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Original Research Article

Nanosponge-based hydrogel preparation of fluconazole for improved topical delivery

Nasir Abbas[1], Kousar Parveen[2], Amjad Hussain[1], Sumera Latif[1], Shaiq uz Zaman[2], Pervaiz Akhtar Shah[1], Muhammad Ahsan[1]*

[1]Department of Pharmaceutics, Punjab University College of Pharmacy, University of the Punjab, [2]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore, Pakistan

For correspondence:* ***Email:* *ahsanshareef105@gmail.com;* ***Tel:*** *+92 3334060054*

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Abstract

Purpose: *To develop polymeric nanosponge based hydrogel system of fluconazole (FZ) for improved delivery for topical application.*
Method: *Six different nanosponge preparations of fluconazole were formulated by oil-in-water (o/w) emulsion solvent diffusion method using various drug to polymer (ethylcellulose, EC) ratios. Polyvinyl alcohol (PVA) and dichloromethane were used to prepare the aqueous and dispersed phases, respectively. The nanosponges (NS) were studied for entrapment efficiency, particle size, structural properties, size and appearance, and in vitro drug release. Furthermore, the hydrogel formulation was evaluated for ex vivo permeation characteristics.*
Results: *Morphological studies revealed porous nanosized particles with the outer surface resembling orange peel. The nanosponges had particle size in the range of 220.2 ± 4.5 to 624.1 ± 10.4 nm. Release studies showed 43.9 ± 3.2 % drug release at 6 h, confirming the sustained release pattern of the drug-loaded nanosponges. Powder x-ray diffraction (PXRD) and Fourier transform infra-red (FTIR) analyses indicate complex formation in the nanosponge structure. Out of six nanosponge formulations prepared, F3 containing FZ and EC in the ratio of 1:0.7 showed optimum physicochemical and release characteristics and, therefore, was selected for hydrogel formulation. Kinetic analysis of the permeation data revealed a Higuchi diffusion pattern. Ex vivo permeation studies indicate that the hydrogel preparation displayed adequate drug permeation through rat abdominal skin.*
Conclusion: *A nanosponge-loaded hydrogel of fluconazole for improved permeation of the drug through skin has been successfully developed. Safety and toxicity tests are required to ascertain its potential suitability for use in humans.*

Keywords: *Fluconazole, Nanosponges, Ethylcellulose, Drug release, Franz diffusion cell, Higuchi diffusion*

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INTRODUCTION

Conventional topical systems such as ointments and creams are less effective for skin permeation due to their poor efficiency and are associated with side effects such as burning, contact dermatitis and stinging sensations owing to uncontrolled release of drug [1,2]. Therefore,

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focus is shifting towards development of particulate carrier systems such as microspheres and liposomes for controlled delivery of drugs to specific skin regions [3]. These systems will presumably control drug input rate and minimize absorption of drug into systemic circulation and consequently adverse reactions. Various studies have shown nano particulate carriers to be viable substitute to liposomal carriers to achieve enhanced cutaneous delivery [4]. Therefore, nano-technology approaches are major areas of interest in the past few decades.
One such novel nano-carrier system which offers topical delivery especially when formulated as hydrogel is nanosponge (NS) based delivery system [5]. NS are porous, spongy, spherical, small sized polymeric structures which release the drug in controlled and predictable manner [6]. They are free-flowing, self-sterilizing, cost effective and stable over range of pH of 1 - 11 and temperatures up to 130°C. Many advantages of NS like improved safety, better product stability, enhanced aesthetic characteristics and non-irritancy make them suitable approach for development of topical preparations [7]. A number of topical agents can safely be incorporated into nanosponges for controlled release [8]. Local anaesthetics, antifungals and anti-acne are the potential categories of drugs that may be easily delivered as topical nanosponge preparations.

Nanosponges can be prepared by solvent method, cross linking of β-cyclodextrins, ultra sound assisted method and emulsion solvent diffusion method which is one of the effective and economical method for preparation of NS [9].

FZ is an anti-fungal agent which is used orally and topically to treat the oropharyngeal and esophageal candidiasis, vaginal candidiasis and cryptococcal meningitis. It is also used for the management of various clinical conditions including peritonitis, candida urinary tract infections, candidemia, and pneumonia [10]. Being an anti-fungal agent, it normally requires a long duration of therapy, which can lead to higher incidence of adverse effects after systemic administration. This requires development of topical FZ preparation to avoid these untoward effects. However, majority of topical delivery systems in the market have insufficient residence time and results into inadequate therapeutic effects [11]. Furthermore, due to their low efficacy as delivery system, these formulations normally need a high amount of active pharmaceutical agent to get desired therapeutic effect. A nanosponge based topical drug delivery system has the potential to reduce the side-effects associated with conventional delivery system. The present research was therefore employed to investigate the permeation properties of FZ by formulating nanosponge based hydrogel delivery system to provide localized delivery to the site of action.

EXPERIMENTAL

Materials

FZ was received as gift sample from Mass Pharma, Lahore, Pakistan. Ethyl cellulose, polyvinyl alcohol (PVA), ethanol, carbopol-940, propylene glycol and triethanolamine were procured from Sigma Aldrich, USA. Dichloromethane was obtained from DH Laboratory, Pakistan. All the other solvents and chemicals were of analytical grade.

Formulation of fluconazole (FZ)-loaded nanosponges

Emulsion-solvent diffusion method was employed for formulation of FZ loaded nanosponges as described earlier [8]. Briefly, six batches of nanosponges using different ratios of drug (FZ) and polymer (EC) were prepared as shown in Table 1. Initially dispersed phase was prepared by ultrasonic stirring of EC and FZ in 20 mL dichloromethane. Then 0.5 % w/v PVA was dissolved in 150 mL of water to prepare aqueous continuous phase by stirring on hot water bath at 60°C. The percentages of PVA and dichloromethane were kept constant in all formulations. The dispersed phase was slowly incorporated in the continuous phase using syringe and was magnetically stirred at 1000 rpm for 2 h. The prepared dispersion was filtered using 0.45 μm filter paper to separate the solid mass. The product was dried in an oven at 40 °C for 2 h and was stored in desiccators for 48 h to evaporate any residual solvent completely. The final products were packed and stored in air tight containers.

Particle size analysis

Average particle diameter of prepared NS was determined using dynamic light scattering technique (zeta sizer, Malvern, ZSP nano) following an earlier described method [12]. Aqueous dispersions of NS were appropriately diluted for scattering intensity at 25 °C. Samples were kept in disposable cuvette and measurements were made at 372.0 kcps (count rate) for 20 s.

Table 1: Composition of FZ nanosponge

Formulation code	Drug (g)	Ethyl cellulose (EC) (g)	Polyvinyl alcohol (PVA) (% w/v)	Dichloromethane (DM) (ml)	Drug/EC ratio (w/w)
F1	1.0	0.3	0.5	20	1:0.3
F2	1.0	0.5	0.5	20	1:0.5
F3	1.0	0.7	0.5	20	1:0.7
F4	1.0	1.1	0.5	20	1:1.1
F5	1.0	1.3	0.5	20	1:1.3
F6	1.0	1.5	0.5	20	1:1.5

Yield (PY)

The yield of FZ nanosponges was determined using Eq 1 [13].

Y (%) = (Wfn/Wi) 100 ………. (1)

where Wfn = weight of drug loaded nanosponges, Wi = weight of raw materials.

Drug loading and entrapment efficiency

These parameters were calculated as in Eqs 2 and 3. Sample of drug loaded NS (10 mg) was dissolved in 10 mL 0.1 N acetate buffer solution (pH 5.5) under sonication for ~5 min. The solution was filtered by using membrane filter (0.45µm) and analyzed for drug content by UV spectrophotometer (Shimadzu, Japan). Absorbance was determined by using a calibrated method for FZ with R^2 value of 0.998 at λ_{max} 260 nm [14].

DL (%) = (DCN/WNS)100 ……… (2)

where DCN = drug content of nanosponges and WNS = weight of nanosponges recovered

EE (%) = (DCN/TDC)100 ………. (3)

where DCN = drug content of nanosponges and TDC = theoretical drug content

Scanning electron microscopy (SEM)

Morphological studies of selected NS formulations were carried out by scanning electron microscopy (Hitachi TM-1000) with auto imaging system. Particles were placed on aluminum stub and coated with gold by using sputter coater (Denton, Desk V HP) operated under vacuum for 25 seconds at 40 mA.

***In vitro* drug release studies**

The release of FZ nanosponges was studied using USP Type II apparatus run at 50 rpm (37°C ± 0.5°C). Dissolution medium was phosphate buffer at pH 5.5 (pH of normal skin). Samples were analysed at 260 nm by withdrawing at specific time intervals and replacing by fresh dissolution medium for the study period of 8 h.

Fourier transform infrared (FTIR) spectroscopy

FTIR analysis of drug and formulated nanosponge samples were carried out using FTIR (Bruker). The spectra were recorded for the frequency in the range from 2000 to 600 cm^{-1}.

Powder x-ray diffraction (PXRD)

X-ray diffraction analysis of powder samples for the pure drug, polymers and selected formulation was carried out using X-ray diffractometer (PAN Analytical) with Cu Kα radiation (λ = 1.54 Å) with a position sensitive detector PSD. Diffraction data were collected in the range 2θ = 20 - 80° with a step size of 0.02^o.

Formulation of nanosponge-loaded hydrogels

Nanosponge based hydrogel was formulated as earlier reported [15]. Initially 150 mg of carbopol 940 was immersed for 2 h in 10 mL of water and was homogeneously dispersed using magnetic stirrer at 600 rpm. Then triethanolamine (1 mL) was added to neutralize the pH and nanosponges of F3 formulation (equivalent to 50 mg of FZ) were incorporated into the dispersion. At this stage, 0.5 mL of permeation enhancer (Propylene glycol) and 0.2 mL of N-methyl-2-pyrrolidone as ethanolic solution was added to the aqueous dispersion. The final dispersion was agitated until smooth gel was formed without lumps.

Evaluation of hydrogel

Physical appearance

The prepared hydrogel formulation was evaluated for appearance and homogeneity by visual observation.

pH determination

The pH of the hydrogel was determined by using a pH meter. For this purpose, the measured quantity (1 %) of the hydrogel was prepared in deionized water and pH measurement was done at 25 °C.

Viscosity

The viscosity of formulated hydrogel was determined using Brookfield viscometer at 100 rpm spindle speed at temperature of 25 °C [16]. The viscosity determination was recorded in triplicate.

Ex vivo permeation studies

Healthy male albino Wistar rats were used to carry out these studies following the protocols of animal ethics committee of Punjab University College of Pharmacy, Lahore (ref no. AEC/PUCP/1055 dated 06/12/2016). The study was performed according to ARRIVE (animal research: Reporting in-vivo experiments) guidelines (17). The rats weighing 200 – 250 g housed in adequate cages were used in the study. The abdominal portion of the rats was carefully shaved with hair remover. Then the rats were sacrificed to excise the dorsal side of the skin. The skin was attached to the Franz diffusion cell in such a way that stratum corneum was towards donor compartment whereas dermis layer was facing the receptor. The prepared gel at specific quantity was employed on the skin surface (donor compartment). The volume to be accommodated in the receptor compartment was 11.0 mL with surface area of 2.26 cm^2. The medium used in receptor diffusion compartment was phosphate buffer (pH 7.4). The contents of diffusion cell were stirred by magnetic bar at 37°C ± 1°C maintained by water circulation [18]. Sample (1 mL) was taken at defined time intervals and analyzed at 260 nm by UV spectrophotometer and sink conditions were maintained. To determine the amount of drug as a function of time and the flux rate (J), Eq 4 was used.

$$J = (V/A)(dc/dt) \quad \ldots\ldots\ldots (4)$$

where A is the surface area, V is the receptor volume, and C is FZ concentration in the receptor compartment at time t.

Kinetic modeling

Frequently used kinetic models including first order, Zero order, Hixson-Crowell model, Higuchi, Korsemeyer-Peppas were fit to analyze the release profile of FZ from the prepared nanosponges [19].

Statistical analysis

Data were statistically analyzed using SPSS software (version 21). Release profiles were compared by applying paired sample *t*-test. The results were considered significant at $p < 0.05$ unless otherwise specified.

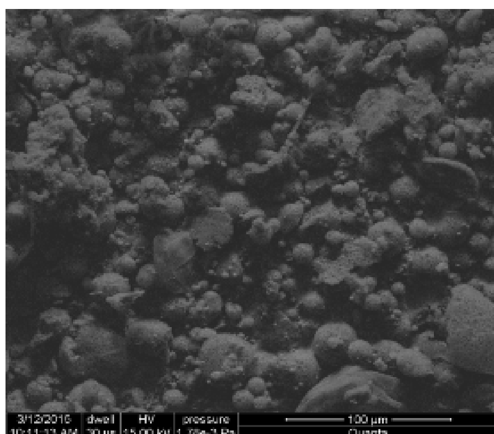
RESULTS

Physicochemical characteristics of nanosponge formulations

The prepared nanosponges analyzed by zeta sizer showed spherical nanosized particles varying from 220 - 725 nm. The average particle diameter was considerably affected by drug to polymer ratio. The relatively smaller particle size is due to lower concentration of polymer providing lesser time for droplet formation. A nanosponge product with size of 358 nm (F3) was chosen for topical gel formulation to avoid gritty feel in the final product [20]. Moreover, the nanosponge of formulation F3 showed promising results in parameters such as percent yield, entrapment efficiency and average particle size (Table 2). SEM analysis of this formulation showed nanosponges with spherical shape, and orange peel like appearance (Figure 1). The entrapment efficiency of the prepared nanosponges was acceptable in the range of 79.32 ± 1.4 to 89.02 ± 0.8% with increasing polymer contents available to entrap the drug. However, percent yield decreased from 92.43 ± 1.2 to 79.54 ± 0.2% with increased polymer quantity causing foaming and aggregation resulting in formation of lesser number of nanoparticles.

Table 2: Physicochemical characteristics of nanosponges

Formulation code	Mean particle size (nm)	Yield (%)	Entrapment efficiency (%)
F1	220.19 ± 4.5	92.43 ± 1.2	79.32 ± 1.4
F2	281.99 ± 12.4	91.03 ± 1.0	83.15 ± 2.3
F3	358.67 ± 7.8	89.14 ± 0.7	82.30 ± 2.6
F4	431.17 ± 11.7	83.09 ± 0.9	84.06 ± 1.5
F5	512.38 ± 9.2	87.21 ± 1.6	86.11 ± 0.6
F6	624.06 ± 10.4	79.54 ± 0.2	89.02 ± 0.8



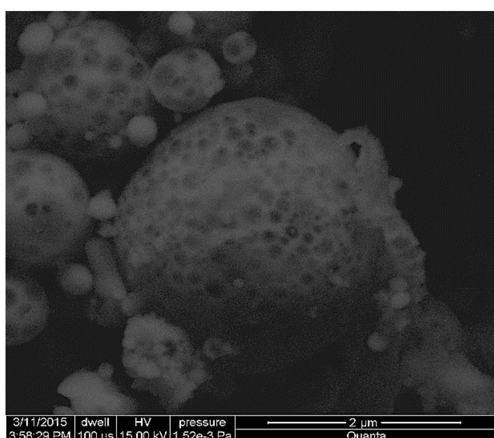


Figure 1: SEM images of F3 nanosponge formulation

In vitro drug release

The release profiles of the formulated FZ nanosponges and pure FZ are illustrated in Figure 3. The overall trend shows decrease in release of FZ from the prepared nanosponges with increasing polymer contents. Theoretically, this slower drug release is ascribed to increased path length for drug diffusion. To explore the pattern of drug release, the results were fit to appropriate kinetic models. The modeling results suggest that the drug is released from nanosponges following Higuchi model.

Statistical investigation of release pattern by paired t-test depicted a non-significant (p = 0.58) difference among nanosponge formulations F-1 and F-2. While release profile of F-3 containing drug to polymer ratio (1:0.7) was statistically significant (p = 0.048) from F-1 indicating considerable effect of polymer on release kinetics. Moreover, the release profiles of all formulations were significantly different from that of pure drug.

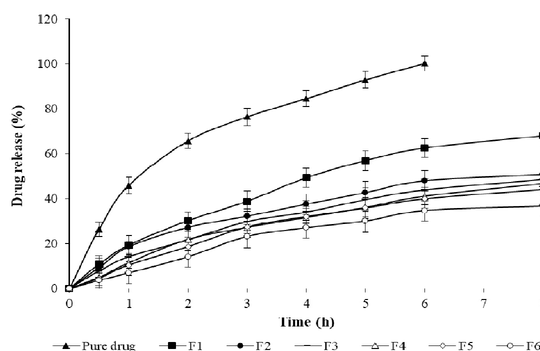


Figure 2: Drug release from formulated nanosponges and pure FZ (mean ± SD)

Fourier transform infrared (FTIR) spectra

Spectra of FZ, physical mixture and nanosponge formulations are shown in Figure 3. The spectrum appears complex due to presence of many groups such as triazolyl and 2,4 difluroubenzyl. Sharp peaks at 965.93 cm^{-1} and at 1135 cm^{-1} are due to triazolyl functional group. Various peaks representing different functional groups at 1074.22 cm^{-1}, 1270.56 cm^{-1} and at 1616 cm^{-1} could be attributed to 2,4 diflurobenzyl group [21].

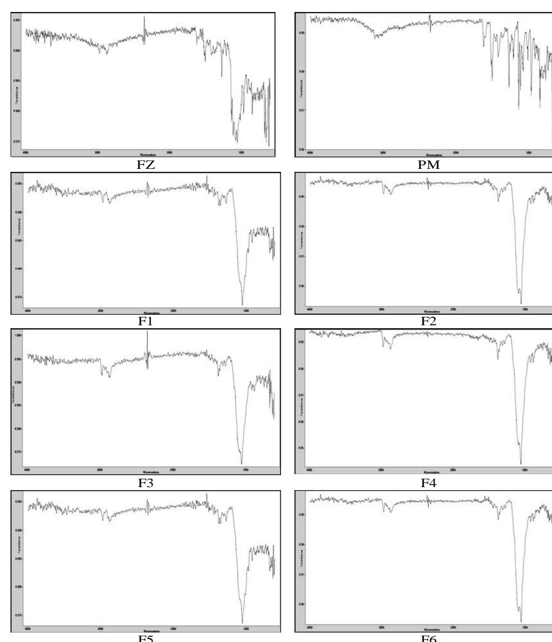


Figure 3: FTIR spectrum of pure drug (FZ), physical mixture (PM), formulations F1- F6

X-ray diffractiograms

Diffraction patterns were obtained for plain drug, physical mixture and the formulated nanosponges (F3). FZ showed distinctive peaks representing the crystalline nature of drug. The intensity of FZ nano-formulations was

considerably lower than that of plain drug. This effect is due to the encapsulation of FZ in amorphous nanosponge core [22].

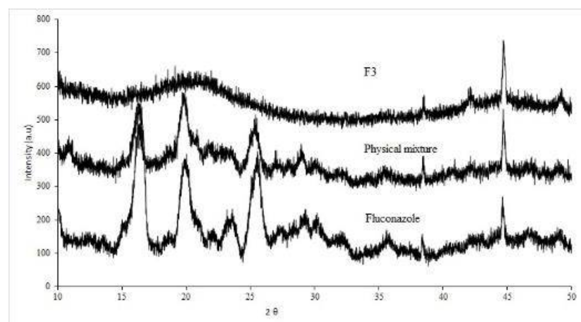


Figure 4: Diffractograms of FZ, physical mixture and nanosponge formulation F3

Hydrogel properties

It is evident from the results that prepared hydrogel had uniform homogeneity and spreadability. The physical appearance of hydrogel was white translucent. The viscosity of product was 21400 cps which was within the desired range. The pH was 5.6 indicating compatibility of product to human skin pH upon application.

Ex vivo permeation and kinetics

The formulation showed a total amount of 4788 μg/cm^2 of the drug diffused across the rat skin at the end of 12 h. The highest value of drug diffusion is attributed to the presence of mixture of N-methyl-2-pyrrolidone and propylene glycol in the gel formulation. Propylene glycol (PG) improved flux of the drug through complex skin membranes by interrupting the lipid configuration within the bilayer of stratum corneum. Furthermore, the maximum permeation of FZ can be ascribed to improved solubility thereby facilitating release of drug from the 3D construct of hydrogel. The *in vitro* permeation data of hydrogel was fit to various models using DD solver software. The permeation profile was best described by Higuchi model which suggest that release of the drug is by diffusion method (Table 3).

Table 3: Release kinetics of nanosponges loaded hydrogel

Kinetic model	R^2	Slope
Zero order	0.939	$K_0 = 0.112$
First order	0.961	$K_1 = 0.004$
Hixon-Crowell	0.811	$K_{HC} = 0.002$
Higuchi	0.974	$K_H = 0.033$
Korsmeyer-Peppas	0.963	n =0.431
Weibull kinetics	0.936	K=0.632

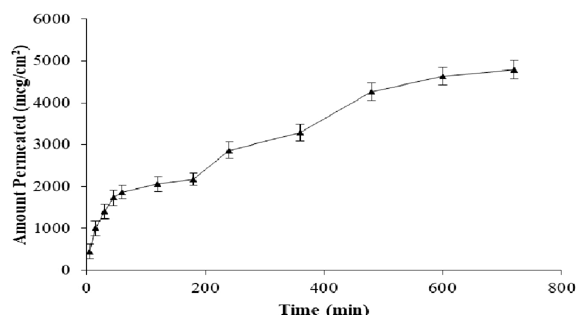


Figure 5: Permeation profile of nanosponge based hydrogel preparation (mean ± SD)

DISCUSSION

Increase in entrapment efficiency by increasing ethylcellulose proportion in nanosponges resulted in an increase in the viscosity of the solution. Higher viscosities resulted into formation of dense network of the polymer preventing the drug from leaving the matrix.

The nature of the drug whether crystalline or amorphous after loading into nanosponges was verified from powder X-Ray diffraction studies. The drug loaded sponges did not show any characteristic peak, which is a proof of crystalline to amorphous transition after nanosizing. The non-appearance of the characteristic crystalline peaks of drug after loading into NS has also been reported [23].

The FTIR spectra of physical mixture of ingredients showed no significant shifts in the positions of wave numbers as compared to that of pure drug. The characteristic peaks of FZ were intact showing no interaction between drug and excipients. Spectra of nanosponge formulations showed broadenings and disappearance of the drug peaks, which is attributed to formation of complexes.

The pure drug dissolved almost completely at the end of 6 h due to solubility in phosphate buffer of pH 5.5. Among all formulations, F1 released higher amount of drug (68.03%) at the end of 8 h which is due to small particle size (220.19 nm) providing large surface area for drug release [24]. F3 nano-formulation released 43.9% drug after 6 h. Similarly, F5 and F6 nanosponge systems further retarded drug release to 39.95 and 34.73% after 6 h, respectively which correspond to reduced diffusion of entrapped drug from polymer matrix.

Ex vivo permeation study is an important parameter for evaluation of permeation characteristics of topical formulation. Our results

showed that the prepared hydrogel had adequate and linear permeation confirmed by higuchi model through skin barrier. This indicated that propylene glycol can effectively be used as permeation enhancer for preparation of nanosponge based hydrogel.

CONCLUSION

Nanosponge-based hydrogel formulations of FZ demonstrate a sustained drug release pattern. *In vitro* drug release showed Higuchi model as the best fit model. The developed topical nanocarrier system possesses potential benefits such as reduction in frequency of application, total dose and systemic side-effects. The better retention ability of nanosponge based hydrogel makes it potentially suitable for the treatment of topical fungal infections which may improve patient compliance.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

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