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Formulation and Characterization of Fluconazole as Topical Gel by Porous Microparticle Based Drug Delivery Systems

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ABSTRACT

Controlled topical release drug delivery system for Fluconazole is potentially useful in improving drug deposition in the skin and reducing the incidence of adverse side effects. The purpose of the present experiment was to produce a topical gel system for the delivery of . Drug loaded microsponges (1–10) were formulated by an emulsion solvent diffusion method. Optimization of the microsponges was selected by drug loading efficiency. The optimized microsponges was formulated as topical gel and evaluated. The *in vitro* drug release, ex vivo drug deposition, primary skin irritancy study and *in vivo* antibacterial activity of loaded formulations were studied. The spherical and porous microparticles were obtained. Moreover, the optimized microsponge possess particle size, entrapment efficiency and production yield and of 84.49 µm, 72.21% and 39.40% respectively. Microsponge loaded gels indicated controlled release, no irritancy to rat skin and antifungal activity. An *in vivo* skin deposition study proved three fold higher retention in the stratum corneum layer as compared with plain gel. Microsponges-based gel formulations showed prolonged efficacy in a rat surgical wound model infected with *Candida albicans*. These results suggest that was stable in topical formulations and amplifying retention in the skin, indicating better potential of the delivery system for treatment of primary and secondary skin infections.

Keywords: Microsponges, Particle size, Entrapment efficiency, Primary skin irritancy, *in vivo* skin deposition *Candida albicans.*

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INTRODUCTION

Fungal infections have become an issue of great concern around the world; it was estimated that over 40 million people do in fact suffer fungal infections both in developed and in growing countries[1]. The occurrence of fungal infections is increasing at an alarming rate, presenting a vast task to health care experts[2]. The delivery of drugs through the most commonly used conventional preparations viz. Creams, gels, lotions, emulsion, etc. limits the effectiveness of actives due to barrier properties, i.e. epidermis of the skin which hinder the drug deposition. Consequently the selection of proper carrier's extremely important by considering the view in the mind that they should increase drug deposition through topical formulations[4]. Topical agents are mainstays in both cosmetics and the treatment of dermatological disorders. Traditional dermatological products provide the active ingredients in relatively excessive concentrations, but for a short duration. This may cause a cycle of short-term over medication followed by long-term under medication[5]. Rashes or different extremely serious side effects can come when a more active ingredient penetrates into the skin[6]. Various controlled drug-delivery systems, such as microcapsules, microspheres, animation, liposomes and niosomes, have been investigated in order to maximize the duration of active ingredients being present either on the epidermis, or within the skin layers, while minimizing their transdermal penetration into the body[7]. However, the release rate of active drugs from microcapsules cannot be controlled as soon as the capsule wall is ruptured. Similarly, liposomes are relatively expensive, tough to formulate and feature a low maintaining capacity of the active drug[8]. One of the novel techniques used to control the release of active ingredients from topical formulations is polymeric microsponge based drug delivery[9]. Microsponges are polymeric, porous and tiny, sponge-like round particles. This system gives maximum efficacy, reduced irritancy, extended product stability, improved formulation flexibility, extended elegance and better aesthetic properties. This delivery system contains a big wide variety of interconnecting voids within a noncollapsible structure that imparts a large porous surface. It can absorb or entrap a wide range of pharmaceutical active ingredients and can be formulated into gels, creams, liquids and powders[10]. Being relatively large in size (5–300 µm), upon topical application, microsponges do not pass through SC and stays on the skin surface. The porous nature of microsponges favours controlled release of the encapsulated drug, leading to minimal accumulation of the drug in the epidermis and dermis[11] is a synthetic triazole antifungal drug for the remedy of superficial and systemic fungal infections has proven activity against aspergillosis, Candida infections, Scedosporium apiospermum and Fusarium spp., Oral as well as parenteral administration of often produces injection site reactions, allergy

reactions; kidney, liver, and pancreas damages. Moreover, oral administration of is reported to interact with a number of medicines, which include rifampicin, rifabutin, carbamazepine, quinidine and ergot alkaloids[12]. Thus, for effective topical antifungal therapy, the cutaneous availability of needs to be modulated; a need that can be met by a suitable drug delivery system. To date, the approach of controlled topical delivery of has not been experimented.

Thus, the intention of the present research was to design microsponges as novel carriers for topical delivery of fluconazole. This research consisted of preparation, characterization and evaluation of microsponges and incorporation of optimized microsponges in semisolid vehicle base to acquire cosmetically acceptable products.

MATERIALS AND METHOD

Materials

Fluconazole was purchased from RMS Research Laboratories, Hyderabad, India. Ethyl cellulose 50 cps was obtained as gift sample from Hyderabad, India. Polyvinyl Alcohol (cold grade) and Carbopol 934 was procured form Himedia Pvt. Ltd Mumbai, India and Loba Chem Pvt Ltd Mumbai India. Triethanolamine, Disodium hydrogen phosphate, Potassium dihydrogen phosphate and solvents like Dichloromethane, Methanol, were obtained from commercial suppliers and were of the highest reagent grade available.

Formulation of Microsponges

Formulation was optimized based on production yield, loading efficiency and particle size. The preparation procedure was as follows. The microsponges were prepared by quasi emulsion solvent diffusion technique with internal and external phases[13]. To prepare the internal phase, different concentrations of and polymer ethyl cellulose were dissolved in ethyl acetate 20 ml. In this procedure, ethyl acetate was an efficient solvent for dissolving both the drug and the polymer. The external phase, which contained the emulsifying agent polyvinyl alcohol (PVA), dissolved in 100 ml of distilled water, was placed in the vessel, and stirred at 1000 rpm, and then the internal phase was gradually added into the stirring external phase. The mixture was then stirred at 1000 rpm for 8 h at room temperature to remove the ethyl acetate from the reaction flask. After that, the formed microsponges were filtered through filter paper with a pore size of 0.45 µm (Millipore, Maidstone, Kent, UK), washed with distilled water, and dried at room temperature.

Table 1:Microsponge preparation with quasi-emulsion solvent diffusion method

Formulation Code	Drug (mg)	Polymer (mg)	EthylAcetate (ml)	Triethyl citrate (ml)	PVA (%w/v)	Water (ml)
1	100	50	10	20	0.75	90
2	100	100	10	20	0.75	90
3	100	150	10	20	0.75	90
4	100	200	10	20	0.75	90
5	100	250	10	20	0.75	90
6	100	300	10	20	0.75	90
7	100	150	10	20	0.45	90
8	100	150	10	20	0.6	90
9	100	150	10	20	0.9	90
10	100	150	10	20	1	90

Drug - Excipient compatibility study

Differential scanning calorimetry (DSC)

DSC of pure drug and physical mixture of the microsponge was recorded using the DSC1, Mettler Toledo system, to identify any interaction between the components of formulation.

FTIR spectroscopy (FTIR)

The FTIR spectra of the drug VZ and physical mixture of the microsponges were obtained in KBr pellets using a Perkin-Elmer FT-IR spectrometer and at mid IR region (wavelength 25 m to 2.5 m, wavenumber from 400cm^{-1} to 4000cm^{-1}), in order to identify the possibility of any interaction between the drug and excipients.

Characterization and evaluation of fluconazole microsponges formulation[14]

Determination of the production yield:

The production yield of the microsponge was determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponges obtained

$$\text{Production yield} = \frac{\text{Practical Mass of microsponges}}{\text{Theoretical Mass (Drug + Polymer)}} \times 100$$

Determination of loading efficiency

A sample of FL microsponges (10 mg) was dissolved in 100 ml of phosphate buffer, freshly prepared (pH 7.4). The solutions were subsequently diluted suitably with the phosphate buffer pH 7.4 and spectrophotometric absorbance was taken at the maximum wave length of FL. The drug content was calculated from the calibration curve and expressed as the loading efficiency

$$\text{Drug Loading} = \frac{\text{Mass of drug present in microsponges}}{\text{Theoretical Mass (Drug + Polymer)}} \times 100$$

Particle size distribution analysis

The particle size distribution of the optimized microsponges formulations of fluconazole was determined by a laser light-scattering technique (Mastersizer 2000; Malvern Instruments Ltd). Before measurement, samples were dispersed in distilled water to ensure that the light scattering signal, as indicated by particles count per second, was within instrument's sensitivity range. The particle size range was set to 0.02 to 2000 µm, and the particle refractive index was set to 1.520. Particle size distribution by volume of fluconazole microsponge was calculated internally. The values (d50) were expressed as mean size range.

Scanning electron microscope (SEM) study

For morphology and surface topography, the prepared microsponges can be coated with gold–palladium under an argon atmosphere at room temperature and then the surface morphology of the microsponges can be studied by scanning electron microscope (JEOL Instrument, JSM-6360, Japan).

Preparation of fluconazole microsponge carbopol gel[15]

0.5% w/w carbopol 934 gel was prepared. The preservative (methyl paraben) was dissolved in a sufficient quantity of pre-warmed water. The carbopol 934 was then added in small amount with vigorous stirring. The dispersion was homogenized using a magnetic stirrer for 1hr and then left for 24 hr for complete swelling. After that, the triethanolamine was added drop by drop with continuous mixing and the final weight was completed to 100 g with water. A weighted amount of FL microsponge was incorporated, so that the final concentration of FL is 1% w/w in the final gel formula. A control formula was prepared by the same procedure using pure FL powder only in a concentration of 1% w/w in the prepared gel.

Physical properties of the prepared gel[16]

The visual examination:

The examination considered a series of visual characteristics (consistency, colour, and homogeneity).

pH determination:

The pH of the prepared gel was measured using pH meter by putting the tip of the electrode into the gel and after 2 minutes the result was recorded

Spreadability

A sample of 0.1g of gel was pressed between 2 slides with 500g weights and left for about 5 min where no more spreading was expected. Diameters of spread circles were measured in cm and were taken as comparative values for spreadability (diameter of the spread circle –initial diameter.

Viscosity

The viscosity of FLZ loaded microsponge carbopol gel was measured in Brookfield viscometer, model-VL2 (Lemis Baltic) with spindle No 4

Determination of FL content in the gel formulation

FL content in the gel was determined by taking required quantity of the gel which is equivalent to 10 mg of fluconazole transferred to 100 ml volumetric flask containing phosphate buffer (pH 7.4) and it allowed to sonicate and filtered. Then, suitably diluted and analyzed at λ max 260.

***In-vitro* skin permeation studies**

Rats 6 to 8 weeks old weighing 120 to 150 gms were killed by inhaling diethyl ether. The hair of test animals were carefully trimmed short to less than 2mm with a pair of scissors and abdominal skin was separated from the underlying connective tissue with scalpel. The excised skin was placed on aluminium foil and dermal side of the skin was gently teased off for any adhering fat and subcutaneous tissue. Cleaned skin was used for in-vitro skin permeation study

Primary skin irritation study[17]

Draize patch test procedure was performed in accordance to the CPCSEA guidelines. The experimental protocol was approved by the Animal Ethical Committee of Vishnu Institute of Pharmaceutical Education and Research. Wister rats weighing 200gms were used. Animals were divided into three groups (n = 6). Group 1: 0.5ml of saline (Control), Group 2: Plain gel containing 1% w/w of Fluconazole Group 3: Microsponge based gel containing 1% w/w of Fluconazole. The back of the animals was shaved free of fur with a depilatory 24 hours before application of the formulations. 0.5 g, were applied on the hair free skin of rats by uniform spreading within the area of 1cm^2. The skin was observed for any visibly change such as erythema (redness) at 24, 48 and 72 h after the application of various formulations and the mean erythemal scores were recorded (ranging from 0 to 4)

Zeta Potential[18]

The zeta potential of FL microsponge formulations were measured using Zetasizer (Malvern instruments, Worcestershire, UK). The measurement was performed at 25^{0}C. A sample of 1 ml was diluted using double distilled water).

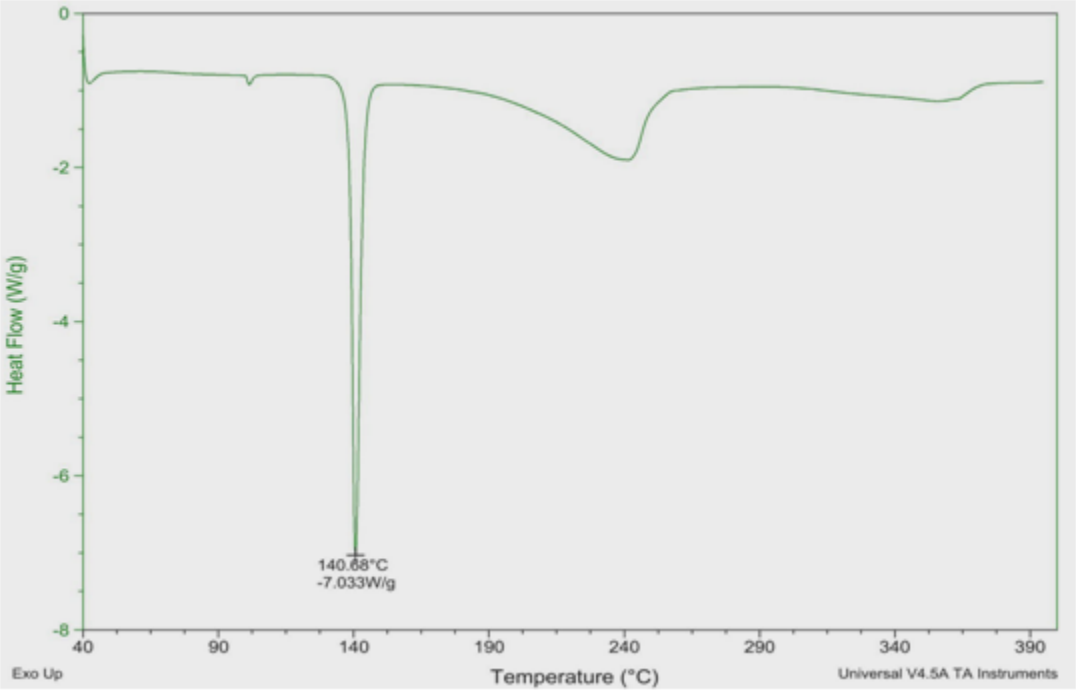
***In-vitro* antifungal studies of microsponge formulations[19]**

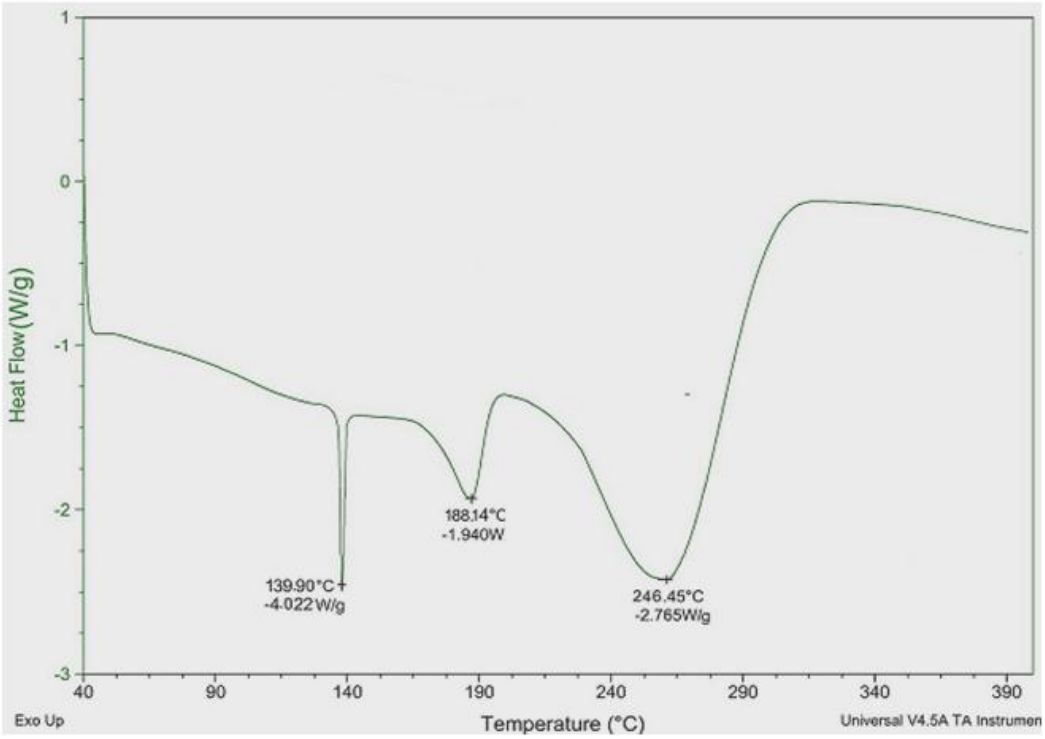
To determine *in vitro* antifungal activity of fluconazole microsponges was carried out by Agar based disk diffusion method (ABDD) against commonly isolated species of dermatophytes by Nweze *et al.* Fluconazole plain gel "A", microsponges 3 "B" and control "C" (equivalent to 2µg/disk) were prepared in lab by dissolving in DMSO & then diluting it to give a final concentration of 1mg/ml then delivering 10µl onto each sterile disk. Sterile disks were also impregnated with 10 µl of 1:100 dilution of DMSO to serve as control "C". The above 3 disks were applied to each inoculated & dried plate & then incubated at 28°C for up to 5 days. When growth took place, the size of zones of inhibition was measured for each antifungal agent.

Preparation of fungal culture

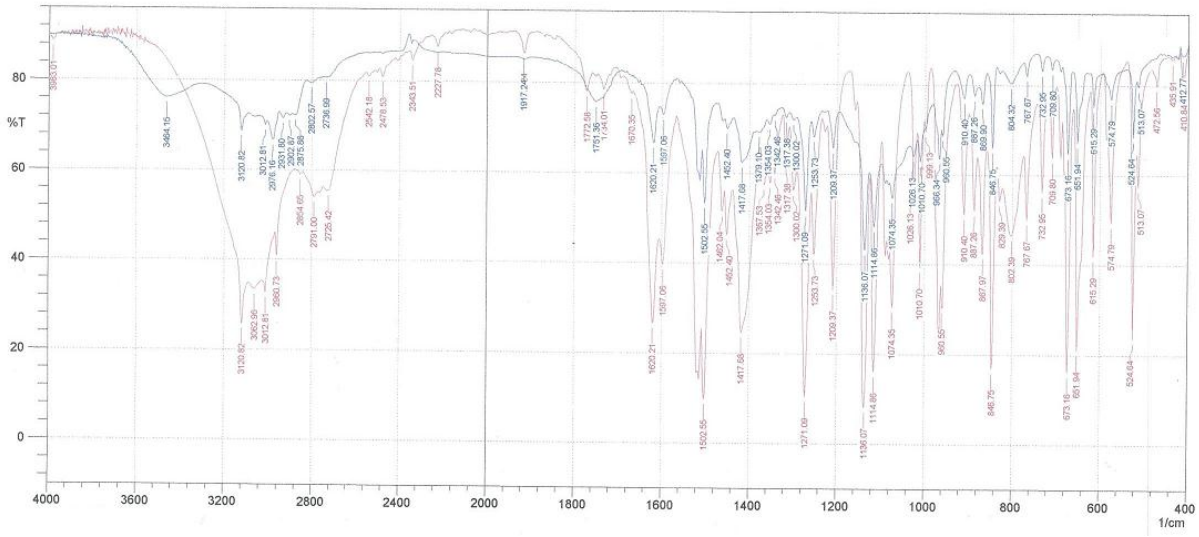
Candida albicans cultures were prepared in sauboaurd dextrose agar plates and further stored in slants as stock cultures. For the experiments, stock culture was prepared by inoculating each culture from slants to flask in sterile Sauboaurd dextrose broth and incubated at 28^0C for 48 h. The stock culture was serially diluted by 10 fold with sterile peptone water and 0.1ml from each dilution was spread over sauboaurd dextrose agar plates and incubated at 28^0C for 48 h. The number of colony forming units (CFU) was counted from plates of each dilution and there by the total CFU was calculated in the stock culture.

RESULT AND DISCUSSION





The characteristics peak of the fluconazole is ~140.68ºC The melting endothermic peak of fluconazole is shifted to ~139.90ºC in the physical mixture with Ethyl cellulose, PVA. However, the minor changes observed in peak values of drugs are not indication of any potential incompatibility.



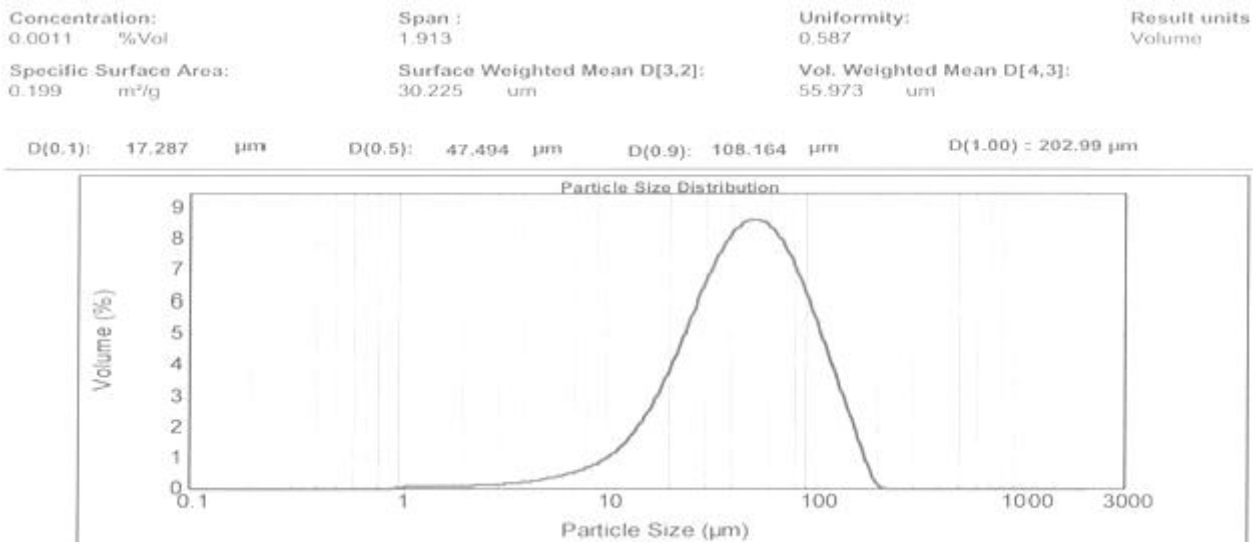
S.No	Functional group	Pure Drug	Physical Mixture	Type of vibration
1	O-H	3120.82	3120.82	Stretching
2	C-H	3012.61	3012.61	Stretching
3	C=O	1620.21	1620.21	Stretching
4	N-H	1597.06	1597.06	Bending
5	C-H	1354.03	1354.03	Bending

The FTIR spectra of the physical mixtures of drug and polymers had all the characteristic peaks and band values of pure fluconazole, confirming that all the functional groups in fluconazole are well preserved. The report clearly indicated absence of chemical interaction between the drug and polymer. Hence, the drug is compatible with all the excipients including polymer used in the present investigation.

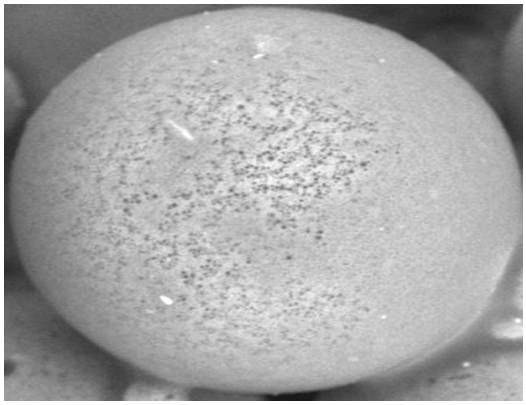
S.No	Formulation code	Production yield (%)	Encapsulation Efficiency (%)
1	1	20.60±0.21	93.44±0.01
2	2	37.41±0.20	87.08±0.02
3	3	50.40±0.39	85.12±0.08
4	4	63.00±0.01	81.28±0.02
5	5	70.13±0.04	76.11±0.04
6	6	79.27±0.02	70.13±0.05
7	7	25.17±0.04	60.24±0.07
8	8	37.12±0.11	68.41±0.07
9	9	20.13±0.17	72.21±0.01
10	10	19.11±0.15	76.11±0.07

Production yield was greatly influenced by drug: polymer ratio and also by the concentration of surfactant. Increasing the drug: polymer ratio resulted in improving the production yield. When drug: polymer ratio was at 1:0.5 the production yield was very less i.e. 20.60%. When drug: polymer ratio has been increased to 1:6 the production yield was remarkably more i.e. 79.27% with the low concentration of surfactant (0.45% w/v), the production yield also found to be very low, i.e. 25.17% for 7. When the surfactant concentration was increased from 0.45% w/v to 0.6% w/v (8) the production yield was also found to be increased 37.52% for 8.When the surfactant concentration was further increased from to 0.9% w/v 9 and 1% w/v 10 the production yield was quite less Table 12. The reason behind the less production may be formation of excessive foam and evaporation while preparing microsponges.

The entrapment efficiency of fluconazole microsponges was noted in the range of 60.24% to 93.44%. The outcome of encapsulation efficiency displayed that the drug payload was based on drug: polymer ratios and high amount of drug entrapment was attained at lower drug: polymer ratios.



The optimized fluconazole microsponges (3) particle size was determined by laser light-scattering technique, which showed that the specific surface area, surface diameter and diameter by volume of the particles were 0.199 m^2/g, 30.22 µm and 0.123 m^2/g 55.97 µm respectively. In Fluconazole microsponges the particle size distribution of d (0.1), d (0.5), d (0.9) and d (1) were 17.28 µm, 47.49 µm, 108.16 µm and 202.99µm.



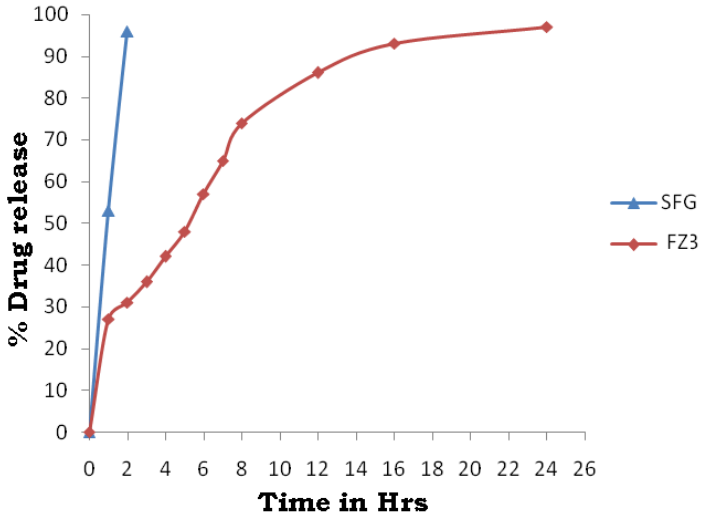
The captured SEM images of fluconazole microsponges are shown in Figure. The SEM results indicated that formed microsponges were highly porous, predominantly spherical and not much drug crystals were observed on the surface of the microsponges. Pores were induced by diffusion of solvent EA from surface of microsponges.

Table: Evaluation of microspongic gel:

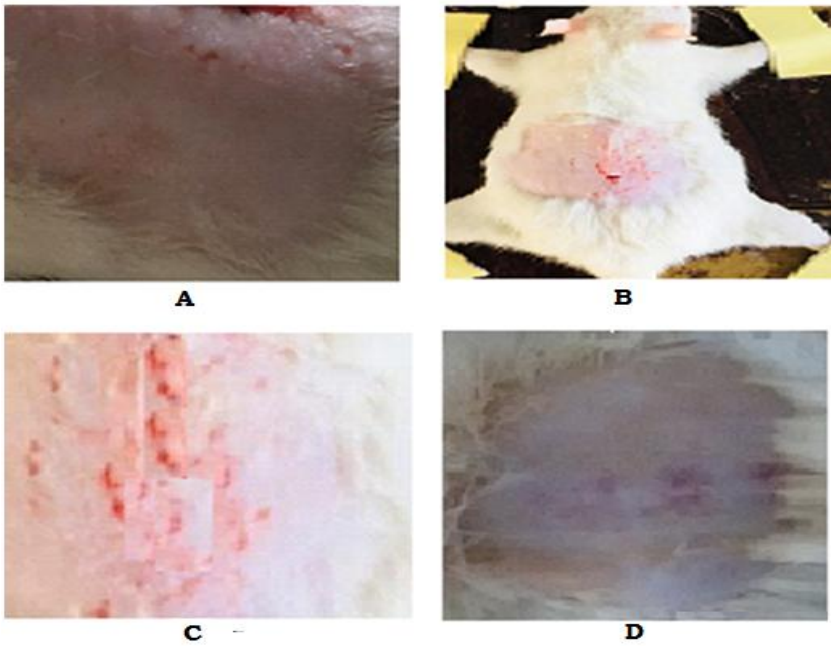
F.Code	pH	Viscosity	Spreadability	Drug content
3	6.8±0.01	34,520±1.78	10.31±0.21	84.03 ±0.39

From these data table we have found that optimized microspongic gel formulation prepared from ethyl cellulose polymer having greater drug content, spreadability and viscosity that are suitable for application on the skin. The drug content was identified

The *in vitro* permeation study of optimized fluconazole microsponge (3) was compared with luconazole plain gel. The results proved that the microspongic gel could sustain the drug release up to 97% over a period of 24hours when compared to 96% release at the end of 2 hour from fluconazole plain gel. The results are showed in figure



Primary skin irritation study:



Photographs of skin irritation studies carried out on Wister rats (A) control (0.5ml of saline); (B) plain Fluconazole gel without microsponge; (C) Placebo microsponge (D) microsponge based Fluconazole gel. The Photographs have been taken after 72 h.

Zeta Potential:

The zeta potential value of fluconazole microsponge gel was found and which lies in ideal limit of ±10 to ±30 mV. Fluconazole microspongic gel showed the zeta potential of – 24.6 mV Figure 47. It indicates that systems may remain stable for longer period.

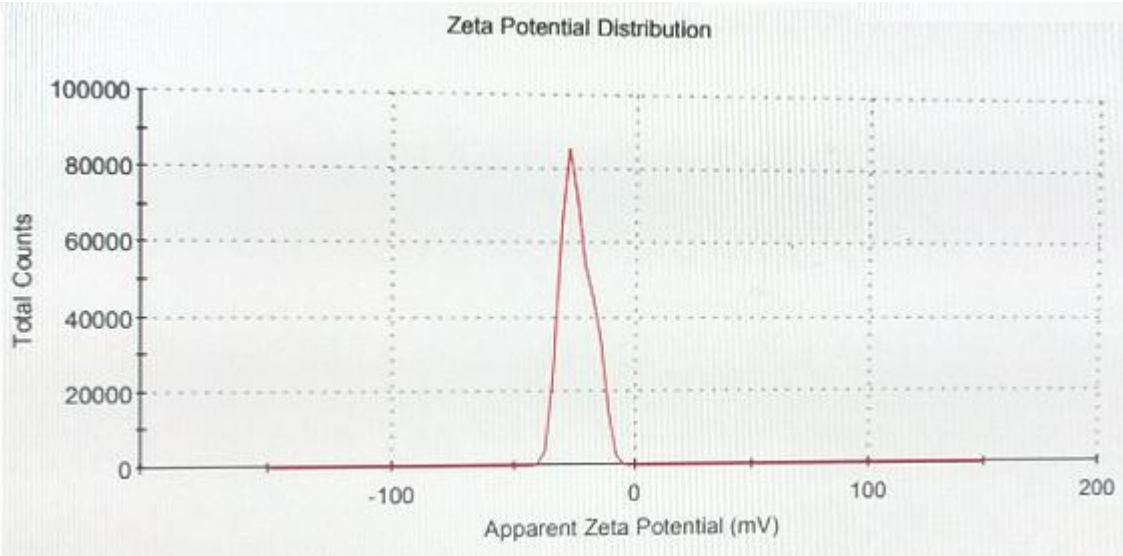


Figure: Zeta Potential of 3 Microspongic gel

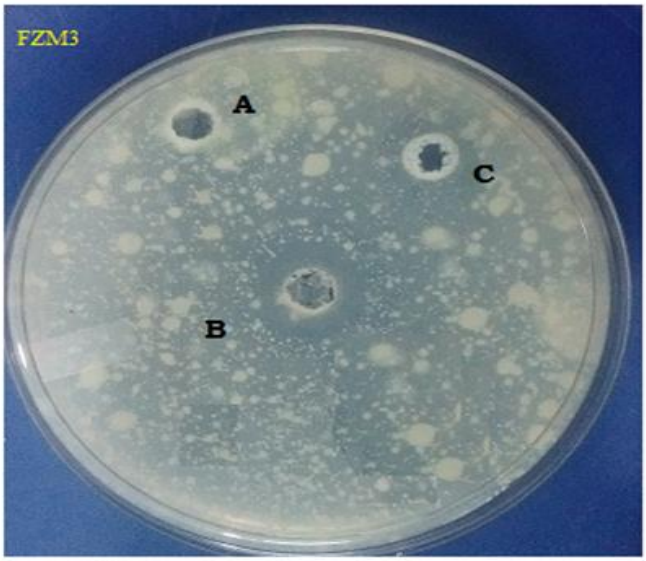
***In-vitro* antifungal activity:**

The zones of inhibition (ZOI) were seen the fungal strains. The zone of inhibition of Fluconazole microsponge formulation was 1.9 mm and no zone of inhibition was seen with disk containing DMSO and plain gels against *Candida Albicans*. The results of zone of inhibition are summarized in Table.

Table: *In-vitro* antifungal activity Microsponge plain gels

Parameter	Micro sponge based gel (Test) MG	Plain gel M	DMSO (Control)
Zone of inhibition ± SD (mm	1.9±0.08	NZOI	NZOI

NZOI = No zone of inhibition



In vitro* antifungal against *Candida Albicans

CONCLUSION

Polymeric porous micro particle based system was developed successfully using quasi-emulsion solvent diffusion method for continual topical delivery up to an extended period so as to reduce application frequency, Implemented method was found to be simple, reproducible and rapid; which led to the formation of highly porous, spherical micro particle with good flow. Varied drug-polymer ratio reflected remarkable effect on drug content, encapsulation efficiency, particle size, and drug release. The FZ3 formulation was chosen for further study on the basis of its superiority in terms of physiochemical characterization, production yield, drug content, entrapment efficiency, morphology, surface topography, intact particles percent, and particle size. The *in vitro* antifungal activity studies revealed that, the microsponge based gels of Fluconazole had shown a significantly higher antifungal activity than plain gels and control (DMSO) formulations as can be seen from the diameter of inhibition zone with good in vitro antifungal activity. Gel based fluconazole microsponges can be used for effective topical drug delivery. Thus, microsponge based delivery system developed and investigated in present research approach was seems to be promising with respect to eradication of candidia albicans infections

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