Available online on 15.06.2024 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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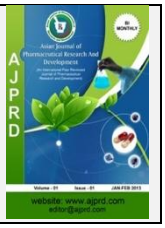

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Research Article

Preparation of flutrimazole micro sponge gel by quasi-emulsion solvent diffusion method

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A B S T R A C T

Microsponge containing Flutrimazole drug with six different proportions of Eudragit RS 100 as polymer were obtained successfully using quasi-emulsion solvent diffusion method. Particle size and physical properties of the formulations were examined. Physical properties showed that microsponge formulations MS IV and MS VI exhibited better material efficiency and yield. Two microsponge formulations were prepared as gels in 0.35% w/w carbomer and examined for pH, viscosity, permeability, drug content, in vitro release, antibacterial activity, and in vivo antifungal activity on Rabbit skin. The viscosity of the microsponge preparation gel MFG 1 is 4390 cps, the transmission is 19.27 g cm/s, and the drug content is 85.2%. Antibiotic research shows that compared with pure drug, the inhibition zone of microsponge formulation gels MFG 1 and MFG 2 is 13.5 mm and 12.0 mm, respectively, and the inhibition zone of pure drug is 18.2 mm. These formulations also showed better antifungal activity on fungal-induced Rabbit skin compared to the no-drug control. The microsponge flutrimazole gel formulation shows a favorable drug release profile and also minimizes gel application in fungal treatment.

Key words: Microsponge, Flutrimazole, Antidandruff, Antimicrobial

A R T I C L E I N F O: Received 10 Feb 2024; Review Complete 28 April 2024; Accepted.18 May 2024; Available online 15 June. 2024

Cite this article as:
Tyagi G, Choudhary S, Preparation of flutrimazole micro sponge gel by quasi-emulsion solvent diffusion method, Asian Journal of Pharmaceutical Research and Development. 2024; 12(3):43-49, DOI: http://dx.doi.org/10.22270/ajprd.v12i3.1393

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INTRODUCTION

Microsponges are porous polymer microspheres widely used for long-term drug delivery. Microsponges are designed to efficiently deliver medicinal ingredients in small and safe doses, reducing side effects and improving drug release. Microsponges can be prepared by various methods using suspension polymerization in emulsion systems or liquid–liquid systems. The most commonly used emulsion system is oil in water (o/w) and microsponges are produced by the emulsion solvent diffusion (ESD) method 1,2 . The results showed that the drug:polymer ratio, mixing speed, and dispersion phase will affect the size and drug release behavior of the produced microsponges, and the presence of emulsifiers is crucial for the production of microsponges3,4. Flutrimazole (FTZ) is a broad-spectrum antifungal agent against many fungi and yeasts. 5. Microsponge gel to study long-term immunity .

MATERIALS AND METHODS

Flutrimazole was supplied as gift sample by Shalby Pharmaceutical Ltd Delhi. Carbopol 940 was supplied by Shree Chemical Ltd Ahmedabad. Eudragit RS 100 , Polyvinyl alcohol was supplied from Sigma (USA) and analytical grade ethanol, triethanolamine, PEG400 from S.D. Fine Chem. Ltd., Mumbai.

Preparation of Microsponges: FTZ-containing microsponges were prepared using the semi-emulsion

solvent diffusion method [4] using different polymers into solution as shown in Table 1. Then pour the inner layer into the PVA aqueous solution. The resulting mixture was stirred at 3000 rpm for 60 min and filtered to separate microsponges. Microsponges were dried in an oven at 40°C for 12 h and weighed to determine [6].

Characterization and evaluation of microsponge formulations:

Fourier transform infrared (FTIR) analysis [7]: FTIR spectra of the FTZ and Eudragit RS 100 weremeasured in potassium bromide disks using Perkin- Elmer Model 1600 FTIR spectrometer (USA).

Scanning electron microscopy [8]: The size of microsponge were observed by scanning electron microscopy. Prepared microsponges were coated with platinum studied by scanning electron microscopy (SEM; JEOL-JSM, 6360, Japan) under vacuum at room temperature.

Particle size studies: Particle size analyses were performed on microsponge by Malvern Mastersizer (Malvern Instruments, Mastersizer 2000, UK). The results are the average of three analyses. The values(*d*50) were expressed for all formulations as mean size range.

Determination of loading efficiency [8]: The drug content in the microsponges was determined spectrophotometrically (λmax = 257 nm). A sample of Flutrimazole microsponges (10 mg) was dissolved in 100 ml of neutralizing phthalate buffer, freshly prepared (pH 5.4). The drug content was calculated from the calibration curve and expressed as loadingefficiency.

$$\text{Drug entrapment} = \frac{\text{Mass of drug present in microsponges}}{\text{Theoretical mass of FTZ}} \times 100$$

Determination of production yield [8]: The production yield of the microsponge was determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponge obtained.

$$\text{Production yield} = \frac{\text{Practical mass of microsponges}}{\text{Theoretical mass (polymer + drug)}} \times 100$$

Preparation of microsponge Flutrimazole gel (MFG): Accurately weighed amount of carbopol 940 was taken and dissolved in water using propeller.In another beaker, microsponges containing Flutrimazole (free or entrapped, equivalent to) drug dissolved in ethanol and added to carbopol solution by stirring, followed by addition of PEG 400. The pH of the final gel formed was determined.

Physical parameters of MFG: Two formulations of microsponges containing Flutrimazole were characterized for pH using pH meter [9], viscosity [9] using a Brookfield digital viscometer (Model DV- III + Rheometer), spreadability [10] and drug content[11].

In vitro diffusion study [12]: The modification was used to study the in vitro release of MFG formulations using cellophane membranes. The dissociation medium used was freshly prepared neutralized phthalate buffer (pH 5.4). Cellophane membrane soaked in dissolution medium overnight is attached to one end of a specially designed glass cylinder (open on both sides). Put one gram of preparation (equivalent to 1000 mg of flutrimazole) into this product. Place the graduated cylinder on the stand and suspend it in 200 ml of isolation medium maintained at 37 ± 1°C. The membrane only contacts the media receptor. Mix the media using Teflon-coated magnetic beads at 100 rpm. Aliquots of 5 ml each were withdrawn at intervals of 120, 180, 240, 300 and 360 min and replaced with an equal amount of receptor medium. Fractions were appropriately diluted in recipient medium and analyzed with a UV-Vis spectrophotometer at 257 nm using neutralized phthalate buffer as blank.

Anti microbial activity [12-14]: This was determined by sabouraud dextrose diffusion test employing "cup plate technique" using previouslysterilized petridish. Solution of gel prepared formulation and pure Flutrimazole as a standard 1mg/ml was poured into cups bored of size 8 mm in to wells of sabouraud dextrose plate previously seeded with test organism (*Candida albicans*). After allowing diffusion of solution for 2 hrs, the plates were incubated at 27^0C for 48 hrs. The zone of inhibition measured around each cup was compared with that of the standard.

***In vivo* study [14-16]:** Male Rabbits (2500-3500 g) were used. The hair was removed from their flanks with electrical clipper. The area of skin (20mm diameter) on each flank was scarified with coarse sandpaper. Scarified skin was infected with few drops of culture of *Candida albicans*. Infected Rabbitswere housed individually in wire bottomcages and were provided food and water *ad libitum*. The fungal infection was induced on the guinea pig for first 3 days, on the 4^{th} day, the skin of guinea pigwas scraped which is shown in figure 5 and was cultivated in sabouraud dextrose agar media plates. The inoculated plates were incubated at 27^0C for 48hrs. The colonies were measured after incubation. On the 4^{th} day, treatment was initiated by topical application to the infected sites with gel formulation for another 4 days. On the 8^{th} and 11^{th} day skin was again scraped and cultured on sabouraud dextrose agar plate respectively and further treatment was done. The inoculated plates were incubated at 27^0Cfor 48 hrs. and examined for growth of colonies.

RESULTS AND DISCUSSION

Formulation of microsponges by quasi-emulsion solvent diffusion method: In quasi-emulsion solvent diffusion method, the formation of the microsponges could be by the rapid diffusion of dichlormethane (good

solvent for the polymer and drug) into the aqueous medium, might reduce the solubility of the polymer in the droplets, since the polymer was insoluble in water. The instant mixing of the dichlormethane and water at the interface of the droplets induced precipitation of the polymer, thusforming a shell enclosing the dichlormethane 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.

Characterization of Microsponges:

Loading efficiency: The loading efficiency of Flutrimazole microsponge formulations are given inTable 2. The loading efficiency calculated for all microsponges ranged from 87.5 to 93 %.The highestloading efficiency was found for the MS VI formulation, where a greater amount of drug was encapsulated. The highest loading efficiency, greaterthe amount of drug was encapsulated.

Scanning electron microscopy: The morphology of the microsponges prepared by quasi emulsion solvent diffusion method and entrapment method were investigated by SEM. The representative SEM photographs of the microsponges are shown in Fig 1. SEM images showed the microsponges porous and spherical in shape.

Fourier transform infrared (FTIR) analysis: FTIR spectra of pure Flutrimazole, Eudragit RS 100and mixture were obtained. Fundamental peaks of Flutrimazole at 1643 (ketonic C=O stretch) cm ¯1 were observed. It clearly indicates that the FTIR spectra of pure Flutrimazole were compatible with Eudragit RS 100 polymer as shown in Fig 2.

Determination of production yield: The production yields of Flutrimazole microsponge formulation are given in Table 2. Production yield calculated for all microsponges ranged from 65.75-77.66 %. From the production yields of Flutrimazole microsponge formulation, it was indicated that increasing the drug: polymer ratio increased the production yield.

Physical parameters of gels: The formulations MFG I and II showed the spreadability of 17.81 and 18.1 g cm/s, viscosity of 4105 and 3970 cps, pH of 6.4 and 7.2 and drug content of 85.2 to 87.6 % respectively. The drug content of the formulations showed that the drug was uniformly distributed in the gels.

***In Vitro* diffusion study**: *In vitro* diffusion profile of microsponges containing Flutrimazole gel, the total amount of drug release was 69.34% and 67.15% obs-erved at different time intervals for a period of 6 hrsfor MFG I and MFG II respectively.

Anti microbial activity: Antimicrobial activities for gels are shown in Table 4. Formulation MFG I and MFG II showed 13.5 mm and 12.0 mm inhibition in comparison with pure drug with 18.2 mm inhibition respectively. Further MFG I and MFG II formulation were used for *in vivo* antifungal activity study on Rabbits for topical study.

***In vivo* study:** The results of the *in vivo* antifungal activity on rabbit skin are shown in Table 4.

Table 1: Microsponge formulation prepared by quasi-emulsion solvent diffusion method

Constituents	Microsponges Formulation					
	MS I	MS II	MS III	MS IV	MS V	MS VI
Ketoconazole (gm)	0.5	0.5	1	1	2	2
Eudragit RS 100 (gm)	0.1	0.2	0.1	0.2	0.1	0.2
Dichloromethane (ml)	5	5	5	5	5	5
Glycerol (ml)	1	1	1	1	1	1
PVA (gm)	0.05	0.05	0.05	0.05	0.05	0.05
Distilled Water (ml)	200	200	200	200	200	200

Table 2: Effect of the Drug to Polymerin Physical Characterization

Formulation Code	Particle Size (µm)	Production Yield (%)	Drug Loading (%)	
MS I	58	65.75	87.5	
MS II	58.2	67.33	87.5	
MS III	55.8	68	90	
MS IV	56.6	71.2	91.5	
MS V	53.8	70.5	90.5	
MS VI	54.8	77.66	93	

Table 3: Formulation of the ketoconazole gel containing microsponges entrapped drug.

Ingredients	**MKG I**	**MKG II**
Ketoconazole Microsponge eqv. to Ketoconazole 1.0% w/w	1.0	1.0
Carbopol 940 (gms)	0.35	0.35
Ethanol (gms)	15	15
PEG 400 (gms)	15	15
Triethanolamine (gms)	5	5
Water q.s(gms)	100	100

Table 4: Results of antimicrobial study and *in vivo* antifungal study on the Rabbits skin. *upto 3rd day infection was induced

Formulation	**Zone of inhibition (mm)**	**Colony***		
		4th day	**8th day**	**11th day**
MKG I	13.5	>50	35	14
MKG II	12	>50	32	12
Pure drug	18.2	-	-	-
Control	-	>50	>50	>50

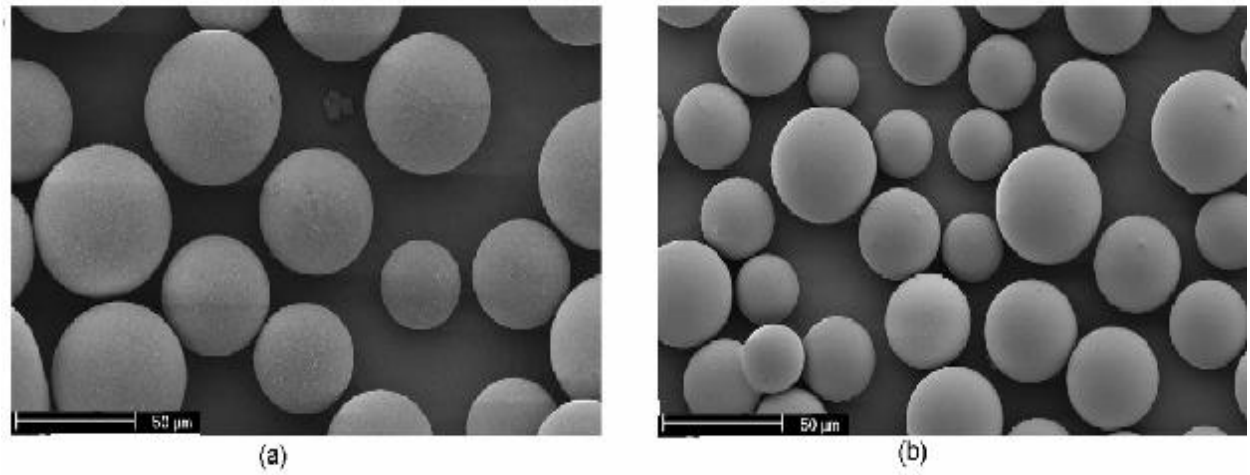


Figure 1: Scanning electron microscopy of (a) MKG I (b) MKG II

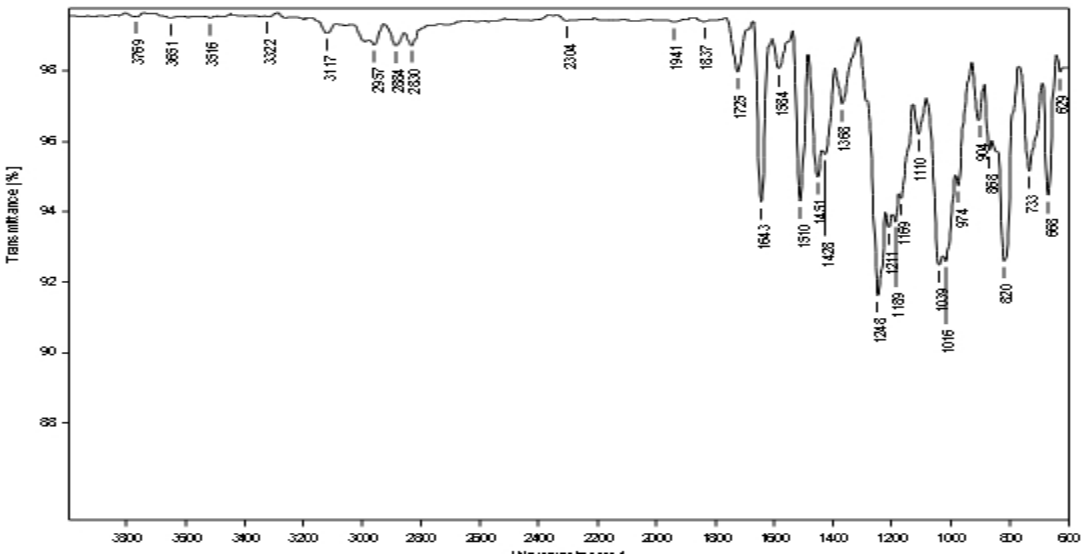


Figure 2: FTIR spectra of Flutrimazole + Eudragit RS 100

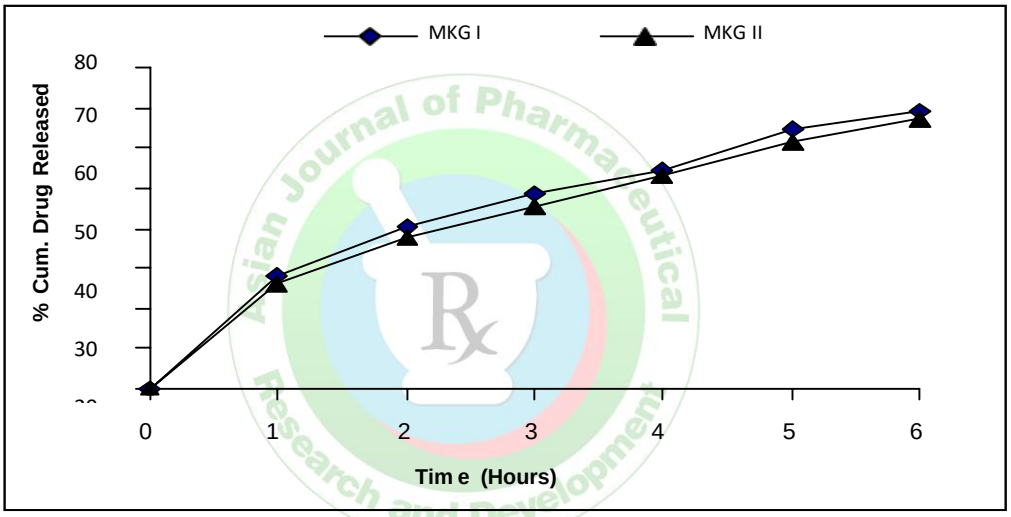
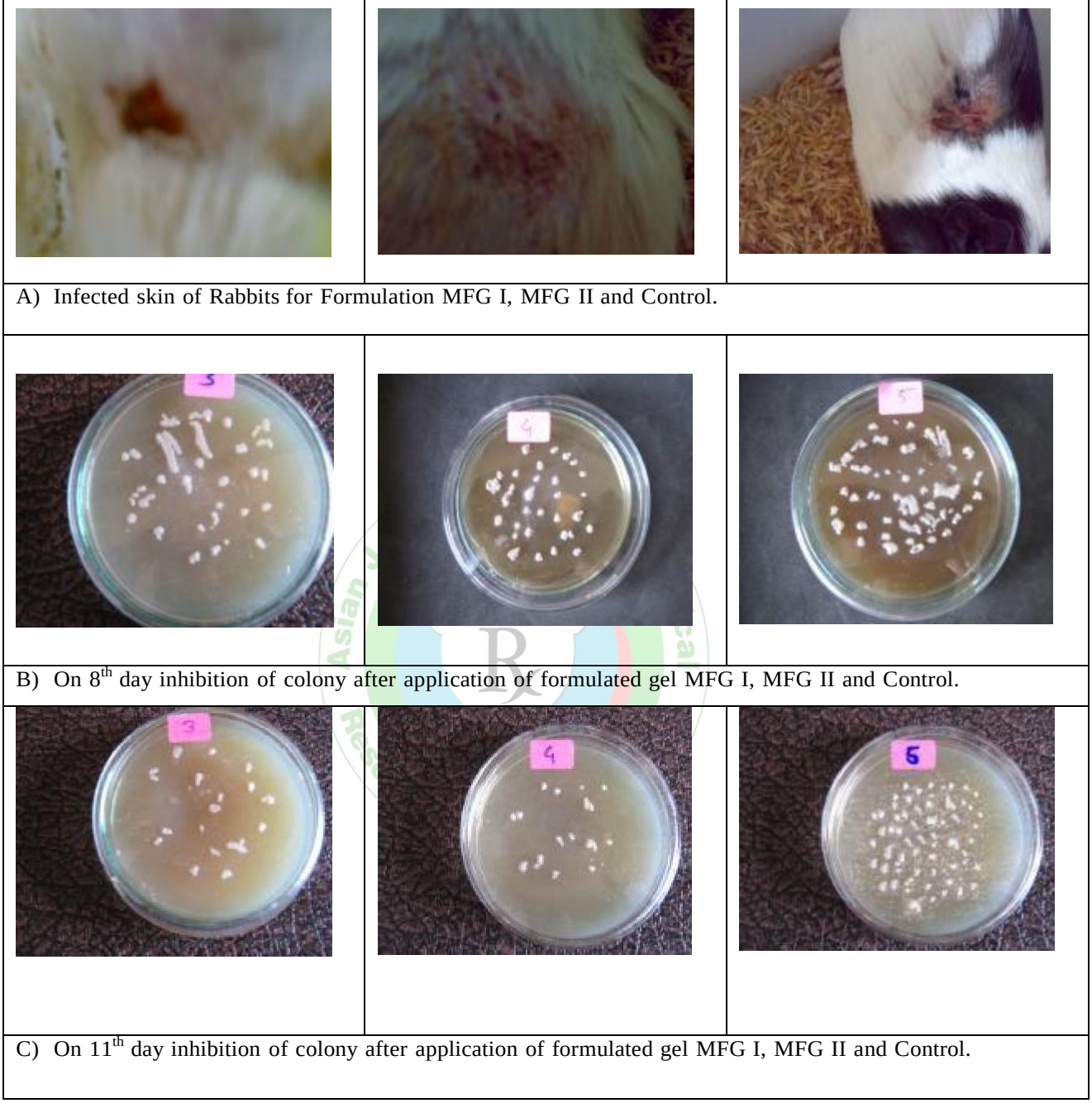


Figure 3: Release profile of MKG I and MKG II

Figure 4: Antimicrob-ial activity of keto-conazole microsp-ongegel of MKG I and MKG II



A) Infected skin of Rabbits for Formulation MFG I, MFG II and Control.

B) On 8th day inhibition of colony after application of formulated gel MFG I, MFG II and Control.

C) On 11th day inhibition of colony after application of formulated gel MFG I, MFG II and Control.

Figure 5: *In vivo* antifungal study on the Rabbits skin**.**

The fungal infection was induced on the guinea pig for first 3 days, on the 4th day, the skin of guinea pig wasscraped and was cultivated in sabouraud dextrose agar media plates and colony count was observed more than 50 for formulation MFG I, MFG II and control respectively. The treatment of the gel formulation was started from 4th day applying for next 4 days and studied with the effectiveness of gel on fungal inhibition. After 8th day the skin of the guinea pig was again scraped and checked for the growth of colony in sabouraud dextrose agar plate. Colony count was observed 35, 32 and more than 50 for formulation MFG I, MFG II and control respectively. Similarly on 11th day the skin of guinea pig was againscraped and checked for the growth of colony in sabouraud dextrose agar plate. Colony count was observed 14, 12 and more than 50 for formulation MFG I, MFG II and control respectively. Antimicrobial study and i*n vivo* study results showed that

formulation MFG I and MFG II showing inhibition of fungal infection in comparison with thecontrol.

CONCLUSION

Microsponge systems are made of biologically inertpolymers. Extensive safety studies have demonstrated that the polymers are non-irritating, non-mutagenic, non-allergenic, non-toxic and non- biodegradable. As a result, the human body cannot convert them into other substances or break them down. This study presents an approach for the production of Flutrimazole containing microsponge gel with prolonged release characteristics. The quasi-emulsion solvent diffusion method used for the preparation of the microsponges was simple, reproducible, and rapid. Furthermore, it was observed that as drug/polymer ratio increases, the particle size is decreased. This is probably due to the fact that at higher relative drug content, the amount of polymer available per microsponge to encapsulate the drug becomes less, thus reducing the thickness of the polymer wall and hence, smaller microsponges. Microsponge formulation MS IV and MS VI showed a good physical parameter study and were used for formulating into gel, incorporated in the 0.35 %w/w carbopol. Microsponges incorporated gels showed a good physical parameter study, *in vitro* drug release and *in vivo* anti fungal activity on guinea pig skin.

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