

## Evaluation of Antifungal Activity of Sustained Release Microsponge Enriched Fluconazole Gel for Penile Candidiasis in Male Rats

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**Abstract:** Objective of the present study is to formulate the controlled release of fluconazole microsponge using emulsion solvent diffusion with different proportions of ethyl cellulose and fluconazole and evaluate its therapeutic efficacy. These microsponge enriched gel formulation were prepared in 2 % and 3% w/w of sodium alginate and characterized as per WHO Good Manufacturing Guideline for its particle size, physical properties, rheological properties, gel strength, spreadability, mucoadhesive force, drug content, diffusion studies, HPLC analysis and scanning electron microscopy. The particle size ranged from 20 to 36  $\mu\text{m}$  microsponge enriched gel was found to be in the range 2500 to 3900 cps. The maximum gel strength of the MEG was found to be (60 sec. ) and maximum mucoadhesion force was found to be (50.56 dynes/cm<sup>2</sup>). The formulations exhibited maximum spreadability (22.14 gm.cm/sec). The optimized formulations were able to release the drug up to 9 h. The pharmacological evaluation showed better therapeutic efficacy in rodents when compared with that of standard marketed fluconazole gel.

**Keywords:** Microsponge enriched gel, *In vivo studies*, Fluconazole, HPLC, Scanning electron microscope.

### Introduction

Candidiasis is the fungal infection that can manipulate the private parts. Genital infection is extremely more frequent in women than men, but when it does occur in males, thrush affects the head of the penis and the foreskin<sup>1</sup>. Efficacy of the formulation based on its pharmacological profile and type of formulation. The penile thrush is most common in men who are taking immunosuppressant, corticosteroids, antibiotics and suffering with metabolic syndrome such as diabetes and living with poor hygiene condition. A man is also recommended to avoid unprotected sex with a candidiasis infected female partner until the woman cleared her infection after the treatment. For the treatment of penile thrush creams and ointments are most commonly used and it's has limitation of duration of action. In addition, the application of topical drug delivery has numerous problems, such as, ointments that are often visually unpleasant, greasiness, stickiness, etc., that often ends in lack of patient compliance. These carriers require high concentrations of drug for effective treatment because of their low efficiency of delivery system, resulting in irritation and allergic reactions in noteworthy users. Additional disadvantages of topical formulations are unrestricted evaporation of the active ingredient, unpleasant odor, and the possible incompatibility of the drugs with the vehicles. Conventional formulations of topical drugs are designed to work on the epidermis of the skin. Predictably, such formulations release their active ingredients upon application, generating a highly concentrated layer of active ingredient that is promptly absorbed. Subsequently the need exists for a system to maximize the amount of time that an active ingredient is present either on the skin surface or within the epidermis, although diminishing its transdermal penetration into the body. Microsponges are spongy microspheres having countless of interconnected holes of particle size varying from 5-150  $\mu\text{m}$ . Microsponge drug delivery system is a distinctive technology which offers controlled release of active ingredients<sup>2,3</sup>. It offers abundant benefits over other technologies like reduced side effects, improved

stability, improved elegance and enhanced formulation flexibility<sup>4,5</sup>. Spongy microspheres are used commonly for topical administration. They can be incorporated into conventional dosage forms such as lotions, creams, powder, ointment, gels and tablets and assign a comprehensive package of advantages and thus affords formulation flexibility<sup>6-8</sup>.

## Experimental

Fluconazole was a gift sample from SM Pharmaceuticals Sdn.Bhd. Sungai Petani (Malaysia). Polyvinyl alcohol, carbomer 934 and Sodium alginate were procured from Esses, (UK) and all other chemicals were of analytical grade.

### Preparation of Microsponges:

Fluconazole microsponges were prepared by emulsion solvent diffusion method<sup>9</sup>. The internal phase of the emulsion was prepared by dissolving fluconazole and ethocel in 10 ml of dichloromethane (DCM), an effectual solvent. A 100 ml of an aqueous solution containing 1% PVA and heated at 60°C with magnetic stirrer (1000 RPM) and then cooled down to room temperature and this solution was used as external phase (Table 1). The internal phase was slowly introduced into external phase drop wise and stirred for 3 hrs at 25°C.

**Table 1: Composition of microspunge**

Formulation code	Inner phase			Outer phase	
	Fluconazole (mg)	Ethocel (mg)	DCM (mL)	PVA ( mg)	D. water (mL)
D1	100	100	10	1000	100
D2	100	200	10	1000	100
D3	100	300	10	1000	100
D4	100	100	10	1000	100
D5	200	100	10	1000	100
D6	300	100	10	1000	100
D7	100	100	10	1000	100
D8	100	100	10	2000	100
D9	100	100	10	3000	100

### Preparation of microsponges enriched gel (MEG):

The microsponges enticed gel of fluconazole was prepared by method described by Schmolka et al. (1972)<sup>10,11</sup>. A 1%, 1.5%, 2% and 3% w/v sodium alginate was taken and dissolved in deionized water, soaked for overnight. The resultant was mixed uniformly with glass rod for 20 min. A 0.13g of microsponges (equivalent to 1% of fluconazole according to assay value) was dispersed slowly in 1g of gel with the help of glass rod (Table 2).

**Table 2: Composition of Gel**

Ingredients (% w/v)	R1	R2	R3	R4
Sodium alginate	1	1.5	2	3
Carbomer934	--	--	0.1	0.2
Methyl paraben	0.001	0.001	0.001	0.001
Deionized water	Upto100 mL	Upto100 mL	Upto100 mL	Upto100 mL

### Viscosity Studies

The rheological studies were conducted by using brookfield programmable DVII+ Model pro II type (USA). The viscosity of MEG was determined at different angular velocities (0.3, 0.6,10, 30, 60 to 100 RPM) and means of two readings were used to estimate the viscosity<sup>12</sup>.

### Particle size studies

Particle size analyses were executed by optical microscopy (DN-117M, USA). The results are the average of three studies. The values (*d*50) were expressed for all formulations as mean size range<sup>12</sup>.

### Scanning electron microscopy

The morphology and size of microsponges were observed by (Phenoworld) scanning electron microscopy. Prepared microsponges were coated with gold and studied under vacuum at room temperature<sup>12</sup>.

### Determination of loading efficiency

The drug content in the microsponges was determined by HPLC method. A sample of drug containing microsponges (10 mg) was dissolved in 100 ml of suitable solvent. HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50 $\mu$ L loop volume. The LC solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile ammonium acetate buffer (pH 3.5 adjusted with orthophosphoric acid (50:50, v/v)), and detection was made at 254.0 nm. The mobile phase was prepared daily, filtered through a 0.45 $\mu$ m membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm  $\times$  4.6mm i.d., 5 $\mu$ ) was used for the separation. The drug content was calculated from the calibration curve and expressed as loading efficiency<sup>13</sup>.

$$\text{Loading Efficacy} = \frac{\text{Actual drug content in microsp sponge}}{\text{Theoretical drug content}} \times 100$$

### Determination of production yield

The production yield of the microsp sponge was determined by calculating accurately the initial weight of the raw materials and the last weight of the microsp sponge obtained.

$$\text{Production yield} = \frac{\text{Practical mass of microsponges}}{\text{Theoretical mass (polymer + drug)}}$$

### Measurement of density and porosity

For density measurement, the solvent displacement method was used<sup>14</sup>. Dried microsp sponge was used for density measurement, which actually showed the apparent density of the microsp sponge. A piece of microsp sponge was taken and weighed in order to determine the mass of the piece. A piece of the polymer was immersed in a predetermined volume of hexane in a graduated cylinder, and the increase in the hexane volume was measured as the volume of the polymer. The density was calculated from following equation:

$$\text{Density} = \frac{M_{\text{Microsp sponge}}}{V_{\text{Microsp sponge}}}$$

Where,  $V_{\text{Microsp sponge}}$  is the volume of solvent displaced by microsp sponge and  $M_{\text{Microsp sponge}}$  is the mass of the microsp sponge.

For porosity measurement, the dried microsp sponge was immersed in hexane overnight and weighed after the excess hexane on the surface was blotted. The porosity was calculated from following equation:

$$\text{Porosity} = V_P/V_T$$

### Diffusion studies

The *in vitro* release of MEG formulations were studied using cellophane membrane using modified apparatus. The dissolution medium used was phosphate buffer, freshly prepared (pH 7.4). Cellophane membrane previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends). One gram of formulation (equivalent to 1% w/w of fluconazole) was accurately placed into this assembly. The cylinder was attached to stand and suspended in 200 ml of

dissolution medium maintained at  $37 \pm 1^\circ\text{C}$ , the membrane just touching the receptor medium surface. The dissolution medium was stirred at 100 RPM speed using teflon coated magnetic bead. Aliquots, each of 5 ml volume were withdrawn periodically at predetermined time interval of 30, 60, 120, 180, 240, 300, 360, 420, 480 and 540 minutes and replaced by an equal volume of the receptor medium. The samples were appropriately diluted and measured by using HPLC method.

### Determination of mucoadhesive force

The mucoadhesive force of all the optimized batches was determined as follows, a section of the chicken mucosa fixed with mucosal side out onto each glass vial using rubber band. The vial with chicken mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. Microsponges enriched gel was added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then weight was kept rising in the pan until vials get detached. Mucoadhesive force was the minimum weight required to detach two vials. The chicken mucosa was changed for each measurement<sup>15</sup>.

Detachment stress ( $\text{dynes/cm}^2$ ) =  $m \text{ g/A}$

Where  $m$  is the weight added to the balance in grams;  $g$  is the acceleration due to gravity taken as  $980 \text{ cm/s}^2$ ; and  $A$  is the area of tissue exposed.

### Measurement of Gel Strength

A sample of 50 gm of microsponges enriched gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at  $37^\circ\text{C}$ . The apparatus for measuring gel strength (weighing 27 gm) was allowed to penetrate in gel. The gel strength, which means the viscosity of the gels at physiological stimuli was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel<sup>15</sup>.

### Spreadability

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability<sup>16</sup>.

$S = ML/T$

Where,

$M =$  weight tide to upper slide (g)

$L =$  length moved on the glass slide (cm)

$T =$  time taken (sec)

### In vivo evaluation of therapeutic efficacy

#### Animals:

Adult Wistar rats ( $280 \pm 10 \text{ g}$ ) of either gender were obtained from SCS College of pharmacy, Harapanahalli. The animals were housed in large, spacious polyacrylic cages at an ambient room temperature with 12-h light/12-h dark cycle. Rats had free access to water and rodent pellets diet. The study was approved by the Institute Animal Ethics Committee of the SCS College of pharmacy, Harapanahalli and all the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Ministry of Environment and Forests, Government of India.

#### Acute toxicity testing:

The female rats were used for the acute toxicity testing. Hair present in the dorsal surface of the animal ( $2 \times 2 \text{ cm}$ ) was removed by applying hair remover and cleaned with alcohol. The screening area was marked ( $1 \times 1 \text{ cm}$ ) and 0.5 g of a microsphere enriched gel was applied to the surface of an animal's skin. During the observation period (14 days), signs such as erythema and edema were assessed<sup>17</sup>.

#### Evaluation of therapeutic efficacy:

The male rats were used for the experiment. The rats were divided into the four groups *viz.*, normal control (group I), *Candida glabrata* control (group II), standard treatment group (group III) and microsponges

enriched gel treatment group (group IV). Group II to IV animals were changed with intravenous methylprednisolone (5mg/kg) for 3 days for induction and maintenance of cell-mediated immunosuppression (Organisms from stock isolates were stored in nutrient agar at 27°C, streaked onto nutrient broth, and incubated at 37°C for 24 h and included culture was used for further experiment).

*Candida glabrata* culture was diluted with PBS and swabbed in smooth muscle of rat pennies and allowed to grow for 3 days until the growth of *Candida* was observed on ischiocavernosus smooth muscle. The colony growth was confirmed by counting colony-forming-unit. The animals which as cfu value of more than 3 cfu/ml were included in the study. The animals were treated for week period and visually observed its physical changes. The swab culture was collected on initial day, 4<sup>th</sup> and 7<sup>th</sup> day of the experiment for microscopical evaluation. End of the experiment the animals were sacrificed and ischiocavernosus smooth muscle was collected from all the experimental animals and preserved in 10% formalin.

**Microscopical evaluation:**

The colony was collected in sterile cotton swab and transferred into 0.5 ml sterile phosphate buffer saline (PBS). The mixture was diluted 10 fold and inoculated in nutrient agar media, incubated for 48 h at 37°C. The yeast count was expressed as log 10 of cfu/ml of PBS.

**Histopathologic analysis:**

The liver and pancreas were dehydrated with alcohol for 12 h each and cleaned with xylene for 15-20 min. The tissue blocks were prepared and the blocks were cut using microtome to get sections of thickness 5 µm. The sections were taken on a microscopic slide on which egg albumin (sticky substance) was applied and allowed for drying. Finally, the sections were stained with eosin (acidic stain) and hemotoxylin (basic stain)<sup>18</sup>.

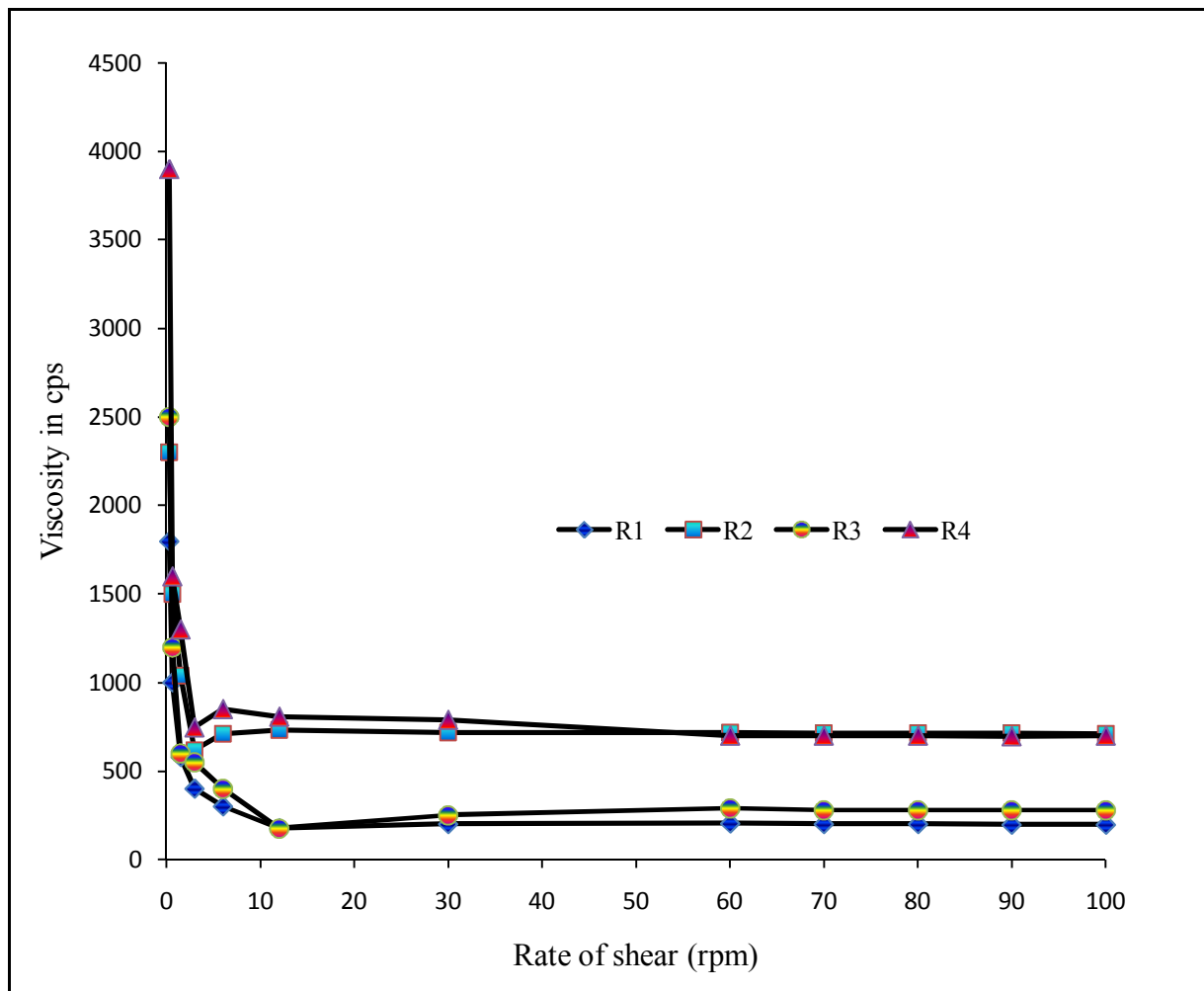


Figure 1: Showing the viscosity of formulations

## Statistical analysis

All the data were expressed as mean  $\pm$  SEM. Statistical significance between the groups were tested using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test post-hoc test. A P less than 0.5 were considered significant.

## Results and Discussion

The rheological properties of gels of various formulations were determined at different shear rates. The viscosity of prepared gel decreased, when the rate of shear increased as shown in fig 1. 3.0% sodium alginate gel having higher viscosity than 1.0% sodium alginate gel. It is found that the 3% sodium alginate gel is the desired candidate with optimum gel viscosity for topical use as compared to 1.0 % sodium alginate gel.

Scanning electron microscopy of the fluconazole microsponge enriched gel and microsponge forms are shown in Fig. 2 and 3. It is perfect from the character that microsponges in gel have predominantly spherical shape and contain orifices (Fig. 2) in comparison with the original fluconazole microsponge (Fig. 3). These orifices caused by the diffusion of the solvent (DCM) from the surface of the microsponge. The concentration of ethocel has a key role to work in the preparation of various size of microsponge. Fluconazole with various concentration of ethocel can be seen from (Fig. 3, 4, 5) that the drug/polymer ratio has considerable impact on the morphology of microsponge. It is clearly shows that the ratio of drug and polymer changed the size of microparticles.

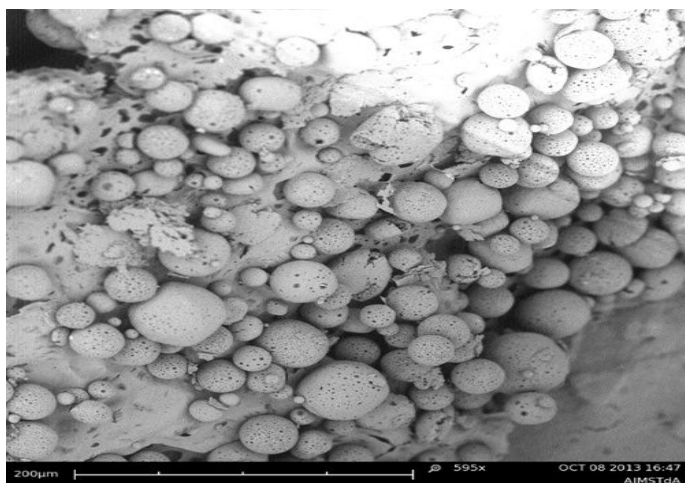


Figure 2. Showing the SEM studies of microsponges enriched gel

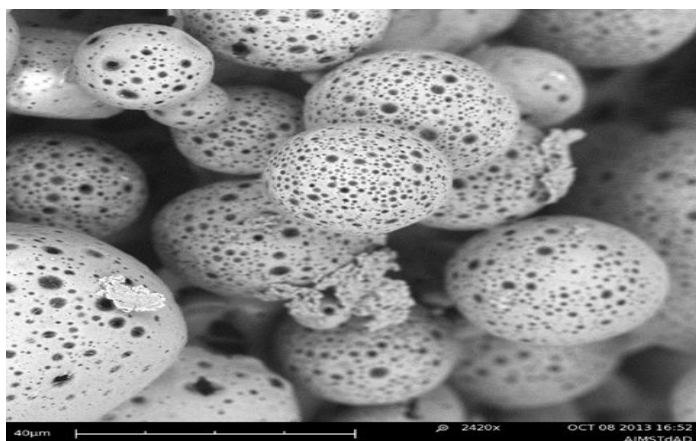


Figure 3. Showing the SEM studies of microsponges with 300 mg of Ethocel



**Figure 4. Showing the SEM studies of of microsponges with 200mg of Ethocel**



**Figure 5. Showing the SEM studies of of microsponges with 100 mg of Ethocel**

Porosity of MEG was shown in Table 3. Ethocel concentration increased, while the porosity of MEG decreased in the presence of PVA. Ethocel prevented the bubbles from escaping from the solution mixture as well as decreased the pore size of microsp sponge, due to the accumulation of Ethocel at the periphery of the pores.

**Table 3: Effect of drug to polymer ratio and surfactant ratio on production yield, drug loading capacity particle size and porosity of fluconazole microsp sponge**

Code	Drug content (%) $\pm$ S.D	Production yield (%) $\pm$ S.D	Loading efficiency (%) $\pm$ S.D	Mean particle size ( $\mu$ m) $\pm$ S.D	% porosity*
D1	97.9 $\pm$ 1.34	72.56 $\pm$ 0.56	80.55 $\pm$ 0.20	36.63 $\pm$ 3.12	0.31 $\pm$ 0.14
D2	98.2 $\pm$ 1.02	73.42 $\pm$ 0.24	82.73 $\pm$ 0.45	24.27 $\pm$ 5.17	0.29 $\pm$ 0.23
D3	97.8 $\pm$ 1.05	68.77 $\pm$ 0.37	81.01 $\pm$ 0.43	20.82 $\pm$ 4.28	0.27 $\pm$ 0.01
D4	97.4 $\pm$ 1.21	72.11 $\pm$ 0.19	80.16 $\pm$ 0.16	36.31 $\pm$ 3.73	0.32 $\pm$ 0.13
D5	98.1 $\pm$ 1.13	74.15 $\pm$ 0.34	81.05 $\pm$ 0.08	25.36 $\pm$ 4.19	0.31 $\pm$ 0.11
D6	99.2 $\pm$ 1.09	68.73 $\pm$ 0.46	82.73 $\pm$ 0.45	24.41 $\pm$ 3.45	0.28 $\pm$ 0.19
D7	98.4 $\pm$ 1.11	72.46 $\pm$ 0.21	80.12 $\pm$ 0.23	36.31 $\pm$ 4.14	0.31 $\pm$ 0.12
D8	97.8 $\pm$ 1.23	74.49 $\pm$ 0.16	83.52 $\pm$ 0.52	34.17 $\pm$ 5.73	0.32 $\pm$ 0.32
D9	98.6 $\pm$ 1.09	73.17 $\pm$ 0.43	81.76 $\pm$ 0.35	35.21 $\pm$ 4.15	0.33 $\pm$ 0.17

\*mean  $\pm$  SD, n=3

The values of spreadability indicate that the gel is easily spreadable by small amount of shear. The spreadability of formulation R3 and R4 (21.89, 22.14 gm.cm/sec) was found to be less as compared to other formulations. This indicates spreadability of MEG having more concentration of SG was good as compared with less concentration of SG.

The gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out from the site of administration. The gel strength of formulation R3 and R4 (55, 60 sec.) exhibited good gel strength among R1 and R2 code formulation which may due to increase in concentration of sodium alginate and carbomer.

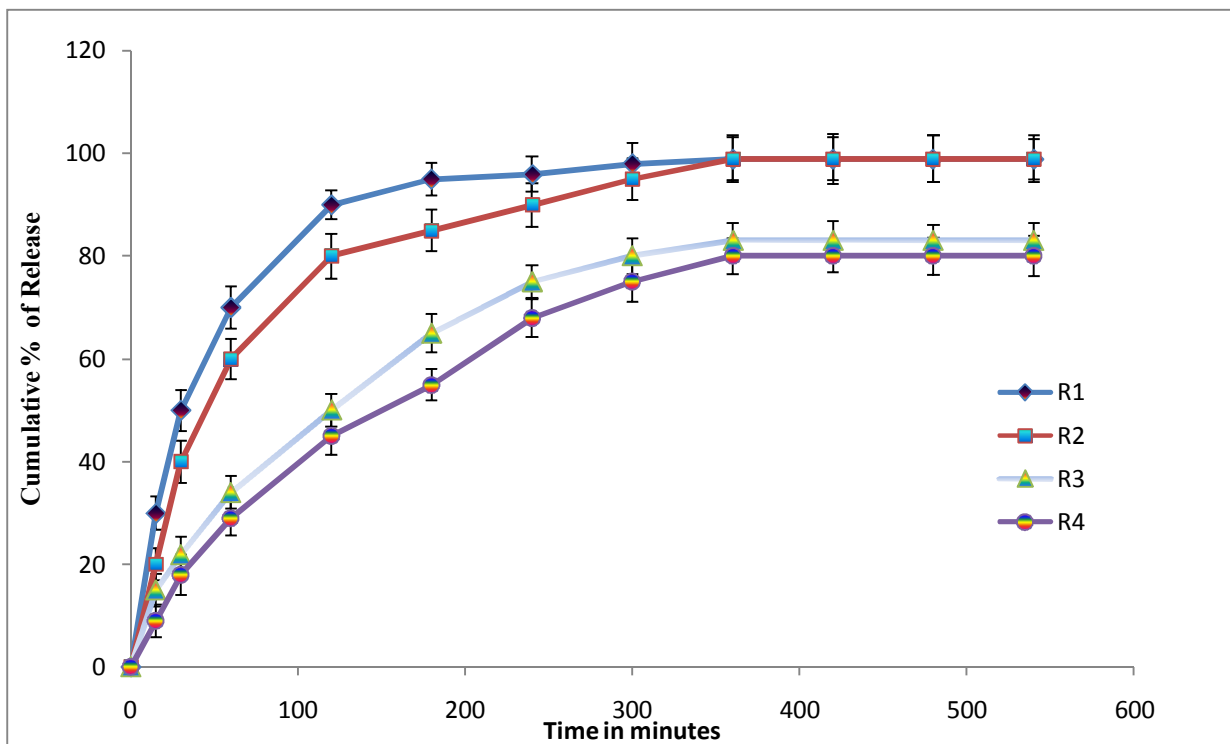
The mucoadhesive force is an important physicochemical parameter of topical application in buccal cavity. The mucoadhesive force was significantly increased from 23.14 dynes/cm<sup>2</sup> to 50.56 dynes/cm<sup>2</sup> for the formula R1 and R4 which consists of 1% of sodium alginate and 3% of sodium alginate: 0.2% of carbomer, as the concentration of mucoadhesive polymer increased as shown in table 4. This also proved that carbomer has better mucoadhesive property than sodium alginate.

**Table 4: Characteristics of various microsponge enriched gel**

Formula code	Mucoadhesive* force(dynes/cm <sup>2</sup> )	Spreadability* (gm.cm/sec.)	Gel Strength* seconds
R1	23.14±1.02	24.13±0.56	45
R2	25.22±1.52	25.92±0.91	50
R3	48.31±1.48	21.89±0.75	55
R4	50.56±1.86	22.14±0.81	60

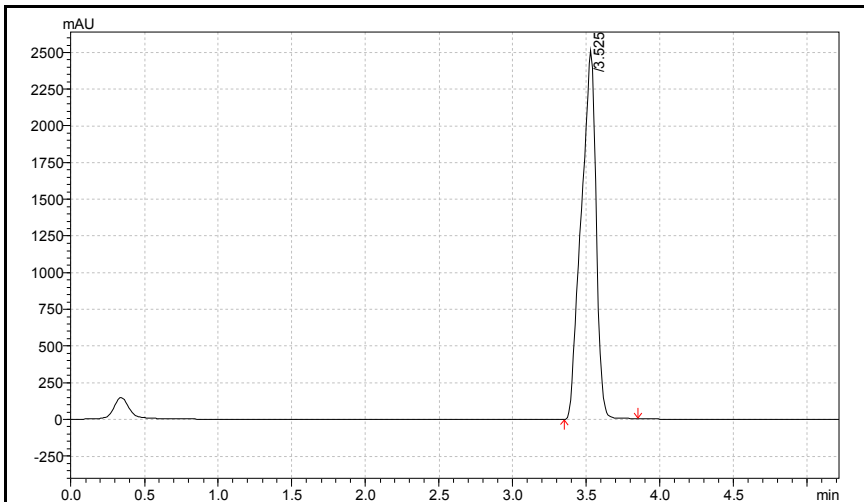
\*mean ± SD, n=3

The *in vitro* diffusion profile of fluconazole from formulations R1, R2, R3 and R4 were conducted in diffusion medium pH 7.4. The formulations R3 and R4 showed 83.21% and 80.31% respectively at 9 hours (Fig. 6). Typical chromatogram of fluconazole sample was shown in Fig.7.



**Figure 6. Showing the Diffusion of optimized formulation**





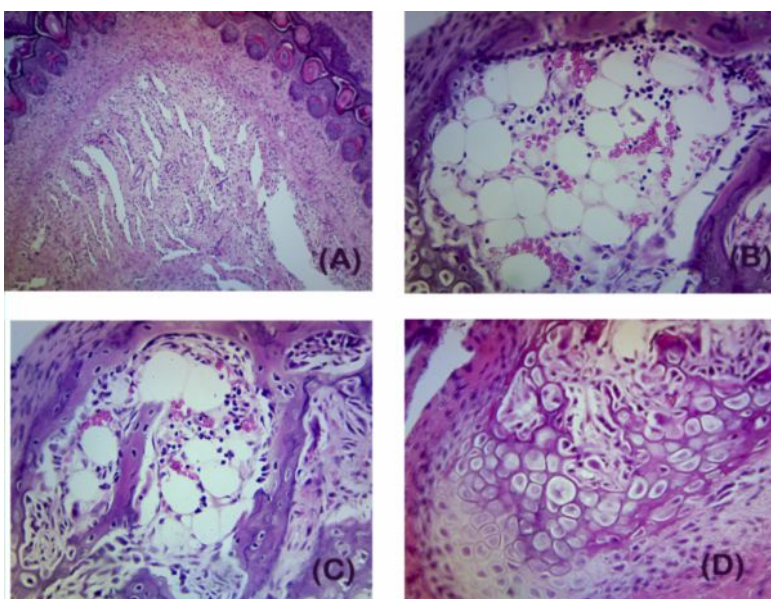
**Figure 7: Typical chromatogram of drug sample**

The therapeutic efficacy of microsponge enriched gel was compared with *Candida glabrata* control by quantitative microbiological analysis and histopathological evaluations (Table –5; Figure 8). MEG and standard marketed formulation treated animals showed significant reduction of CFU count on 4<sup>th</sup> day of the treatment onwards. The efficacy of the MFG is comparable with standard marketed formulation.

**Table 5: Quantitative microbiological analysis of the *Candida glabrata* in rat penal smooth muscle surface.**

Mean	Initial day	4th day	7th day
Control	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02
Negative control	3.33 ± 0.05	3.49 ± 0.07	3.38 ± 0.11
Standard	3.35 ± 0.04	2.59 ± 0.11**	0.50 ± 0.16***
Treated with MEG	3.34 ± 0.09	2.30 ± 0.05***	0.15 ± 0.04***

P < 0.05, \*\* P < 0.01 and \*\*\*P < 0.001 compare to control group (One-way ANOVA followed by tukey's multiple comparison test)



**Figure 8. Histological Analysis. Rat penal smooth muscle section from (A) control showed normal articheture and (B) showed *Candia* microorganism infection in smooth muscle surface (C) and (D) showed reduction in growth of *Candia* due to antifungal effect of standard and MEG, H&E, 400X.**

## Conclusion

Fluconazole, a triazole antifungal drug used in the treatment and prevention of penile thrush, was successfully formulated as micro sponge enriched gel (1% w/w) using sodium alginate as a gelling agent in combination with carbomer as a mucoadhesive agent. The micro sponge enriched gel afforded sustained drug release over a 9-h period and the formulation was therapeutically efficacious. The *in vivo* animal studies for penile thrush which was conducted in adult Wistar rats revealed that the fungal burden at the end of 7<sup>th</sup> day was found to be ( $\log_{10}$  0.15 CFU/ml) for the animals treated with the MEG in comparison with standard. The MEG formulation is a viable alternative to conventional dosage forms such as lotions, creams, powder, ointment by virtue of its ability to enhance bioavailability through its longer residence time and ability to sustain drug release. The MEG comfort for administration and decreased frequency of administration succeeding in better patient acceptance.

## References

1. CDC. Genital candidiasis: Statistics. Centers for Disease Control and Prevention. Published online, accessed 7th October 2013.
2. Jain NK. Advances in controlled and novel drug delivery. New Delhi: CBS Publishers and Distributors; 2003: 89-91.
3. Kydonius AF, Controlled release technologies: Methods, Theory and Applications, Boca Raton: CRC; 1980 Press,; 21-49.
4. Nacht S, Katz M. The micro sponge; a programmable delivery system. In Osborne, DW, Aman AH, Topical drug delivery formulations. New York: Marcel Dekker; 1990: 299-325.
5. Embil K, Nacht S. The micro sponge delivery system (MDS): a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for release of actives. J. Microencapsul 1994; 13:575-588.
6. Kim W, Hwang S, Park J, Park H. Preparation and characterization of drug loaded polymethacrylate microspheres by an emulsion solvent evaporation method. J. Microencapsul 200; 6: 811-822.
7. Kawashima Y, Niwa T, Hand T, Takeuchi H, Iwamoto T. Control of prolonged drug release and compress ion properties of ibuprofen microspheres with acrylic polymer by changing their intra - particle porosity. Chem. Pharm Bull 1992; 40: 196-201.
8. D'souza JI, More HN. Topical Anti-Inflammatory Gels of Fluocinolone Acetonide Entrapped in Eudragit Based Microsponge Delivery System. Res J Pharm Tech 2008;4:502-506.
9. Yazici E, Kas HS, Hincal AA. Microsponges, Farmasotik Bilimler Dergisi (Turkey) 1994;19:121-8 .
10. Schmolka IR. Preparation and properties of Pluronic PF -127 gels for the treatment of bruns. J Biomed Mater Res 1972;6: 571 – 582.
11. Chaudhari P, Ajab A, Malpure P, Kolsure P, Sanap D. Development and *in-vitro* evaluation of thermo reversible nasal gel formulations of Rizatriptan benzoate, Indian J Pharm Edu Res 2009;43: 55-62.
12. Jaya raja Kumar, Muralidharan S, Ramasamy S. Microsponges enriched gel (MEGs): A novel strategy for ophthalmic drug delivery system containing ketotifen. J. Pharm. Sci. & Res 2013; 5: 97-102.
13. D'souza JI, More HN. Topical anti-inflammatory gels of fluocinolone acetonide entrapped in eudragit based microsponge delivery system. Res J Pharm Tech 2008; 1:502-506
14. Tang CL, Yin CH, Pei YY, Zhang M., Wu LF. New superporous hydrogels composites based on aqueous carbopols solution (SPHCs): synthesis, characterization and *in vitro* bioadhesive force studies. Eur Polymer J 2005; 41:557-62.
15. Jaya raja Kumar, Jayachandran E, Srinivas GM, Giridhar B, Nair R, Jayakandan M. Formulation of thermoresponsive and buccal adhesive in situ gel for treatment of oral thrush containing itraconazole. J. Pharm. Sci. & Res 2010; 2: 116-122.
16. Evelyn D, Wooi CC, Jaya raja Kumar, Muralidharan S, Dhanaraj SA. Development and evaluation of microemulsion based gel (MBGs) containing econazole nitrate for nail fungal infection. J Pharm Res 2012;5: 2385-2390.
17. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother 2011; 2:74-9.
18. Petchi RR, Vijaya C, Parasuraman S. Antidiabetic activity of polyherbal formulation in streptozotocin - Nicotinamide induced diabetic Wistar rats. J Tradit Complement Med 2014; 4:108-17.