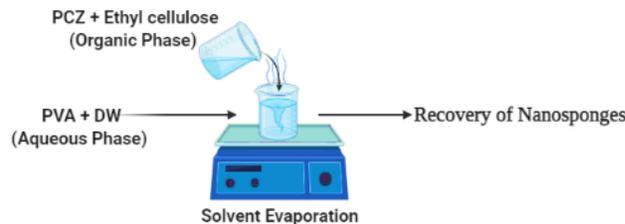
MATERIALS AND METHODS

Materials

Posaconazole powder supplied by Zhengzhou Fushi Technology, China, Ethylcellulose, Carbopol 934, and polyvinyl alcohol polymers were obtained from Zhengzhou Fushi Technology, China, Dichloromethane obtained from Merck KGaA, Germany. All other materials used in this study were of analytical grade (Figure 2).

Preparation of Posaconazole (PCZ)-loaded Nanosponges

PCZ-NS was prepared by emulsion-solvent diffusion method Table 1. Briefly, The internal organic phase consisted of different amounts (ratios) of PCZ and ethylcellulose; all dissolved in 20 mL dichloromethane (DCM) (Organic or dispersed phase); while PVA dissolved in 100 mL water as an external or aqueous continuous phase. The organic phase was emulsified dropwise into the aqueous phase using a mechanical stirrer for 2 hours at room temperature Figure 3. The prepared dispersion was filtered using a vacuum funnel to separate the solid mass. The product was then dried in an oven at 40°C

**Figure 3:** Emulsion-solvent diffusion method [Created with BioRender.com]

for 24 hours and was stored in air-tight containers for further analysis.^{7,8}

Formulation of Nanosponge-Loaded Hydrogels

1 gm of (Carbopol 934) a gel-forming polymer was immersed in 100 ml water for 12 hours. Then dispersed by agitation using a magnetic stirrer to get a uniform dispersion. The dispersion was allowed to settle for 15 minutes so that all the entrapped air was expelled. Propylparaben was dissolved in a sufficient quantity of water pre-warmed and incorporated to be used as a preservative. Then triethanolamine was added drop by drop with continuous mixing to neutralize the pH of the polymer aqueous solution. Finally, pure PCZ powder or lyophilized PCZ NS were incorporated in the polymer aqueous solution, forming two hydrogels as represented in Table 2.⁹

Evaluation of the Prepared Nanosponges

Particle Size Analysis

Particle size and polydispersity index (PDI) performed by using the NanoBrook 90 Plus (Brookhaven Instruments, USA) particle size analyzer; which is a dynamic light scattering, works by measuring the intensity of light scattered by the molecules in the sample as a function of time, All the samples were tested in triplicate (n = 3).⁶

The In-vitro Dissolution Profile of Prepared Nanosponges

Drug release from the nanosponges was conducted using a pretreated dialysis membrane bag (Molecular weight cutoff: MwCO: 8000-14000, USA). The dialysis membrane was soaked in buffer pH 7.4 medium for 8 hours; An aqueous dispersion of nanosponge, which contains an equivalent amount of 600mg,300mg respectively, of the drug was placed on a USP type II dissolution apparatus (fixed at 100 rpm, 37.5°C). PCZ-Loaded NS was evaluated by immersing in (900 mL phosphate buffer pH 7.4). Drug release was studied for 7 hours, and the volume of sample was 3 mL of the dissolution medium was withdrawn and replaced with an equal volume of fresh medium to maintain a constant level. The samples were filtered and analyzed using a UV spectrophotometer.¹⁰

Freeze-drying (Lyophilization) of the Selected PCZ Nanosponges

The suspension of the selected formula was centrifuged at 3000 rpm for 10 min to separate the un-entrapped drug as a residue below the colloidal supernatant. The supernatant layer was then filtered using a filter paper and then freeze-dried using a lyophilizer (Copley, Germany) to obtain PCZ loaded nanosponges.¹¹

Scanning Electron Microscopy (SEM)

SEM can be used to study morphology, and surface topography of the selected NS, and the difference in morphology state of the pure drug and the product by using a Vega/TESCAN scanning electron microscope (Tescan Orsay Holding, Brno, Czech Republic).¹²

Saturation Solubility of Pure Drug Versus Lyophilized Powder

Excessive PCZ and the lyophilized powder were dispersed in 10 mL of phosphate buffer pH 7.4 separately and shaken in a water bath for 72 hours, then filtered and analyzed using UV spectroscopy.¹³

Powder X-ray Diffraction (PXRD)

The data obtained from PXRD was used to determine whether newly formed compounds are crystalline or amorphous, the following conditions were used for the measurement: target metals Cu, filter K, 40kV voltage, and 30 mA current. Samples were scanned over a two-degree range of 10–90°C with a 0.2° phase scale.¹⁴

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of pure PCZ and selected formula of NS were recorded and interpreted for the possible chemical interactions. The transparent pellets of these samples were prepared by mixing each of these components with potassium bromide, and FTIR spectra were recorded in the region of 4000–400 cm⁻¹ (FTIR-7600 Lambda scientific, United states).¹⁵

Evaluation of Prepared PCZ Loaded-Hydrogel

Physical Appearance and pH of Hydrogel Formulas

The prepared hydrogel formulations were evaluated visually for appearance and homogeneity; also, the pH of the hydrogel was determined by using a pH meter at 25°C.⁷

Viscosity

The viscosity of prepared PCZ-NS loaded hydrogel formulation was measured using a digital viscometer (Model: Myr Rotational) with spindle no. R7, at 20–200 rpm, at room temperature; before the viscosity measurement, the hydrogel samples were allowed to settle for about 15 minutes, an experiment was performed in triplicate (n = 3).¹⁶

Drug content of PCZ in the Hydrogel Formulas

PCZ content in the prepared hydrogel was determined by dissolving a specific amount of the prepared gel, which is equivalent to 10 mg of PCZ and transferred to a 100 mL volumetric flask containing phosphate buffer (pH 7.4), then filtered and detected at λ max of PCZ using a UV spectrophotometer, and DC determined using the following equation.¹⁵

$$\% \text{ Drug content (DC)} = (\text{Actual amount of}) / (\text{Theoretical drug amount of drug}) \times 100 \dots (1)$$

In-Vitro Drug Release Study of the Prepared Hydrogel Formulas

The *in-vitro* release of PCZ from NS-loaded hydrogel and pure PCZ hydrogel formulas was performed by using dissolution apparatus-II (900 mL dissolution media phosphate buffer pH 7.4, at 37 ± 0.5°C, Stirring speed of 100 rpm). 3.3 g hydrogel was placed in the dialysis bag (Molecular weight cutoff: MwCO: 8000-14000, USA). Drug release was studied for 7 hours, and the samples of 5 ml were collected from the media and replaced by the same volume of the fresh media to keep the volume constant. The samples were filtered and analyzed using a UV spectrophotometer.⁹

Statistical analysis

The results were statistically analyzed using GraphPad Prism 9 software (version 9.1.0). The experiments were conducted in triplicate and expressed in the form of mean ± SD. An independent t-test and ANOVA were used to assess the degree of statistical significance between various groups.

RESULT AND DISCUSSION

The effect of different parameters on the particle size was studied using eight different formulations. The mean particle size for formulations varied in the range from 65.3 ± 33.6 nm to 1398.7 ± 324.9. The particle sizes for different formulations of different parameters are summarized in Table 3.

Table 3: The mean particle size of different formulations.

<i>Formula code</i>	<i>Mean Particle size(nm) ± SE*</i>
F1	201.6 ± 29.9
F2	133.8 ± 73.7
F3	137.5 ± 64.1
F4	65.3 ± 33.6
F5	70.1 ± 20.7
F6	1398.7 ± 324.9
F7	4904.7 ± 540.4

*SE standard error, n = 3

The average particle diameter was considerably affected by the amount of EC and the dose of the drug, as shown in Figures 4 and 5. The particle size decreases as the amount of EC decreases because less time is required for droplet formation. In addition, particle size showed a significant decrease ($p < 0.05$) as the dose of PCZ decreased. This can be explained by the less viscous organic phase formed during the processing; Bohrey *et al.* reported the same result.^{8,17}

Particle size was decreased insignificantly ($p > 0.05$) with PVA concentration increased as seen with F2 and F6 in Figure 6. The decrease was due to the increase in PVA concentration leading to an increase in the viscosity of the aqueous phase providing stability of the emulsion Miladi *et al.* reported the same results.¹⁸

The results showed that stirring speed influenced the particle size, as seen in Figure 7. The mean particle size was significantly decreased ($p < 0.05$, paired t-test) from 490.47 ± 540.4 nm to 133.8 ± 73.7 nm when the stirring speed increased from 800 to 1000 rpm. Therefore, high mechanical shear was applied, resulting in rapidly splitting the formed droplets, providing less chance for bigger droplets to coalesce.¹⁹ In addition, the mean particle size increased from 133.8 ± 73.7 nm to 1398.7 ± 324.9 nm, when the stirring speed was increased from 1000 to 1500 rpm although this increment was insignificant ($p > 0.05$, paired t-test) but this was because of intense stirring speed destroyed the repulsive force between particles and caused the particles aggregation (Zahid *et al.*, 2016) reported the same results.²⁵ In comparison to the low and high stirring speeds, a moderate stirring rate (1000 rpm) was observed to be optimum for providing good mixing and a more uniform environment for NS formation.

In-vitro Dissolution Profile of Prepared Nanosponges

The release profiles of the formulated PCZ nanosponges are illustrated in Figure 7. The overall results revealed a significant ($p < 0.05$) increase in the release of PCZ from the prepared nanosponges with decreasing the amount of EC. The slower drug release was attributed to increased path length for drug diffusion (Abbas *et al.*, 2019) reported the same results.⁸

The formulas 3 and 5, as seen in Figure 8; revealed that as the amount of drug increased; the *in-vitro* drug release reduced significantly ($p < 0.05$); Bohrey *et al.* observed the same results; and explained it by the fact that increasing amount of drug results in a more viscous organic phase (dispersed phase), making complex the mutual dispersion of the phases and bigger nanoparticles formation.¹⁷

The presence of PVA molecules stabilized the emulsion nanodroplets and prevented them from aggregation with one

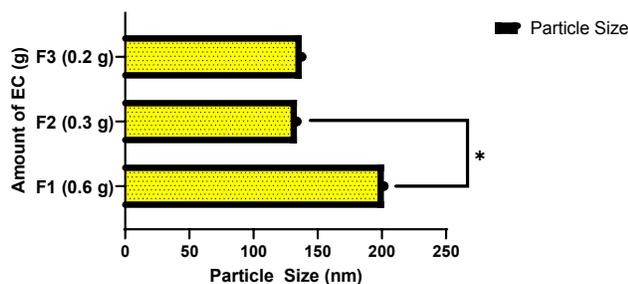


Figure 4: Effect of the amount of EC on particle size * ($p < 0.05$)

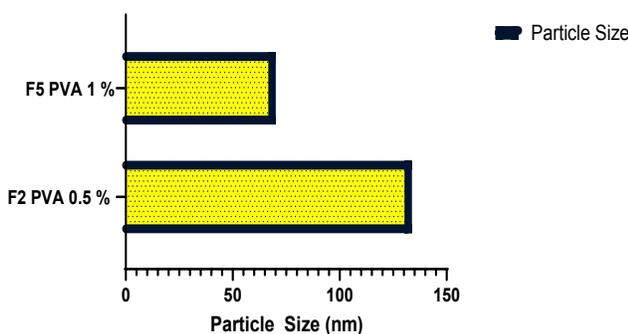


Figure 6: Effect of PVA concentration on particle size.

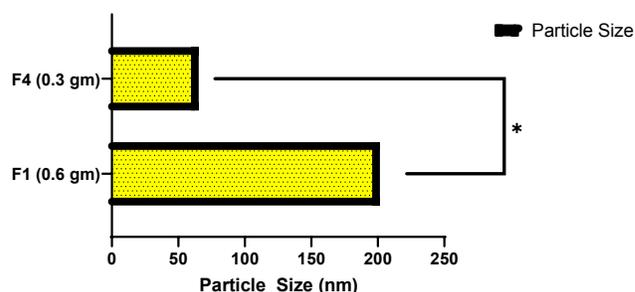


Figure 5: Effect of the dose of the drug on particle size. * ($p < 0.05$)

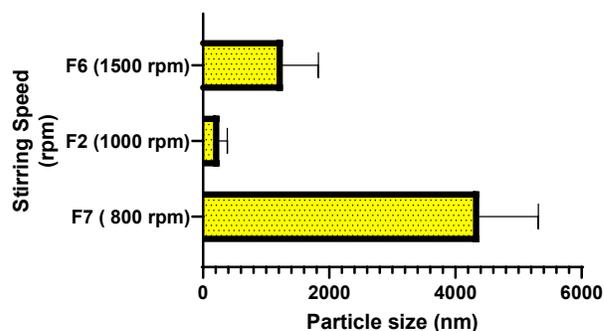


Figure 7: Effect of stirring speed on particle size.

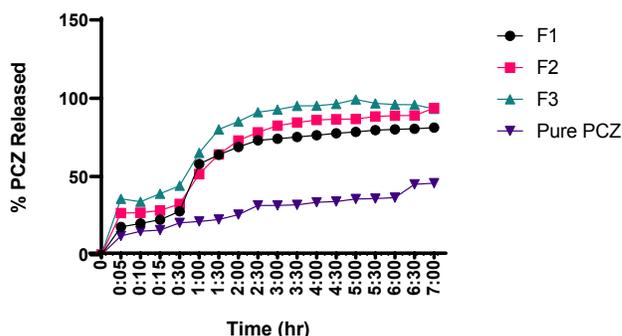


Figure 7: Dissolution profile of formulas F1, F2, and F3 in PBS at pH 7.4

another, changing the amount of PVA from (0.5% in F2) to (1% in F5) caused an insignificant decrease ($p > 0.5$) on *in-vitro* drug release as seen in Figure 9.

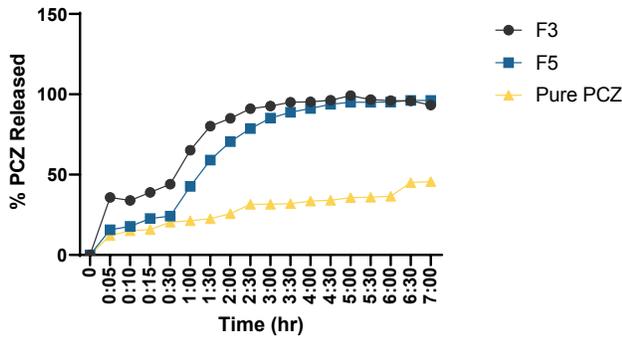


Figure 8: Dissolution profile of formulas F3 and F5 in PBS of pH 7.4

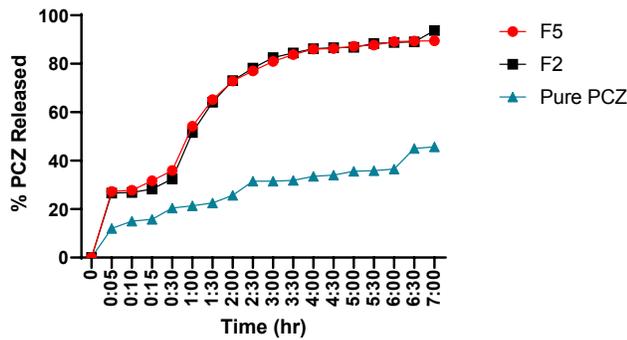


Figure 9: Dissolution profile of formulas F2 and F5 in PBS of pH 7.4

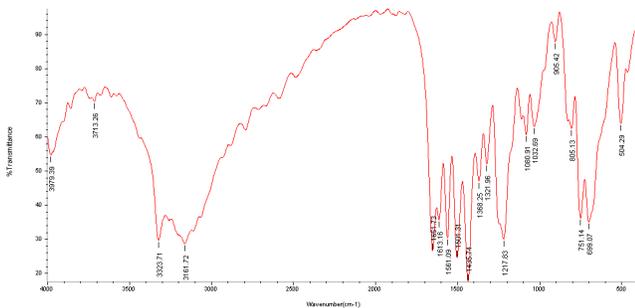


Figure 10A: FTIR spectrum of pure PCZ

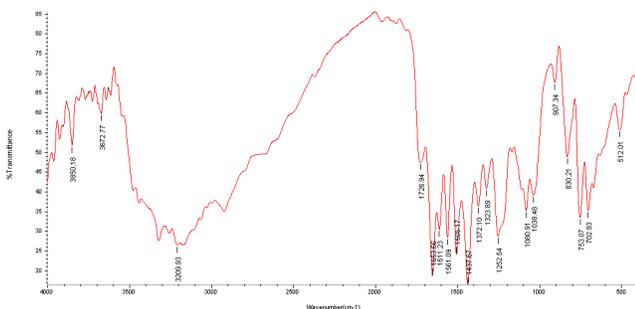


Figure 10B: FTIR spectrum of PCZ loaded NS lyophilized powder

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR of pure PCZ has sharp peaks of alkene stretching ($=C-H$ and CH_2) vibration at 3979.39 cm^{-1} and 3323.71 cm^{-1} and exhibited $C=O$ stretch at 1651 cm^{-1} due to saturated ketone and $C=O-NH$ stretching at 1631 cm^{-1} , FTIR of the selected formula shows no significant shifts in the positions of wave numbers as compared to that of pure drug this indicates there is no interaction between drug and excipients used.²⁰ The FTIR spectrum of pure PCZ and lyophilized powder is given in Figure 10A, B.

Powder X-ray Diffraction (PXRD)

Powder X-ray diffraction (PXRD) patterns of pure drug and selected nanosponge formula are shown in Figures 11A and B, respectively. The diffraction patterns of pure PCZ showed intense and sharp peaks indicating the crystalline nature of the drug, while the diffraction pattern of PCZ nanosponge

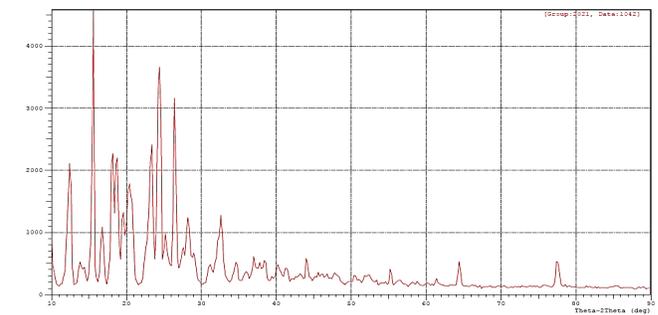


Figure 11A: PXRD of pure PCZ.

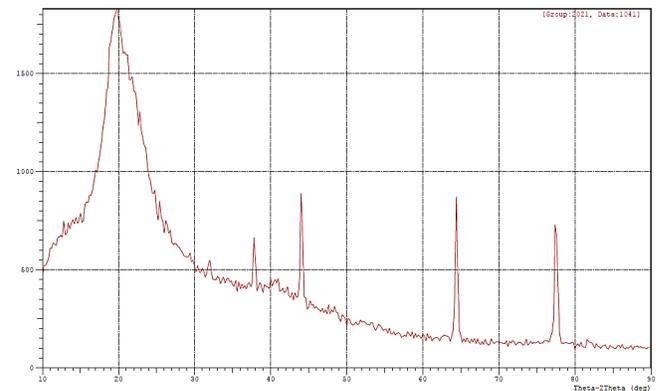


Figure 11B: PXRD of selected formula.

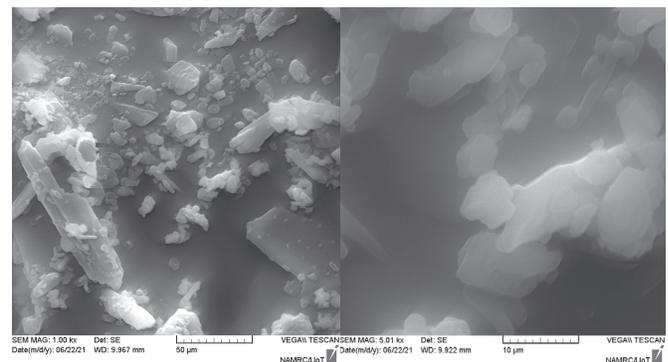


Figure 12A: SEM images of the pure drug; at 1.00 Kx and 5.01 Kx

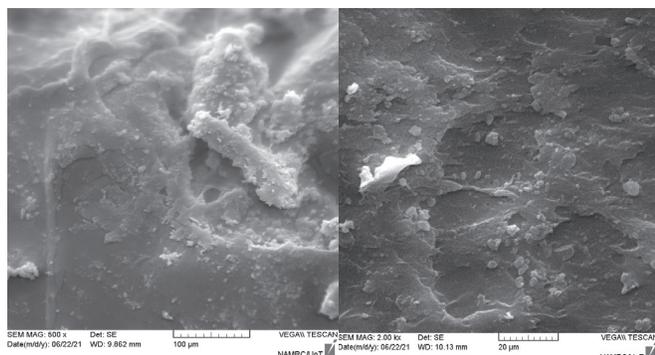


Figure 12B: SEM images of F5 nanosponge formulation; at 2.00 Kx and 500 x magnifications

showed a decrease in the intensity of the peaks, which suggests the successful encapsulation of the PCZ within the core of the nanosponge.²¹

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy of PCZ loaded nanosponges showed irregular shapes. The surface of NS was rough with porous structure as shown in Figure 12 (Omnya *et al.*, 2019), reported the same results.²²

Saturation Solubility of Freeze-Drying Nanosponges

The saturation solubility of the lyophilized powder of PCZ loaded nanosponges was increased significantly ($p < 0.5$) from that of pure PCZ solubility as seen in Figure 13; the same result was reported by Rao *et al.*²³

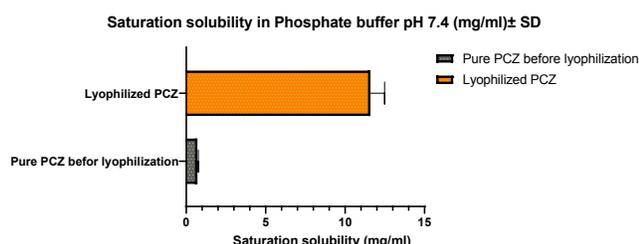


Figure 13: Saturation solubility in Phosphate Buffer pH 7.4 (mg/mL) ± SD

Physical Appearance and pH of Hydrogel Formulas

The prepared hydrogel was translucent, smooth, and uniform homogeneity. The pH of PCZ NS-loaded hydrogel was 6.43 ± 0.152 , referring to the compatibility of the product. The results agreed with that of the research on fluconazole-loaded nanosponge hydrogel.

Viscosity

PCZ loaded NS hydrogel showed approximate viscosity between (4,300 -24,200) CP while for Pure PCZ, hydrogel showed viscosity between (3,500–19,000) CP; (Figure 14). The viscosity value of PCZ-loaded NS hydrogel was excellent because the preparation was easily removed from the container and smooth texture upon application on the infected area; there was a significant difference ($p < 0.05$) between formulations.²⁴

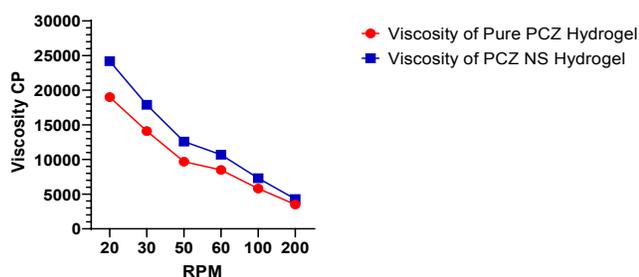


Figure 14: The rheogram of nanosponges hydrogel and pure PCZ hydrogel

Determination of PCZ Content in the Hydrogel Formulas

The PCZ NS contents in the hydrogel were 96.06%. While in pure PCZ, hydrogel contents are 89.455%. From this, it can be concluded that PCZ was uniformly distributed in Both hydrogel formulas (Table 4).

The In-vitro Dissolution Profile of Prepared Hydrogels Formulas

From the release profile, Figure 15; PCZ NS loaded hydrogel significantly improved the dissolution rate, which was significantly higher ($p < 0.05$) than that of pure PCZ hydrogel. *In-vitro* release of PCZ nanosponge from hydrogel showed fast and completed in comparison to pure PCZ hydrogel, the same result observed with; Aldawsari *et al.*; they prepared a topical hydrogel of lemongrass-loaded NS; faster release rates is due to the high porosity of the larger particles that allow for the leakage of the PCZ to the hydrogel during preparation.²⁵

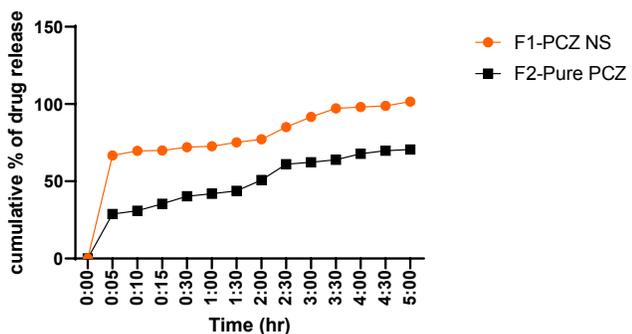


Figure 15: Dissolution profile of PCZ-NS hydrogel formula and pure PCZ hydrogel in PBS (pH 7.4)

CONCLUSION

The present study aimed to make the PCZ-NS so that the drug can be released in a controlled fashion and also to enhance the bioavailability of the drug. The method used to prepare PCZ-NS was the emulsion solvent diffusion method using EC as a polymer. This method was rapid, reproducible, and simple. Nanosponge-based hydrogel formulations of PCZ revealed a sustained released pattern of the PCZ. This system provided potential benefits such as reduced frequency of application,

Table 4: Saturation solubility of PCZ before and after lyophilization

Saturation solubility in Phosphate buffer pH 7.4 (mg/mL) ± SD	
Pure PCZ before lyophilization	0.653 mg/mL ± 0.655
Lyophilized PCZ	11.156 mg/mL ± 0.786

leading to increased patient compliance because nanosponge-based hydrogel offered better drug retention ability and made PCZ more efficient for the treatment of fungal infections topically.

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