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FORMULATION AND EVALUATION OF EUDRAGIT RS 100 LOADED MICROSPONGES OF FLUTRIMAZOLE

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ABSTRACT: Flutrimazole is a wide spectrum antifungal drug. It is used for the topical treatment of superficial mycoses of the skin. Conventional topical products typically provide active ingredients in relatively high concentrations but with a short duration of action. Microsponge Systems are designed to allow a sustained rate of release of the active ingredients, offering a potential reduction in side effects while maintaining their therapeutic efficacy. Therefore, the aim of the present study was to produce microsponges containing flutrimazole which were able to control the release of drug to the skin. Compatibility of drug with formulation components were established by Differential Scanning Colorimetry (DSC) and Fourier Transform Infra-Red (FTIR). Microsponges were prepared by using previously optimized emulsion solvent diffusion method. Shape and surface morphology were examined using Scanning Electron Microscopy (SEM). Drug content, particle size analysis and loading yield were determined in the prepared microparticles. The micrograph revealed microporous nature of microsponges. It was shown that the drug: polymer ratio, stirring rate, volume of dispersed phase influenced the particle size and drug release behavior of the formed microsponges. Flutrimazole microparticles were then incorporated into standard vehicles for release studies. Kinetics of drug release from microsponge itself followed Higuchi matrix model. Drug release was observed controlled as compared with the cream containing free drug.

INTRODUCTION: Numerous antifungal drugs have been prescribed to kill or inhibit the growth of fungi. Even though the therapeutic efficacy of these drugs has been well established, inefficient delivery could result in inadequate therapeutic index and local and systemic side effects including cutaneous irritation, peeling, scaling and gut flora reduction.

Flutrimazole is a new wide spectrum antifungal drug. It is used for the topical treatment of superficial mycoses of the skin. Flutrimazole is an imidazole derivative. It interferes with the synthesis of ergosterol by inhibiting the activity of the enzyme lanosterol-14-demethylase [1, 2].

Release of active ingredients from conventional topical formulations over an extended period of time is quite difficult [3]. These vehicles require high concentrations of active agents for effective therapy because of their low efficiency of delivery system, resulting into irritation and allergic reactions in significant users.

In contrast, Microsponge technology allows an even and sustained rate of release, reducing irritation while maintaining efficacy. Their high degree of cross-linking results in particles that are insoluble, inert and of sufficient strength to stand up to the high shear commonly used in manufacturing of creams, lotions, and powders.

Their characteristic feature is the capacity to adsorb or "load" a high degree of active materials into the particle and on to its surface. Its large capacity for entrapment of actives, up to three times its weight, differentiates microsponge products from other types of dermatological delivery systems [4, 5].

The purpose of the present investigation was to;

(a) Prepare Flutrimazole microsponges using Eudragit RS 100 with different drug: polymer ratios using an emulsion solvent diffusion method,

(b) Study the effect of drug: polymer ratio, stirring rate, inner phase solvent volume on the physical characteristics of the microsponges, and

(c) Compare the release rate from microsponges incorporated in topical formulations with that of the same formulations prepared with pure Flutrimazole.

MATERIALS AND METHODS:

Materials: The following materials were used in the study:, Eudragit Rs 100, poly vinyl alcohol (PVA), Ethanol, Sodium lauryl sulphate (SLS), Triethanolamine, Stearic acid, Liquid paraffin, White bees wax, Sodium hydrogen phosphate, Potassium dihydrogen phosphate, Sodium Chloride all obtained from M/s Loba Chemie Ltd. Mumbai. Flutrimazole was provided by Ajanta Pharmaceuticals, Mumbai. Eudragit RS 100 was a gift from Evonic India Pvt. Ltd, Mumbai. All other chemicals and solvents were of analytical grade.

Preparation of Microsponges: The microsponges containing flutrimazole were prepared by quasi-emulsion solvent diffusion method using different polymer amounts. To prepare the inner phase, Eudragit RS 100 was dissolved in ethyl alcohol. Then flutrimazole was added to solution and dissolved under ultrasonication at 35°C. This inner phase was poured into the PVA solution in water (outer phase). The mixture was stirred for 60 min to remove ethanol from the reaction flask. The mixture was filtered to separate the microsponges. The microsponges were dried in an air-heated oven at 40°C for 12 hr and weighed to determine production yield [6].

For the evaluation of the effect of drug: polymer ratio on the physical characteristics of microparticles, different weight ratios of drug to ethyl cellulose (1:1, 3:1, 5:1, 7:1, 9:1, 11:1, 13:1) were employed. For optimizing the preparation method, volume of organic solvent, Stirring speed and time and volume of aqueous phase were changed, and the characteristics of the prepared microparticles were evaluated.

Compatibility studies: Compatibility of drug with reaction adjuncts was studied by Fourier Transform Infra-red spectroscopy (FT-IR). Effect of process of entrapment on crystallinity of the drug was studied by Differential Scanning Colorimetry (DSC).

Determination of production yield and loading efficiency: The production yield of the microsponges was determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponge obtained [7].

Production Yield =

$$\frac{\text{Practical mass of Microsponges}}{\text{Theoretical Mass (Polymer + Drug)}} \times 100 \ \ldots\ldots (1)$$

The loading efficiency (%) of the microsponges was calculated according to the following equation:

Loading Efficiency =

$$\frac{\text{Actual Drug Content in Microsponges}}{\text{Theoretical Drug Content}} \times 100 \ \ldots (2)$$

Scanning Electron Microscopy: The morphology of microparticles was examined with a scanning electron microscope (SEM-JEOL Instrument, JSM-6360, Japan) operating at15 kV. The samples were mounted on a metal stub with double adhesive tape and coated with platinum/palladium alloy under vacuum [8].

Particle Size Analysis: Particle size and size distribution studies of microsponge particles were done by using particle size analyzer (Mastersizer 2000, Version 2.0, Malvern Instruments Ltd, UK). The results are the average of three analyses. The values ($d50$) were expressed for all formulations as mean size range.

Drug release from Microsponges: Accurately weighed loaded microsponges were placed within 50 ml of ethanol in glass bottles. The later were horizontally shaken at $37^{o}C$ at predetermined time intervals. Aliquots samples were withdrawn (replaced with fresh medium) and analyzed UV spectrophotometrically at 218 nm. The content of Flutrimazole was calculated. The solutions were protected from light throughout the assay [9].

Preparation of cream containing Flutrimazole Microsponges: Cream formulation containing 1% w/w of flutrimazole was prepared. Creams were prepared using a standard reverse emulsification method. Aqueous phase containing 5 g triethanolmine was added dropwise into an oil phase containing stearic acid 20 g, liquid paraffin 5 g and white bees wax 3 g while the mixture was stirred.

The temperature of the aqueous phase and oil phase was adjusted to $70^{o}C$ and $65^{o}C$ respectively. The mixture was stirred, while allowing it to cool to room temperature and whilst a cream formed. At this point either flutrimazole powder or flutrimazole microsponges was added to the formulation and mixed to prepare a homogenous cream.

Drug release studies from Cream: The release study was conducted using Franz diffusion cell. Cellophane membrane was soaked overnight in phosphate buffer pH 7.4 containing 0.1% SLS and fitted into the place between two chambers of cell. The receptor phase composed of phosphate buffer pH 7.4 with 0.1% SLS and temperature was maintained at $37^{0}C$.

Preliminary experiments showed that there was no interaction of receptor phase with membrane or the formulation placed on the donor side. The receptor phase was stirred at 500 rpm during the study. A predetermined amount of cream mounted on the donor side of Franz cell. Samples were withdrawn at different time intervals and these were analyzed spectrophotometrically at 209 nm.

***In-vitro* Drug Release Kinetic study** [10]: To analyze the mechanism of drug release from the microsponges and cream containing microsponges, release data were fitted to the following equations:

a) **Zero – order equation:**

$$Q_t = Q_0 + K_0 t$$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time

b) **First order equation:**

$$\log C = \log C_0 - Kt / 2.303$$

Where C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of $-K/2.303$

c) **Higuchi's equation:**

$$Q = K_H t^{1/2}$$

Where, K_H is the Higuchi constant. The data obtained were plotted as percentage drug release versus square root of time

RESULTS AND DISCUSSION:

Preparation of Flutrimazole Microsponges: Free flowing powder particles of MDS were obtained by quasi-emulsion solvent diffusion method with Eudragit RS 100 in ethyl alcohol. The quasi-emulsion solvent diffusion method used for the preparation of the microsponges was simple, reproducible, and rapid. Surface morphology by SEM observed in **fig. 1** revealed the microporous nature of microsponges.
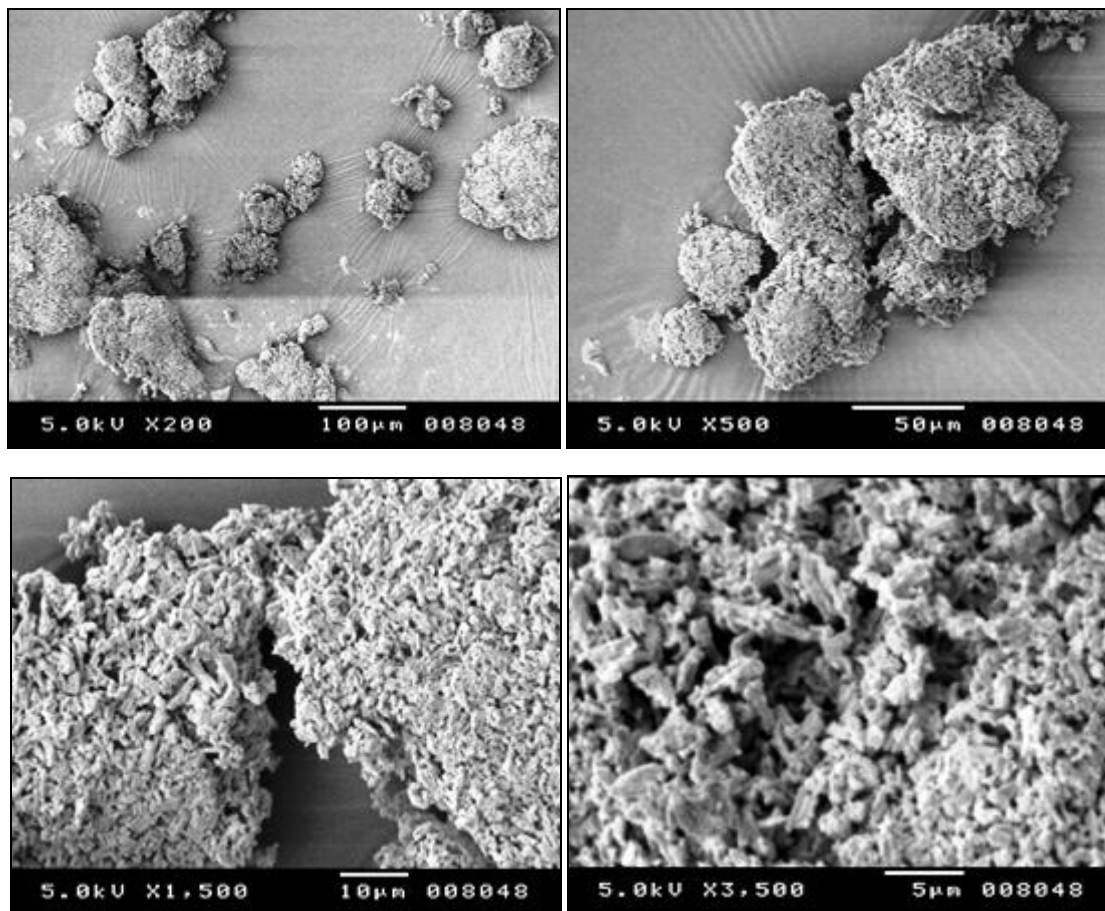
FIGURE 1: SEM OF MICROSPONGE FORMULATIONS

Compatibility studies: Fig. 2 gives the FTIR spectra of Flutrimazole, physical mixture, Eudragit RS 100 and MDS 7. The entire major drug peaks (functional group) at 1224.80 cm-1 (s) [C-F stretch], 3121.22(w) [C-H stretch], [C=C stretch] 1603.06(s), [C-N (aromatic) stretch] 1274.66 (s) were observed retained. Characteristic peaks of FLZ were observed in the spectra of MDS 7 formulation. These results showed that there was no chemical interaction or changes during microsponge preparation and FLZ was stable in all microsponge formulations [11].

DSC provides information about the physical properties of the sample as crystalline or amorphous nature and demonstrates a possible interaction between drug and other excipients in microsponges [12]. The thermal behavior of Flutrimazole, physical mixture, Eudragit RS 100, & formulation MDS 7 containing drug are shown in **fig. 2**.

Endothermic peak at 171.85°C corresponding to the melting point of drug in the crystalline form. Physical mixture of Flutrimazole, Eudragit RS 100, PVA, was found to contain one peak at 167.99°C

and formulation MDS 7 was found to contain one peak at 166.92°C. This peak does not deviate much from peak of standard Flutrimazole. Therefore polymer and drug were found compatible with each other and drug was in its proportionate crystalline form as in pure drug.
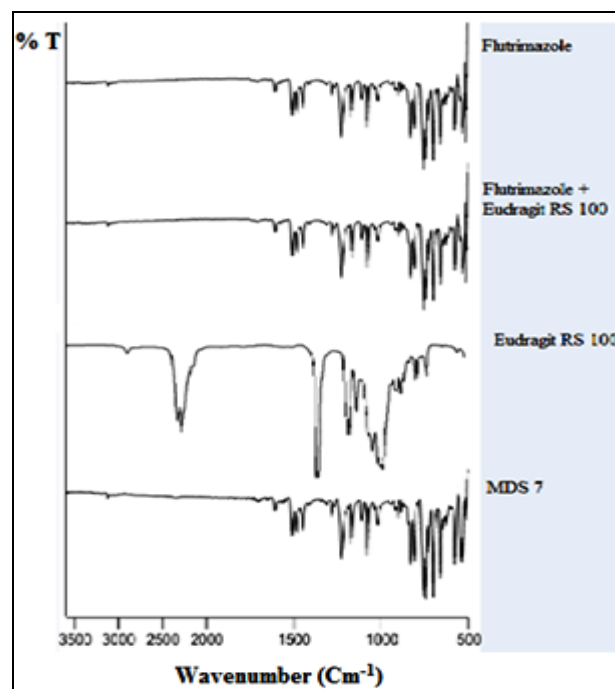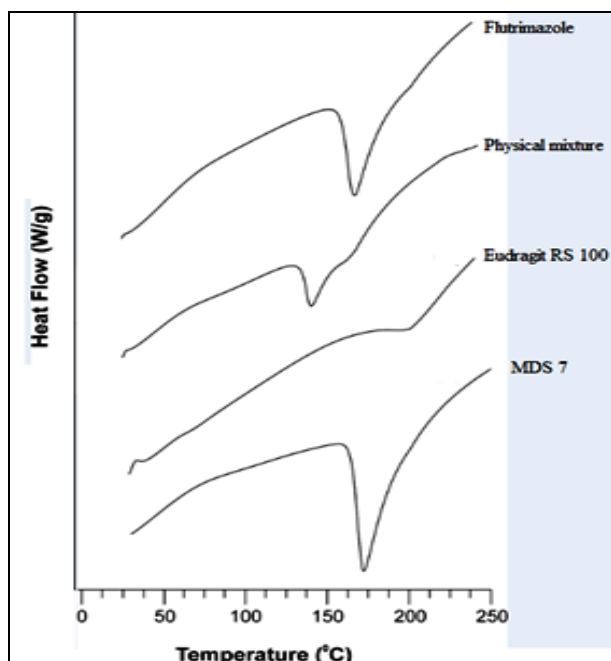
FIG. 2: FTIR SPECTRA (LEFT) AND DSC THERMOGRAMS (RIGHT) OF FLUTRIMAZOLE, PHYSICAL MIXTURE, EUDRAGIT RS-100, AND MICROSPONGE FORMULATION (MDS 7)

Production yield, loading efficiency and particle size analysis: The production yield, loading efficiency and mean particle size of Flutrimazole

microsponge formulations are given in **Table 1**. When drug: polymer ratio was 1:1, the production yield was very low (less than 20%), and therefore, no further investigations were carried out on this ratio. Increase in drug: polymer ratio increased the production yield.

However when drug: polymer ratio increased above 13:1, the increase in the production yield was not significant. This showed that the highest production yield was obtained when the ratio of drug: polymer was 13:1. The reason for increased production yield at high drug: polymer ratios could be due to the reduced diffusion rate of ethanol from concentrated solution into aqueous phase. This provides more time for droplet formation and may improve the yield of microsponges.

The results showed that increasing drug: polymer ratio increased actual drug content and encapsulation efficiency of microsponges [13]. It was found that by increasing drug: polymer ratio, the mean particle size was decreased.

TABLE1: PRODUCTION YIELD, DRUG CONTENT, ENCAPSULATION EFFICIENCY, AND MEAN PARTICLE SIZE OF THE MICROSPONGE FORMULATIONS (±SD, N=3)

Formulation code	Drug : polymer ratio	Production yield (%+SD)	Theoretical drug content (%)	Actual drug content (%+SD)	Encapsulation efficiency (%+SD)	Mean particle size (µm+SD)
MDS 1	1 :1	< 20 %	-	-	-	-
MDS 2	3 :1	58.5+1.57	74.9	57.2+0.89	76.36+0.92	436.413+5.67
MDS 3	5 :1	60.2+2.11	83.33	65.6+0.45	78.72+2.87	388.66+7.78
MDS 4	7 :1	66.9+1.89	87.49	71.3+1.15	81.49+2.69	369.554+6.56
MDS 5	9 : 1	71.9+3.45	90	76.1+1.67	84.55+1.54	308.367+8.34
MDS 6	11 : 1	73.86+1.12	91.66	82.9+0.23	90.44+3.43	278.061+5.87
MDS 7	13 : 1	76.9+2.56	92.85	86.3+0.43	92.94+1.79	211.127+6.32

Drug release from Microsponges: The release profiles obtained for the microsponge formulations are presented in **figure 3**. The present study showed that increase in the ratio of drug: polymer resulted in a increase in release of FLZ from microsponges. This could be due to formation of a thinner matrix wall (due to decrease in the amount of polymer) in microsponges with higher drug: polymer ratios lead to a shorter diffusion path, and consequently increased drug release rates [14]. Different kinetic models (zero-order release, first order release, Higuchi equation) were employed to fit the data relating to the kinetics of the release of Flutrimazole from microsponges. The results as observed in **table 2** showed that the release kinetics

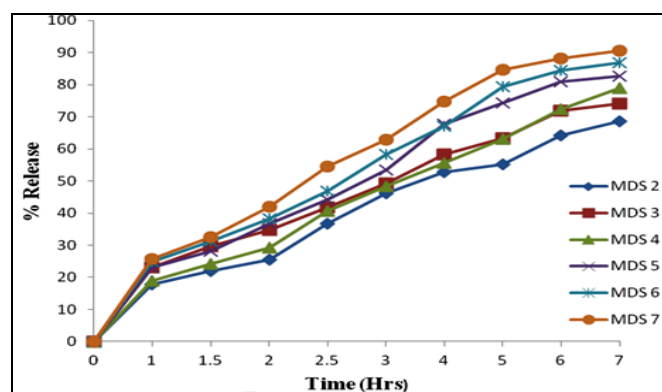on the basis of the highest r^2 values best fitted the Higuchi matrix model.

FIG. 3: IN VITRO DRUG RELEASE PROFILES OF FLUTRIMAZOLE FROM MICROSPONGES

TABLE 2: KINETIC DATA FROM *IN VITRO* DRUG RELEASE MODELS FOR THE MICROSPONGE FORMULATIONS

Formulation code	Zero order		First order		Higuchi	
	r^2	K_0 (mg/hr)	r^2	K (hr^{-1})	r^2	K_h (mg/$hr^{-1/2}$)
MDS 2	0.8812	0.590	0.9431	1.3622	0.9673	0.372
MDS 3	0.8747	0.624	0.9118	1.802	0.9688	0.433
MDS 4	0.8773	0.616	0.8990	1.9221	0.9462	0.411
MDS 5	0.8776	0.609	0.9167	1.9201	0.9472	0.374
MDS 6	0.879	0.604	0.8880	2.013	0.9514	0.387
MDS 7	0.889	0.553	0.9225	2.784	0.94	0.280

***In-vitro* release study from Cream:** 1% weight of Flutrimazole and Flutrimazole microsponges were incorporated into the cream formulation and the drug release from the resultant formulations was studied. The effect of drug: polymer ratio on the release of the drug from cream formulations is shown in **Fig. 4**. Cream containing MDS formulations retard the drug release as compared to cream having free drug. Hence, drug release was observed controlled. The cumulative amount released increased with decrease in concentration of polymer in the formula
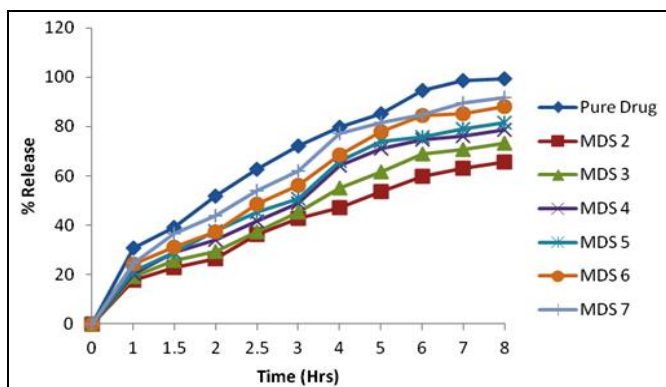
FIG. 4: IN VITRO DRUG RELEASE PROFILES OF FLUTRIMAZOLE FROM CREAM FORMULATION CONTAINING PURE DRUG AND MICROSPONGES.

Effect of various formulation parameters on produced microsponges

1. **Effect of drug: polymer ratio:** Drug: polymer ratio had an effect on the production yield, actual drug content, encapsulation efficiency and size of microsponges with increase in drug: polymer ratio leading to increase in production yield & encapsulation efficiency and decrease in mean particle size as shown in Table 1. Decrease in particle size is probably due to the fact that in high drug to polymer ratios; the amount of polymer available per microsponge was comparatively less. Probably in high drug-polymer ratios less polymer

amounts surround the drug and microsponges with smaller size were obtained [15].

2. **Effect of stirring speed:** The results of stirring rate on the drug content and production yield are listed in **Table 3**. The results showed that increasing stirring rate from 500 to 1500 rpm decreased the production yield but the drug content increased from 79.86 to 87.52%. This indicates that the drug loss was decreased as the stirring rate was increased. It was observed that at the higher stirring rates employed, due to turbulence created within the external phase, polymer then adhered to the paddle and production yield decreased [16].

3. **Effect of inner phase solvent amount:** Table 3 shows the effects of internal phase solvent volume on the production yield, and drug content of MDS 7 formulation. The production yield and drug content of microsponges were decreased due to the lower concentration of the drug in the higher volume of ethanol. The result show that increasing solvent volume (ethanol) decreases particle size. This could be due to decrease in viscosity of solvent.

CONCLUSIONS: This study presents an approach for production of Flutrimazole microsponges with prolonged release characteristics. Ease manufacturing, simple ingredients and wide range actives can be entrapped along with a programmable release make microsponges extremely attractive. Production yield, drug content and encapsulation efficiency of MDS 7 showed that flutrimazole can be loaded in form of microsponges in very high percentages and this is supported by mean particle size value. *In-vitro* drug diffusion study concluded that the microsponges prepared by quasi-emulsion solvent diffusion method controls the release of Flutrimazole for longer period of time when compared to drug itself.

TABLE 3: EFFECT OF STIRRING RATE AND INNER PHASE SOLVENT VOLUME ON THE DRUG CONTENT, PRODUCTION YIELD AND PARTICLE SIZE ON FLUTRIMAZOLE MICROSPONGES PREPARED USING A DRUG: POLYMER RATIO 13:1 (MDS 7)

Parameters	Stirring rate (rpm)			Inner phase solvent volume (ml)		
	500	1000	1500	5	10	15
Mean diameter (µm)	436.454	211.127	132.078	532.80	211.127	89.361
Drug content (%)	79.86	86.3	87.52	86.88	86.3	82.80
Production yield (%)	80.65	76.90	75.46	79.11	76.90	70.96
Theoretical drug content (%)	92.85	92.85	92.85	92.85	92.85	92.85

The release profile of drug from cream and respective microsponge batches does not differ significantly. This was due to no deformation of structure of microsponges during cream preparation. From the present study, it was concluded that smaller drug: polymer ratio shows slower drug release due to formation thicker matrix wall in microsponges. From the in vitro drug release kinetic study, it was concluded that microsponges & cream containing microsponges releases Flutrimazole by following Higuchi matrix model.

MDS holds a promising future in various pharmaceutical applications in the coming years by virtue of their unique properties like small size, efficient carrier characteristics enhanced product performance and elegancy, extended release, reduced irritation, improved thermal, physical, and chemical stability so flexible to develop novel product forms.

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