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Formulation and In-Vitro Evaluation of Fluconazole Loaded Microsponge Gel For Topical Sustained Delivery

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***Abstract**: The objective of present work was to formulate and evaluate Fluconazole (FLZ) microsponges using quasi emulsion solvent diffusion technique and microsponge gel by using carbopol. Microsponges containing FLZ were obtained successfully with different proportions of ethyl cellulose polymer (EC). The formulations were studied for particle size and physical characterization. The physical characterization of the microsponge formulations showed better loading efficiency and production yield. The formulations were prepared as gel in 0.5%w/w carbopol and studied for pH, viscosity, spreadability, drug content, and in vitro release. All three microsponge gel formulations (i.e. FM7, FM8 and FM9) showed better results like pH between 6.5-7.0, viscosity between 62,800-62,768 cps, spreadability 2-6 cm/s and drug content of 76.20±0.02% to 96.41±0.01%. In-vitro diffusion studies of formulations were performed in Franz diffusion cell. Surface morphology by scanning electron microscopy showed micro-porous nature of microsponges. Sustained release was observed when compare with control formulation.*

***Keywords**: Fluconazole, microsponge, carbopol, quasi emulsion solvent diffusion, Franz diffusion cell.*

I. Introduction

A topical conventional delivery drug suffers some problems such as aesthetically unappealing, greasiness and stickiness that often leads to lack of patient compliance. In the formulation point of view, uncontrolled evaporation of active ingredient, unpleasant odour and incompatibility of drugs with the vehicles are the notable drawbacks faced by Pharmaceutical scientists [1]. Thus, huge demand for novel drug delivery systems. Microsponges drug delivery (MDS) is one of the potential and promising drug delivery systems to encounter those hurdles. Moreover, it may improve stability, reduce side effect of active ingredients from topical formulations, further it helps to modify drug release favorably [2]. The MDS is a patented polymeric system consisting of porous microspheres and they are tiny sponge like spherical particles that consist of a myriad of interconnecting voids within a noncollapsible structure with a large porous surface through which active ingredient are released in a controlled manner [3]. Microsponges (MSP) are prepared by two general methods, namely; emulsion and suspension. The most commonly used is emulsion solvent diffusion (ESD) method [4]. Fluconazole (FLZ) is a synthetic triazole antifungal drug for the treatment of superficial and systemic fungal infections. FLZ is a selective inhibition of the fungal cytochrome P450 system and also an inhibition of the C-14 α astral demodulation process, avoiding in this way the membrane ergosterol synthesis. [5] Commercially Fluconazole is available as tablets and injections and oral administration of FLZ often produces gastric irritation, heartburn, vomiting and sometimes patients can develop ulceration and there is less patient compliance with long term therapy. Furthermore, oral FLZ is reported to interact with a number of Co administered drugs, especially oral hypoglycemics [6]. Thus aim of the present investigation is to formulate and evaluate microsponge containing Fluconazole and evaluate the in-vitro performance for prepared formulae.

II. Materials and Methods

2.1 Materials

Fluconazole was obtained as a gratis sample from RMS Research labs Pvt Ltd Hyderabad, India. Ethyl cellulose-N50 was gifted by Dr. Reddy's laboratories Hyderabad, India. Polyvinyl alcohol, methanol and ethyl acetate were purchased from Emerck (India) Ltd., Mumbai. All other chemicals and solvents used were of analytical grade.

2.2 Compatibility studies

In order to check the integrity (Compatibility) of drug in the formulation, IR spectra of the selected formulation along with the drug and other excipients were obtained and compared. In the present study, potassium bromide (KBr) pellet method was employed. The samples were thoroughly mixed with dry powdered potassium bromide and scanned from 400-4000cm-1 using FT-IR spectrophotometer (Model number 02437 Shimadzu, India) [7]

2.3 Preparation of FLZ loaded Microsponges

FLZ microsponges were prepared by an Emulsion solvent diffusion method (ESD). The inner phase, Ethylcellulose (EC) was dissolved in Ethyl acetate (EA) and then drug was added to solution under sonication at 35°C and was then gradually added into external phase, which contained poly vinyl alcohol (PVA) as emulsifying agent. This mixture was stirred mechanically at 1000 rpm for 3 hours at room temperature to remove Ethyl acetate from the reaction flask. The formed microsponges were filtered, washed with distilled water and dried at room temperature [8]. Microsponges were weighed, and production yield (PY) was calculated. Drug concentration and stirring speed of 1000 rpm for a period of 3 hours was kept constant for all the formulations and effect of different variables such as surfactant concentration, polymer concentration was observed. The formed microsponges were evaluated for their physical characteristics, % entrapment efficiency, drug content and particle size.

2.4 Determination of production yield (PY), Actual FLZ content, and Entrapment Efficiency (EE %)

FLZ Microsponges equivalent to 100 mg of drug was dissolved in 2 ml methanol; 30 ml of pH 5.5 Phosphate Buffer Saline (PBS) solution was added and stirred for 30 min with a magnetic stirrer. The mixture was heated at 50–55°C for 45 min in a water bath to remove methanol. After that, the volume was adjusted to 50 ml with fresh PBS solution (pH 5.5) heated at 50–55°C. The solution was cooled, filtered, and an aliquot, after suitable dilution, was analyzed spectrophotometrically at 260 nm. Each experiment was carried out in triplicate actual drug content, entrapment efficiency and Production yield of the microparticles was calculated according to the following equation [9]:

Loading efficiency (%) $M_{act}/M_{the.}$ x100 (1)

$$\text{Production yield \%} = \frac{\text{Practical mass of Microsponges}}{\text{Theoretical Mass (Polymer + Drug)}} \times 100 \quad (2)$$

Where M_{act} is the actual quantity of Fluconazole in the weighed quantity of microparticles,
M_{ms} is the weighed quantity of the microsponges
M_{the} is the theoretical amount of Fluconazole in the microsponges.

Table. 1 Formulation of Fluconazole Microsponges

Formulation	FM 1	FM 2	FM 3	FM 4	FM 5	FM 6	FM 7	FM 8	FM 9
FLZ(mg)	100	100	100	100	100	100	100	100	100
Ethyl Cellulose(mg)	300	200	100	300	200	100	300	200	100
PVA (%w/w)	0.25	0.25	0.25	0.5	0.5	0.5	0.75	0.75	0.75
Ethyl Acetate(ml)	20	20	20	20	20	20	20	20	20
Distilled Water(ml)	80	80	80	80	80	80	80	80	80

2.5 Scanning Electron Microscopy

The morphology of microparticles was examined with a scanning electron microscope (SEM-JEOL Instrument, JSM- 6360, Japan) operating at 15 kV. The samples were mounted on a metal stub with double adhesive tape and coated with platinum/palladium alloy under vacuum [10]. The obtained photograph was recorded at x500 magnification

2.6 Particle Size Determination

The mean particle size of FLZ microsponge determined by using the microscope fitted with an ocular micrometer and stage micrometer [11]

One division of the stage micrometer = 0.01mm = 10 µm
C= (SM X 100) / EM
Where C= correction factor
SM= Reading of stage micrometer which coincides with reading of eye-piece micrometer (EM).
The average particle size was determined using the equation
D (Mean) =Σnd/Σn
Where n= No of MSP observed, d= mean size range

2.7 Preparation of FLZ Loaded Microsponge Carbapol Gel

0.5% w/w carbopol 934 gel was prepared. The preservative (methyl paraben) was dissolved in a sufficient quantity of water pre warmed to 40°C. 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 it for 24 hr for complete swelling. Next, the triethanolamine was added drop wise with continuous mixing and the final weight was completed to 100 g with water. Calculated amounts of FLZ microsponge formula was incorporated, so that

the final concentration of FLZ is 1% w/w in the final gel formula [(12)]. A control formula was prepared by the same procedure using pure FLZ powder only in a concentration of 1% w/w in the prepared gel.

2.8 Physical properties of the prepared gel

2.8.1 The visual examination, pH determination and viscosity

The test considered a series of visual characteristics (consistency, color, and homogeneity) The pH of the prepared FLZ loaded microsponge gel was measured using pH – meter by putting the tip of the electrode into the gel and after 2 minutes the result was recorded 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 The viscosity of FLZ loaded microsponge carbopol gel was measured in Brookfield viscometer, model- VL2 (Lemis Baltic) with spindle No 4[13,14]

2.8.2 Determination of FLZ Content in the Gel Formulation

FLZ content in the gel was determined by taking required quantity of the prepared gel which is equivalent to 10 mg of Fluconazole and transferred to 100 ml volumetric flask containing phosphate buffer (pH 7.4), it allowed to sonicate and filtered. Then, suitably diluted and analyzed at λ max260 of FLZ [15]

2.8.3 *In-vitro* diffusion study of FLZ loaded Microsponge Gel

The in vitro release of FLZ microspongic gel was performed by the membrane diffusion technique a sample of 1g of the preparation was spreaded on a cellophane membrane previously soaked overnight in the release medium. The loaded membrane was firmly stretched over the edge of a glass tube of 2 cm diameter; the membrane was tied up with a rubber to prevent leakage. Tubes were then immersed in the dissolution vessel which contained 50 ml of the release medium, phosphate buffer pH 5.5, and maintained at 37ºC ± 0.5ºC. The shafts were rotated at 50 rpm and aliquots each of 2 ml were withdrawn from the release medium at specified time intervals. Withdrawn samples were replaced by equal volumes of fresh release medium. The samples were assayed spectrophotometrically at λmax 260 nm and the concentration of the drug was determined from the previously constructed calibration curve [16]. The in vitro diffusion studies were recorded for a 10 hour period.

III. Results and Discussion

3.1 Compatibility Studies

The FT-IR spectrum of pure FLZ, physical mixtures are given in figure. The spectrum of pure FLZ showed characteristic peaks at 3120.82cm-1, 1620.21cm-1 and 1209.37cm-1. The spectrums of physical mixtures were equivalent to the spectrum of the drug, polymer and PVA indicating no chemical interaction or complexation occurs Fig 1

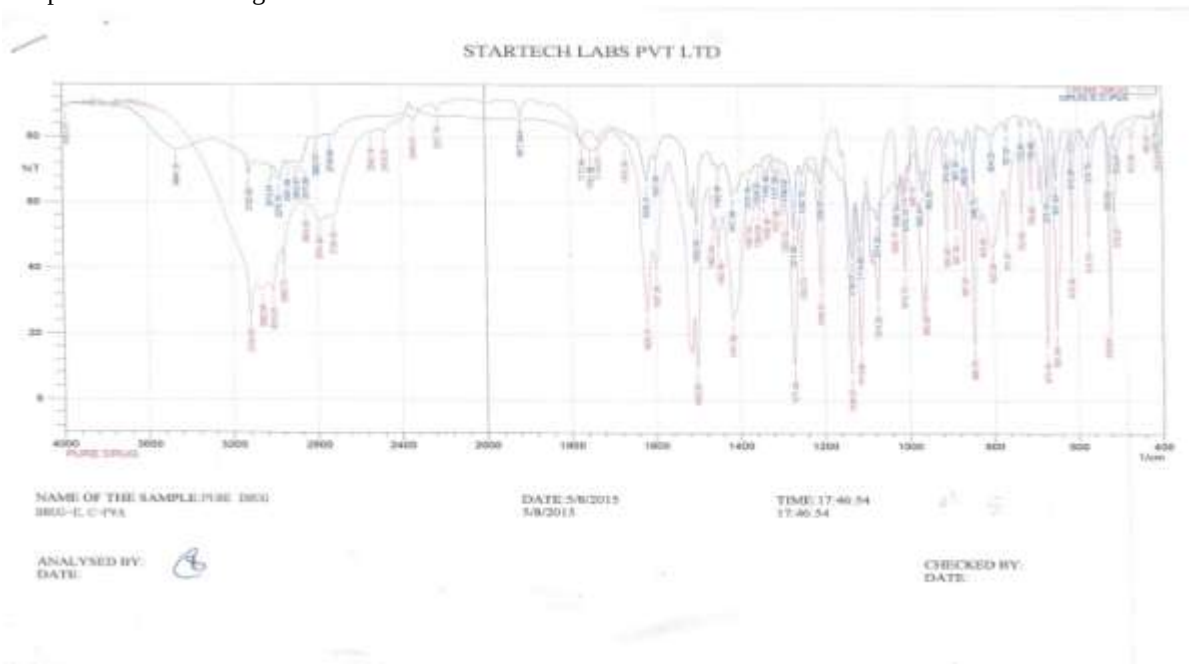

Fig 1. FTIR studies of Pure drug and mixture of drug, Ethyl cellulose and PVA

3.2 Preparation of FLZ Loaded Microsponges

ESD method was used to formulate FLZ microsponges because of its reliability, simplicity and reproducibility, cost effective and avoiding solvent toxicity [17]. In this method, the formation of microsponges could be by the rapid diffusion of Ethyl acetate into the aqueous medium, might reduce the solubility of the polymer in the droplets, since the polymer was insoluble in aqueous media. The instant mixing of the Ethyl acetate and water at the interface of the droplets induced precipitation of the polymer, thus forming a shell enclosing the Ethyl acetate and the dissolved drug. The finely dispersed droplets of the polymer solution of the drug were solidified in the aqueous phase via diffusion of the solvent

Table. 2 Production Yield, Entrapment Efficiency and Particle size

F.Code	Formation of MSP	%Yield (±S.D)	EE % (±S.D)	Particle Size µm (±S.D)
FM1	*	-	-	-
FM2	*	-	-	-
FM3	*	-	-	-
FM4	**	51.04±0.00	34.18±0.05	310.84±0.02
FM5	**	49.20±0.02	32.10±0.01	303.80±0.03
FM6	**	48.21±0.03	30.21±0.02	300.21±0.01
FM7	***	45.40±0.01	34.11±0.01	312.15±0.03
FM8	***	43.11±0.04	33.10±0.04	318.24±0.03
FM9	***	40.25±0.01	31.41±0.04	320.10±0.01

* No MSP was formed, ** Unstable MSP was formed, ***stable MSP was formed

The concentration of surfactant has a major role to play in the formation of microsponges. Based on the above reports we observed that the minimum concentration of surfactant required to bring about the formation of uniform microsponges was found to be 0.5% w/v of external phase which resulted in the particle size range of between 300 µm to 310 µm and also we noticed that the formed microsponges were not stable after 2days this may be due to insufficient concentration of surfactant. Whereas the surfactant concentration 0.75% w/v showed stable microsponges the particle size range of between 312 µm to320 µm. When the concentration of emulsifier was increased, the production yield and entrapment efficiency decreased whereas the mean particle size of microsponges was increased Table 2

3.3 Evaluation of Surface Morphology by Scanning Electron Microscope (SEM)

SEM picture of the selected formula of X7 was presented in figure at x 500 magnification. It was observed by SEM analysis that the microsponges were finely spherical, smooth, and porous. Fig 2. The surface topography reveals that FLZ microsponges contained tiny pores. The pores were induced by the diffusion of the volatile solvent (Ethyl Acetate) from the surface of the microparticles and the pore size was in the range of 1.18µm to 5.58 µm Fig 3. The appearance of the particles was such that they were termed microsponges. [18]

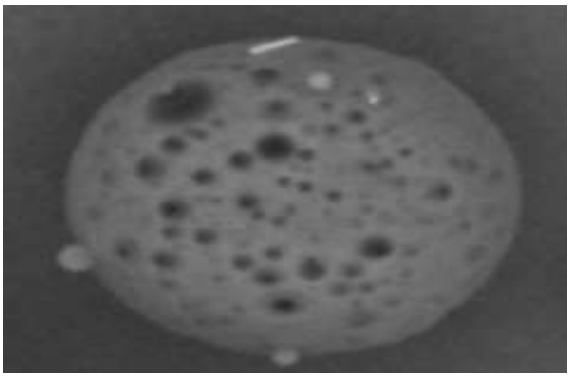

Fig 2. Surface Morphology

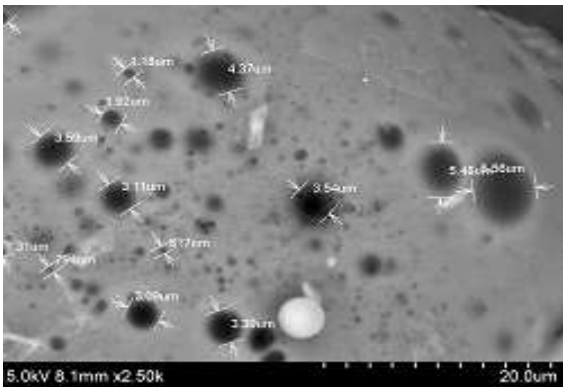

Fig 3. Pore size of Microsponge

3.4 Characteristics of FLZ Microsponge loaded carbapol gel

For preparation of FLZ loaded microsponge carbapol gel, we select only the formulations of FM7, FM8 and FM9 and the obvious reason was its stability. The prepared gel formulations of FLZ loaded microsponges were inspected visually for their colour, consistency and homogeneity. All three formulations showed non-transparent white gel with smooth texture and of good homogeneity without lumps. The control formulation showed transparent gel with bubbles.

The pH of all the three FLZ loaded microsponge carbapol gel and control formulation was found in the range of 6.5–7.0, due to neutralization of formula by triethanolamine. Spreadability is an important characteristic of topical preparations and is responsible for correct dosage transfer to the target site and ease of application on the substrate. All three formulations and control formulation was showed spreadability in the range of 2-6 cm. Viscosity holds a major contribution in deciding the drug content and its release from prepared gel formulation. All three formulations showed approximate viscosity between 62,800-62,768 cps, whereas the control formulation showed 45,000cps. The FLZ content of the gel was in the rage of 76.20±0.02% to 96.41±0.01. The drug content of the formulations showed that the drug was uniformly distributed in the gels.

Table 3. Characterization of Microsponge incorporated gels

Parameters	FM 7	FM 8	FM 9	Control
Visual Appearance	*	*	*	**
pH	6.5	6.5	6.8	7.0
Spreadability (gm.cm/sec)	2	3	4	6
Viscosity (cps)	62,800	62,789	62,768	45,000
% Drug content	76.20±0.02	80.12±0.01	84.10±0.01	96.41±0.01

* No phase separation, Non-transparent and No lumps ** Transparent with air bubbles

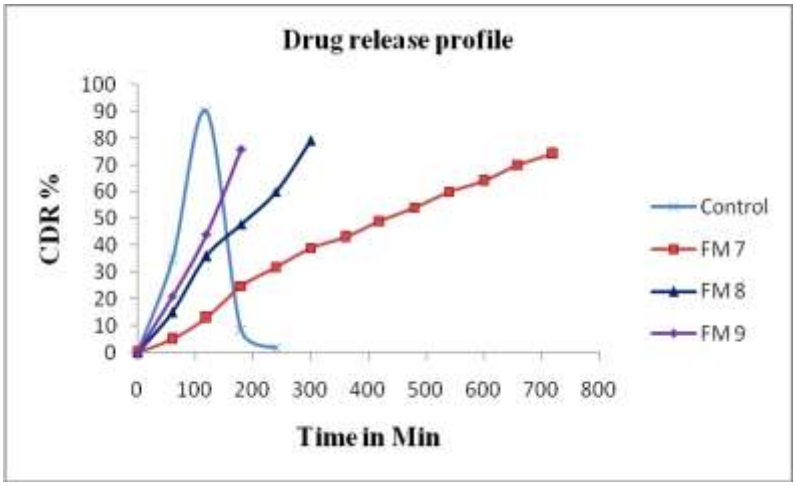

Fig 4. Drug release profile of control & FLZ –MSP gel (FM7-FM9)

It is showed from the release profile Fig 3 that FLZ loaded carbopol gels has produced a great influence in the diffusion rate which is significantly higher than that of pure FLZ gel. According to the results obtained, it was noted that, release of fluconazole from control formulation (Carbopol 0.5% w/w) showed an immediate release of drug than FLZ loaded microsponges. The percentage release of FLZ from FM 7 at the end of 12hrs

was found to be 74% it indicated that microspongic drug delivery system shows it influences to sustain the release of medicament whereas; the other two formulations sustained the release between 3-5 hrs this may be due to less concentration of polymer in microsponge

IV. Conclusion

In the present work a topical polymeric microsponge formulation of a locally acting anti fungal agent, fluconazole has been developed which includes formulation and evaluation of fluconazole microsponges. The concentration of the polymer required to produce stable microsponges with good physical and morphological characteristics was found to be in the range of 100-300mg. The minimum requirement of the emulsifier to produce microsponges was found to be 0.5% w/v.

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