

## FORMULATION AND EVALUATION OF KETOCONAZOLE MICROSPONGE GEL BY QUASSI EMULSION SOLVENT DIFFUSION

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**Abstract:** *Microsponge containing Ketoconazole drug with six different proportions of Eudragit RS 100 as polymer were obtained successfully using quasi-emulsion solvent diffusion method. These formulations were studied for particle size and physical characterization. The physical characterization showed that microsponge formulation MS IV and MS VI showed a better loading efficiency and production yield. These two microsponge formulation were prepared as gel in 0.35 %w/w carbopol and studied for pH, viscosity, spreadability, drug content, in vitro release, antimicrobial activity and in vivo antifungal activity studied on guinea pig skin. The microsponge formulation gel, MKG 1 showed viscosity 4390 cps, spreadability of 19.27 g cm/s and drug content of 85.2%. The antimicrobial studies showed zone of inhibition with 13.5 mm and 12.0 mm for microsponge formulation gel MKG 1 and MKG 2 respectively when compared with pure drug, zone of inhibition 18.2 mm. These formulations also showed better antifungal activity on fungal induced guinea pig skin when compared with control group without application of drug. The microsponge ketoconazole gel formulations showed an appropriate drug release profile and also bring remarkable decrease on gel application for fungal treatment.*

**Key words:** Microsponge, Ketoconazole, Antidandruff, Antimicrobial

### INTRODUCTION

Microsponges are porous, polymeric microspheres that are mostly used for prolonged topical administration. Microsponges are designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, reduce side effects, and modify drug release profiles. Microsponges are prepared by several methods utilizing emulsion system or by suspension polymerization in a liquid-liquid system. The most common emulsion system used is oil-in-water (o/w), with the microsponges being produced by the emulsion solvent diffusion (ESD) method [1,2]. It was shown that the drug: polymer ratio, stirring rate, volume of dispersed phase influenced the particle size and drug release behaviour of the formed microsponges and that the presence of emulsifier was

essential for microsponge formation [3,4]. Ketoconazole (KTZ) is a broad spectrum antifungal agent active against a wide variety of fungi and yeasts [5]. In present investigation the formulation of Ketoconazole microsponge gel with different concentration of carbopol 940, ethanol and PEG 400 were used to study antifungal activity for long duration.

### MATERIALS AND METHODS

Ketoconazole was supplied as gift sample by Shalaks Pharmaceutical Ltd Delhi. Carbopol 940 was supplied by Shree Chemical Ltd Ahmedabad. Eudragit RS 100 was from Degussa-Rohm GmbH & co. Germany. Polyvinyl alcohol was supplied from Sigma (USA) and analytical grade ethanol, triethanolamine, PEG 400 from S.D. Fine Chem. Ltd., Mumbai.

**Preparation of Microsponges:** The microsponges containing KTZ were prepared by quasi emulsion solvent diffusion method [4] using different polymer ratio as shown in Table 1. The inner phase, Eudragit RS 100 was dissolved in dichloromethane and then added drug to solution under ultrasonication at 35°C. The inner phase is then poured into PVA solution in water. The resultant mixture was stirred at speed of 3000 rpm for 60 min, and filtered to separate the microsponges. The microsponges were dried in an air heated oven at 40°C for 12 hrs., and weighed to determine the yield [6].

### Characterization and evaluation of microspunge formulations:

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

**Scanning electron microscopy [8]:** The morphology and size of microspunge 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 microspunge 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 ( $\lambda_{\text{max}} = 257 \text{ nm}$ ). A sample of ketoconazole 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 loading efficiency.

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

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

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

**Preparation of microspunge ketoconazole gel (MKG):** Accurately weighed amount of carbopol 940 was taken and dissolved in water using propeller. In another beaker, microsponges containing ketoconazole (free or entrapped, equivalent to) drug dissolved in ethanol and added to carbopol solution by stirring, followed by addition of PEG 400. Neutralized the carbopol solution by slowly adding triethanolamine solution with constant stirring until the gel is formed. The pH of the final gel formed was determined.

**Physical parameters of MKG:** Two formulations of microsponges containing ketoconazole 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 *in vitro* release of MKG formulations were studied using cellophane membrane using modified apparatus. The dissolution medium used was neutralizing phthalate buffer, freshly prepared (pH 5.4). 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 1000 mg of Ketoconazole) 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 pre-determined time interval of 120, 180, 240, 300, 360 min and replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV-Viible spectrophotometer at 257 nm using neutralizing phthalate buffer as blank.

**Anti microbial activity [12-14]:** This was determined by sabouraud dextrose diffusion test employing “cup plate technique” using previously sterilized petridish. Solution of gel prepared formulation and pure ketoconazole as a standard 1 mg/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°C for 48 hrs. The zone of inhibition measured around each cup was compared with that of the standard.

**In vivo study [14-16]:** Male guinea pigs (250-350 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 guinea pigs were housed individually in wire bottom cages and were provided food and water *ad libitum*. The fungal infection was induced on the guinea pig for first 3 days, on the 4<sup>th</sup> day, the skin of guinea pig was scraped which is shown in figure 5 and was cultivated in sabouraud dextrose agar media plates. The inoculated plates were incubated at 27°C for 48 hrs. The colonies were measured after incubation. On the 4<sup>th</sup> day, treatment was initiated by topical application to the infected sites with gel formulation for another 4 days. On the 8<sup>th</sup> and 11<sup>th</sup> 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°C for 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, thus forming 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 ketoconazole microsphere formulations are given in Table 2. The loading efficiency calculated for all microsponges ranged from 87.5 to 93 %. The highest loading efficiency was found for the MS VI

formulation, where a greater amount of drug was encapsulated. The highest loading efficiency, greater the 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 Ketoconazole, Eudragit RS 100 and mixture were obtained. Fundamental peaks of Ketoconazole at 1643 (ketonic C=O stretch) cm<sup>-1</sup> were observed. It clearly indicates that the FTIR spectra of pure Ketoconazole were compatible with Eudragit RS 100 polymer as shown in Fig 2.

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

**Physical parameters of gels:** The formulations MKG 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 ketoconazole gel, the total amount of drug release was 69.34% and 67.15% observed at different time intervals for a period of 6 hrs for MKG I and MKG II respectively.

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

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

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

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

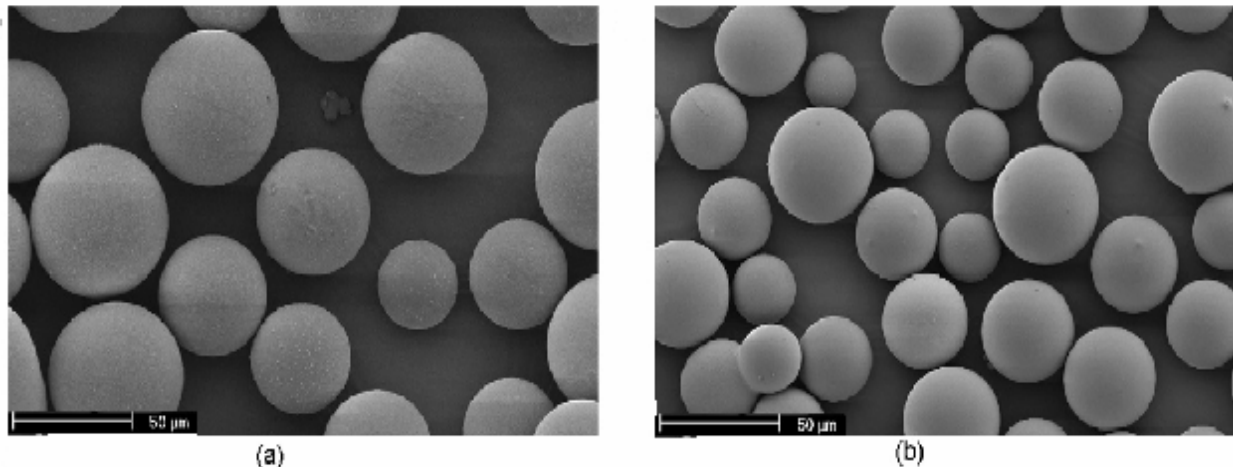
**Table 2:** Effect of the Drug to Polymer in Physical Characterization

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

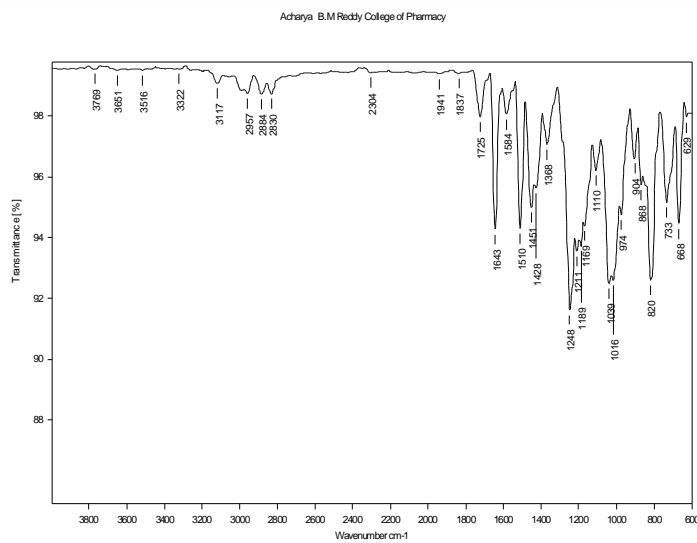
**Table 3:** Formulation of the ketoconazole gel containing microsponges entrapped drug.

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

**Table 4:** Results of antimicrobial study and *in vivo* antifungal study on the guinea pig skin. \*upto 3<sup>rd</sup> day infection was induced

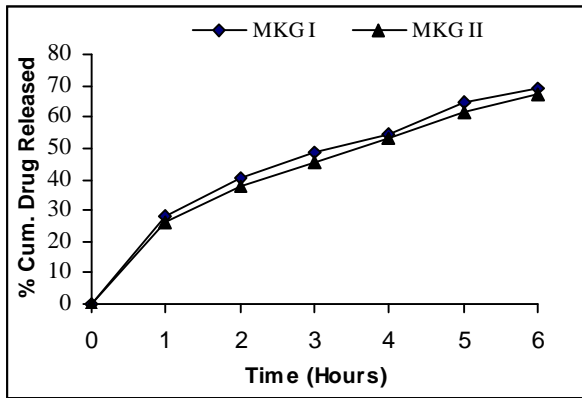


**Fig 1:** Scanning electron microscopy of (a) MKG I (b) MKG II



**Fig 2:** FTIR spectra of Ketoconazole + Eudragit RS 100

File Name- KETOCONAZOLE+ EUDRAGITRS 100.0

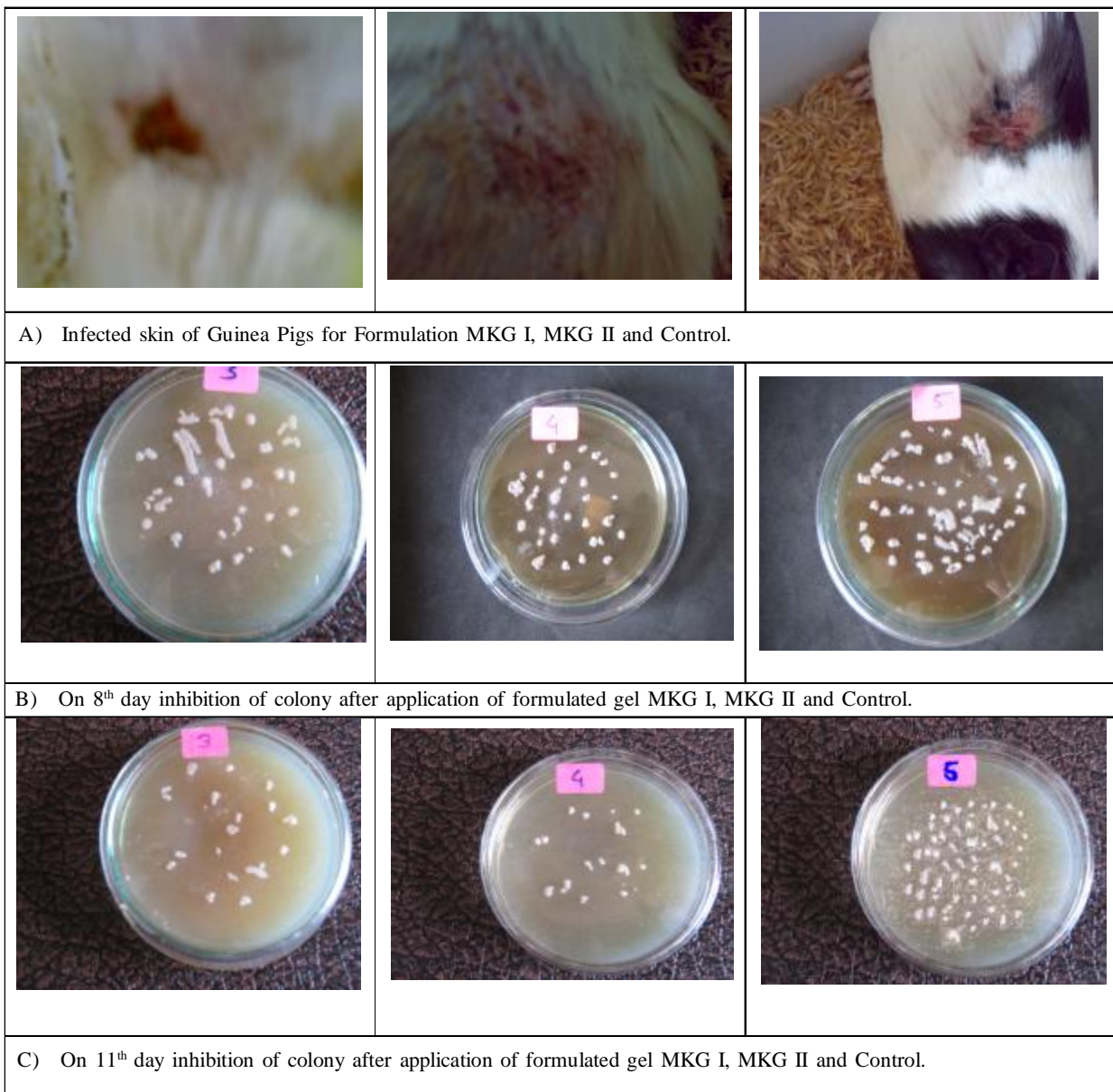


**Fig 3:** Release profile of MKG I and MKG II



**Fig 4:** Antimicrob-ial activity of keto-conazole microsponge gel of MKG I and MKG II

**Fig 5:** *In vivo* antifungal study on the guinea pig skin.



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

### CONCLUSION

Microsponge systems are made of biologically inert polymers. 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 ketoconazole 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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