International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 3, March-April 2021 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Formulation Development and *In-Vitro* Evaluation of Microsponge Drug Delivery System of Antifungal Drug

Vilas S. Bhagat[1], Sanjay R. Arote[2]

[1]Shri Anand College of Pharmacy, Pathardi, Maharashtra, India
[2]Matoshri Institute of Pharmacy, Dhanore, Yeola, Maharashtra, India

ABSTRACT

A Microsponge Delivery System (MDS) is patented, highly cross-linked, porous, polymeric microspheres that can entrap wide range of actives and then release them onto the skin over a time and in response to trigger. It is a unique technology for the controlled release of topical agents and consists of microporous beads, typically 10-25 microns in diameter, loaded with active agent. In this study, Ketoconazole microsponges were prepared by using quasi-emulsion solvent diffusion method. The microsponges thus prepared, were evaluated for production yield, loading efficiency, particle size analysis, *in-vitro* release study of microsponges, and stability. Hence, the present work concluded that MDS has a great potential in topical delivery of drugs like Ketoconazole, with added advantage of reduction in irritation profile due to the controlled release and possible enhancement in the activity due to amorphization of the drug.

KEYWORDS: *Microsponge, microporous beads, particle size analysis*

How to cite this paper: Vilas S. Bhagat | Sanjay R. Arote "Formulation Development and In-Vitro Evaluation of Microsponge Drug Delivery System of Antifungal Drug" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-3, April 2021, pp.654-661, URL: www.ijtsrd.com/papers/ijtsrd39870.pdf

IJTSRD39870

INTRODUCTION

The Philosophy behind development of a novel drug delivery system is to make the therapeutic agent do its best when administered. This means, a high therapeutic efficacy with minimal toxicity. Conventional topical drug delivery systems suffer from many serious drawbacks of drugs and vehicles like: unwanted systemic absorption leading into serious side effects; aesthetically unappealing appearance; low efficacy of vehicle as delivery system; uncontrolled evaporation of the active ingredients; unpleasant odour; incompatibility of one or more drugs with each other or vehicle etc. Hence there is a need to develop a topical drug delivery system that can overcome the abovedrawbacks.[1]

Microsponges are uniform, spherical, porous polymeric microspheres having myriad interconnected voids of particle size range 5-300 µm. These microsponges have the capacity to entrap a wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens and anti-infective, etc. and then release them onto the skin over a time in response to a trigger. Microsponge Delivery System (MDS) can be used to resolve the problem associated with these conventional approaches. The drug entrapped microsponges can be incorporated into a formulated product, such as a gel, cream, liquid or powder. Microsponge is as tiny as a particle of talcum powder. Although they are microscopic in size, these systems are too large to pass through the stratum corneum thus preventing excessive accumulation of drugs within the epidermis and dermis, and hence systemic entry of the drugs. They increase the rate of solubilization of poorly water soluble drugs by entrapping such drugs in pores of the microsponges. As these pores are very small, the drug is in effect reduced to microscopic particles, significantly increasing surface area and thus greatly increasing the rate of solubilization. When microsponge delivery system is applied to the skin, the release of drug can be controlled through diffusion or other variety of triggers, including rubbing, moisture, pH, friction, or ambient skin temperature.

Microsponges consisting of non-collapsible structures with porous surface through which active ingredients are released in a controlled manner. When applied to the skin, the MDS releases its active ingredient on a time mode and also in response to other stimuli (rubbing, temperature, pH, etc). MDStechnology is being used in cosmetics, over-the-counter (OTC) skin care, sunscreens and prescription products. 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 absorb 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. The active payload is protected in the formulation by the microsponge particle; it is delivered to skin via controlled diffusion. Hence the present

work was aimed towards, screening microsponge drug delivery system in topical application to the skin of Ketoconazole.[2]

MATERIALS & METHODS

Materials

The materials used were Ketoconazole(FDC Limited, Raigad), Carbopol 934 NF (Lubrizol Advanced Materials India Pvt. Ltd., Mumbai), Divinyl benzene and Ethyl vinyl benzene(Thermax Ltd., Pune),Eudragit RS 100 (Degussa-Rohm GmbH & Co., Germany) acetone and methanol (Fine Chemicals, Mumbai, India). All other chemicals and solvents were of analytical grade.

Methods

Preformulation Studies

Preformulation testing is the first step in the rational development of dosage forms of a drug. It can be defined as an investigation of physical and chemical properties of drug substance, alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass-produced.[3]

A thorough understanding of physicochemical properties may ultimately provide a rationale for formulation design or support the need for molecular modification or merely confirm that there are no significant barriers to the compounds development. The drugs were tested for organoleptic properties such as appearance, colour, taste, etc.

Spectroscopic Studies

UV spectroscopy: (Determination of λ max)

The stock solutions (100µg/mL) of the drugs were prepared in methanol(Ketoconazole). The stock solutions were appropriately diluted with the respective solvents to obtain a concentration of 20µg /mL. The UV spectrum was recorded in the range of 200-400 nm on Schimadzu 1700 UV spectrophotometer to find the λ_{max}.

IR Spectroscopy

The spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}. A dry sample of the drug and potassium bromide were mixed uniformly and filled into the die cavity of sample holder and an IR spectrum was recorded using diffuse reflectance FTIR spectrophotometer.

Construction of Calibration Curve for Drugs

The stock solution (100 µg/mL) was prepared by dissolving 10 mg of the drug in methanol (Ketoconazole) in a 100 mL volumetric flask. From the stock solution, solutions containing 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/mL of the drugs were prepared by appropriate dilutions. Absorbance of these solutions were measured at 238 nm for Ketoconazole against respective blank solvents.[4]

Drug-Excipient Compatibility Studies

Drug-excipients compatibility studies were carried out for one month. The drug with excipients Eudragit RS 100 & PVA were subjected to storage at room temperature and elevated temperature at 45°C/ 75% RH in stability chamber for one month. After 7, 14, 21 and 30 days the samples were taken to check the following parameter.[5]

Physical change

The samples were checked for physical changes such as discoloration, odor etc.

FTIR study

The dry sample of drug and potassium bromide were mixed uniformly and filled into the die cavity of sample holder and an IR spectrum was recorded using diffuse reflectance FTIR spectrophotometer.[6]

Preparation of Ketoconazole Microsponges by Liquid-liquid Suspension Polymerization method

Ketoconazole was found to be sensitive to reaction conditions of suspension polymerization techniques. Hence quasi-emulsion solvent diffusion method was chosen to prepare Eudragit basedmicrosponges.[7]

Quasi-emulsion Solvent Diffusion method: (Eudragitmicrosponges)

The processing flow chart is presented in Figure 1. To prepare the inner phase, Eudragit RS 100 was dissolved in 3 mL of methanol and triethylcitrate (TEC) was added at an amount of 20% of the polymer in order to facilitate the plasticity. The drug was then added to the solution and dissolved under ultrasonication at 35°C. The inner phase was poured into the PVA (72000) solution in 200 mL of water (outer phase). The resultant mixture was stirred for 60 min, and filtered to separate the microsponges. The microsponges were washed and dried at 40°C for 24h.

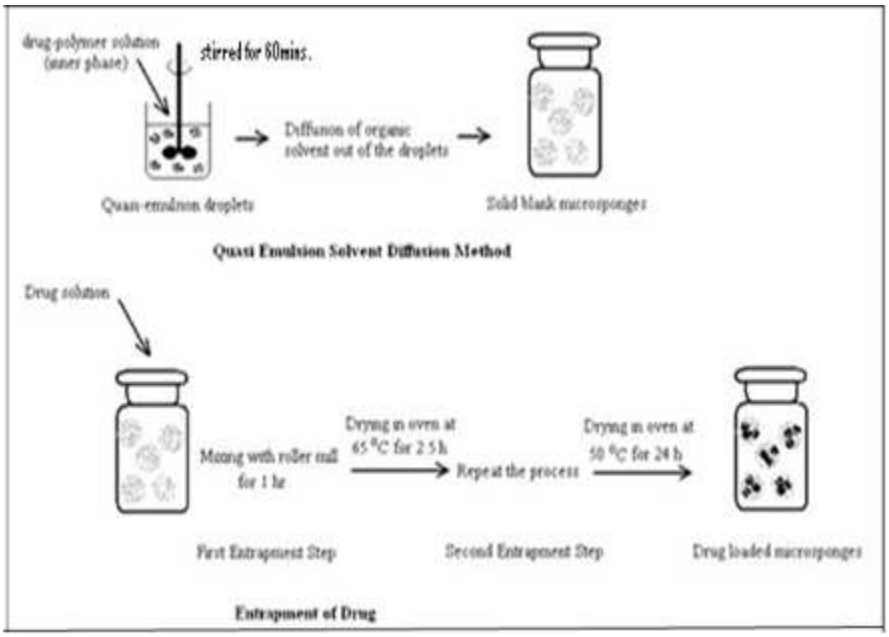

Figure 1: Preparation of microsponges by quasi- emulsion solvent diffusion method

Seven different ratios of drug to Eudragit RS 100 (1:1, 3:1, 5:1, 7:1, 9:1, 11:1 and 13:1) were employed to determine the effects of drug: polymer ratio on physical characteristics and dissolution properties of microsponges. Agitation speed employed was 500 rpm using three blade propeller stirrers.[8-11]

Table 1: Microsponge formulations using Eudragit RS100

Constituents	Ketoconazole Microsponges						
	F10	F11	F12	F13	F14	F15	F16
Inner phase							
Ketoconazole/ Oxiconazole nitrate	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Eudragit RS 100 (g)	2.5	0.83	0.50	0.36	0.28	0.23	0.19
Methanol (mL)	3	3	3	3	3	3	3
Outer phase							
Distilled water (mL)	200	200	200	200	200	200	200
PVA 72000 (mg)	50	50	50	50	50	50	50

Evaluation of Microsponges

Determination of Production Yield and LoadingEfficiency[12-14]

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

$$\text{Production Yield} = \frac{\text{Practical Mass of Microsponges}}{\text{Therotical Mass (polymer + drug)}} \times 100$$

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

$$\text{Loading Efficiency} = \frac{\text{Actual Drug Content in Microsponges}}{\text{Therotical drug Content}} \times 100$$

Scanning Electron Microscopy

For morphology and surface topography, prepared microsponges were coated with platinum at room temperature so that the surface morphology of the microsponges could be studied by SEM.[15]

The SEM, a member of the same family of imaging is the most widely used of all electron beam tools. The SEM employs a focused beam of electrons, with energies typically in the range from a few hundred eV to about 30 keV, which is rastered across the surface of a sample in a rectangular scan pattern. Signals emitted under this electron irradiation are collected, amplified, and then used to modulate the brightness of a suitable display device which is being scanned in synchronism with probe beam.[16]

***In-vitro* Release Study of Microsponges**

Accurately weighed loaded microsponges (5 mg) were placed in 50 ml of methanol in 100 ml glass bottles. The later were horizontally shaken at 37°C at predetermined time intervals. Aliquot samples were withdrawn (replaced with fresh medium) and analysed UV spectrophotometrically at 238 nm for Ketoconazole. The contents of drugs were calculated at different time intervals up to 6hrs.[17-18]

RESULTS AND DISCUSSION

Preformulation Study

Table 2: Characterization of Ketoconazole PureDrug

Sr. No.	Characters	Specification	Result
1.	Description	White to off-white, crystalline powder	White tooff-white, crystallinepowder
2.	Melting point	148-152°C	149-151°C
3.	Solubility	Freely soluble in dichloromethane; soluble in chloroform and in methanol; sparingly soluble in ethanol (95%); practically insoluble in water and in ether	Freely soluble in dichloromethane; soluble in chloroform and in methanol; sparingly soluble in ethanol (95%); practically insoluble in water and in ether

Spectroscopic Studies

UV Spectroscopy: (Determination of λ_{max})

The UV spectrum of Ketoconazole, in methanol was scanned and $\lambda_{max}$was found to be 238 nm.

IR Spectroscopy: IR Spectra of Ketoconazole, in their pure form was recorded.Results are depicted in Figure No. 2 and Table No. 3 respectively.

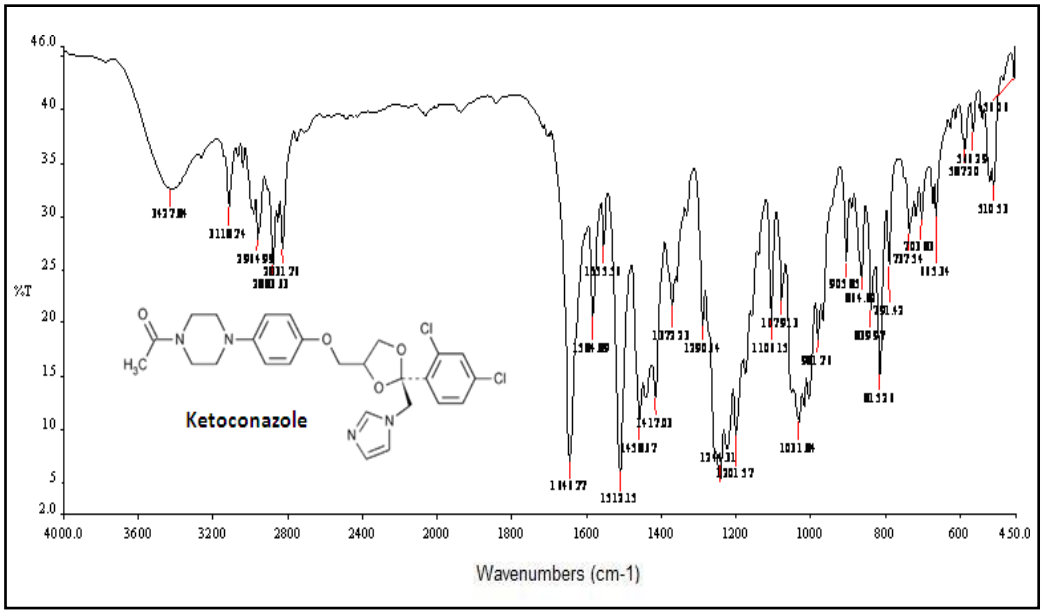

Figure 2: IR Spectra of Ketoconazole

Table 3: IR spectrum interpretation of Ketoconazole

Functional group	Wave number observed (cm^{-1})
C=O (carbonyl group)	1646.77
C-O (aliphatic ether group)	1031.84
C-O (cyclic ether)	1244.31

Construction of calibration curve

Table 4: Calibration curve data for Ketoconazole

Sr. No.	Concentration (µg/mL)	Absorbance at 238 nm*
1.	2	0.056± 0.0031
2.	4	0.09±0.0025
3.	6	0.128±0.0024
4.	8	0.176±0.0012
5.	10	0.195±0.0036
6.	12	0.233±0.0014
7.	14	0.263±0.0047
8.	16	0.302±0.0024
9.	18	0.333±0.0071
10.	20	0.37±0.0031

*Each value is average of three separate determinations ±SD

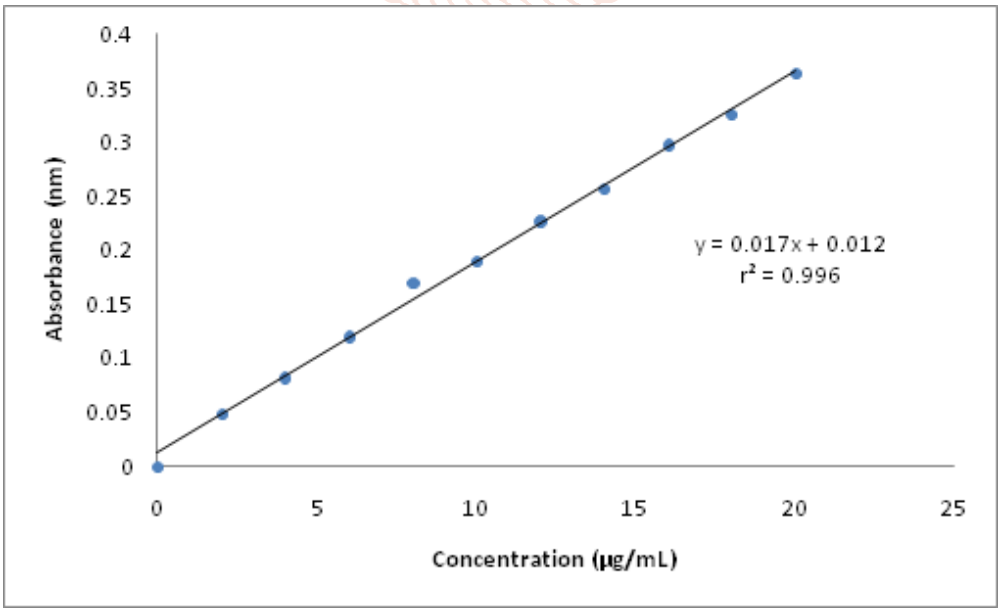

Figure 3: Calibration curve of Ketoconazole

Drug-excipient compatibility studies

Physical Change

No physical changes such as discoloration; change in texture etc were observed during compatibility study.

FTIR Study

FTIR spectra of all the three 'pure drugs' and 'drug entrapped microsponges' were compared to study incompatibility of drugs with excipients and reaction conditions. Principal peaks of microsponge-entrapped drugs were compared with peaks of pure drugs to know about whether they are concordant with each other. Overlay FTIR spectra of pure and entrapped drugs are shown in Figure 4. Principle peaks of drugs were observed retained; broadening of peaks may be due to overlapping of peaks of polymer system and drug in microsponge formulation.

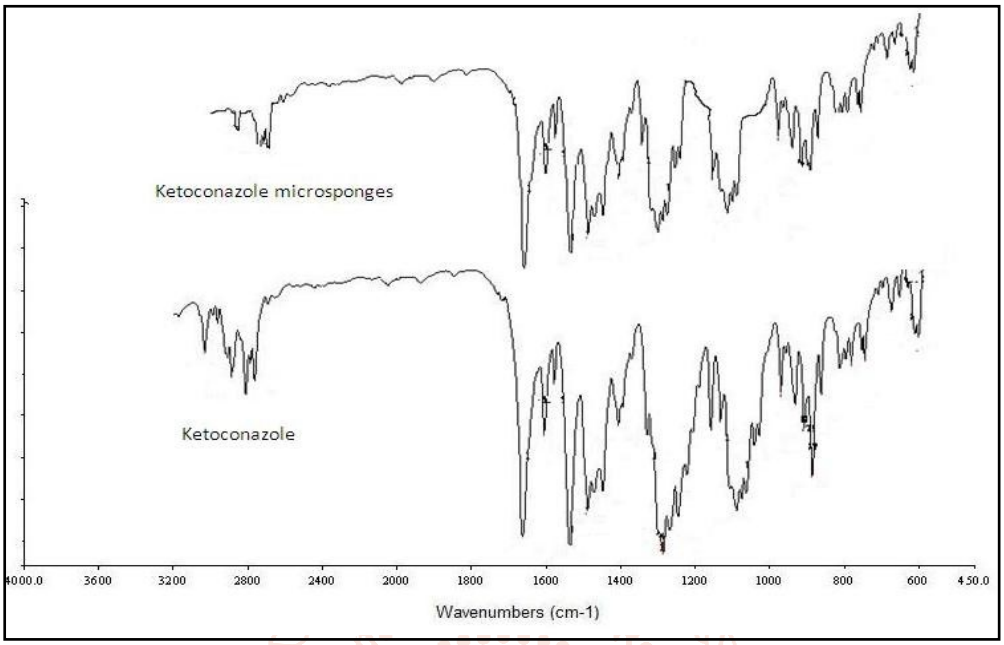

Figure 4: Compatibility study of Ketoconazoleby IR

Evaluation of Microsponges

Production Yield

Table 5: Production yield of Ketoconazolemicrosponge

Formulation code	Production yield (%)
F10	77.19±2.13
F11	80.23±1.17
F12	82.21±1.23
F13	85.12±2.01
F14	87.25±1.14
F15	89.14±1.90
F16	90.17±2.16

*Each value is average of three separate determinations ±SD

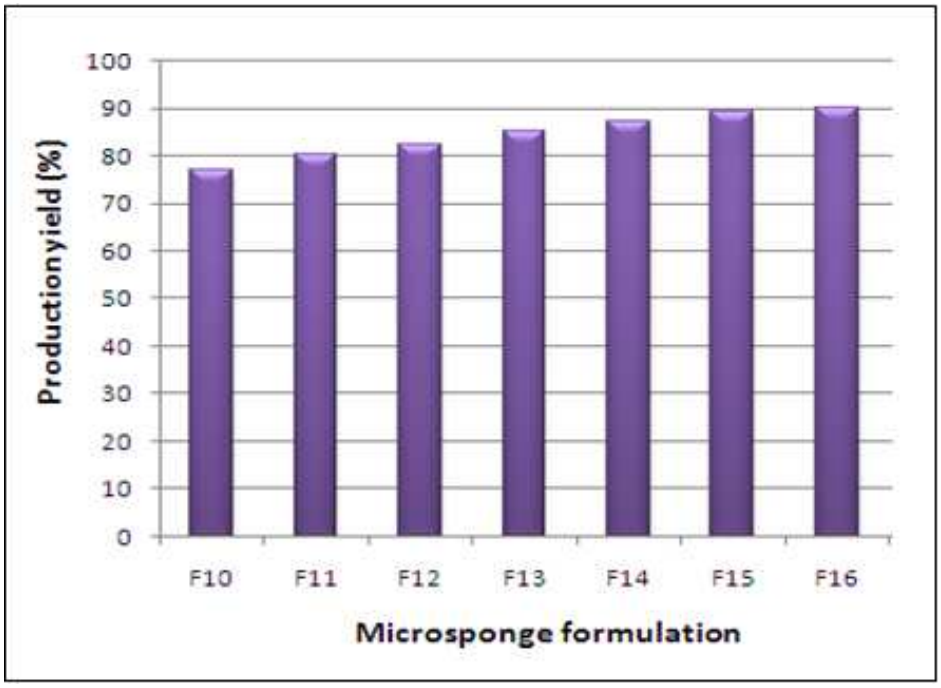

Figure 5: Production yield of Ketoconazolemicrosponge formulations

Production yield of Ketoconazole microsponges were between 77.19 to 90.17 % (Table 5). In case of Eudragit RS 100 microsponges, it was revealed that, by increasing drug: polymer ratio there is increase in the production yield of the microsponges.

Drug Loading Efficiency

Table 6: Drug loading efficiency of Ketoconazolemicrosponge formulations

Formulation code	Drug Loading efficiency (%)
F10	85.36±1.32
F11	86.45±0.69
F12	88.49±2.01
F13	90.86±0.27
F14	92.38±1.26
F15	94.21±0.39
F16	94.89±0.16

*Each value is average of three separate determinations ±SD

Figure 6: Loading efficiency of Ketoconazolemicrosponge formulations

The loading efficiency was found to be 85.36 to 94.89 % in Ketoconazole microsponges. In case of Eudragit RS 100 microsponges, it was found that as drug: polymer ratio increases, drug loading efficiency also increases.

Scanning Electron Microscopy

The morphology of the microsponges prepared were investigated by SEM. The representative SEM photographs of the microsponges are shown in Figure 7. Closer view of a microsponge revealed the characteristic internal pores on surfaces. Eudragit RS 100 microsponges prepared by quasi-emulsion solvent diffusion method (Ketoconazole) were comparatively less spherical.

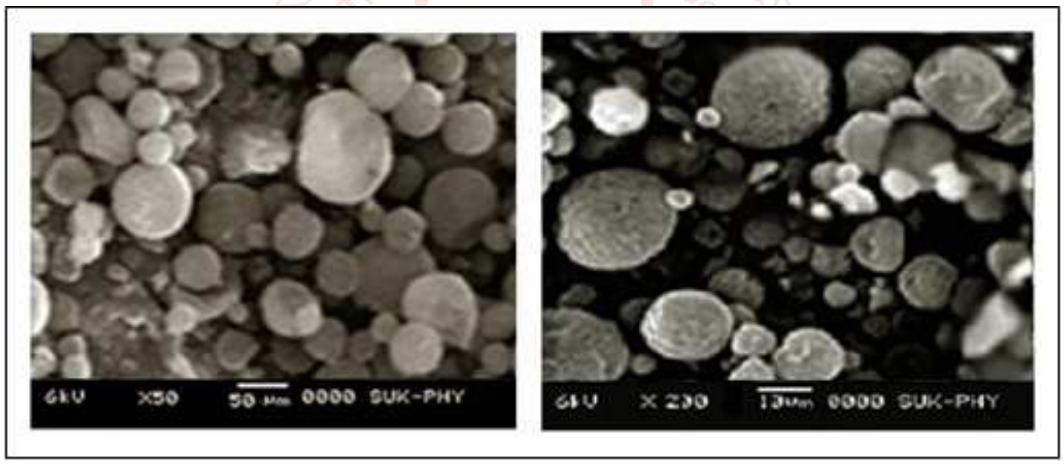

Figure 7: SEM Photographs of Ketoconazolemicrosponges

***In-vitro* Release Study of Microsponge**

Table 7: In-vitro release study of Ketoconazolemicrosponges

Time (Min)	Cumulative % drug release						
	F10	F11	F12	F13	F14	F15	F16
0	0	0	0	0	0	0	0
15	19.96±1.52	22.13±1.13	19.36±1.53	19.65±1.58	22.18±1.27	23.83±1.60	18.35±0.40
30	27.63±1.16	29.48±1.52	26.35±1.30	26.54±1.12	29.54±1.70	31.52±1.66	26.52±1.33
45	36.48±1.53	35.41±1.50	35.87±1.57	33.65±1.52	37.56±1.49	36.84±1.73	38.47±1.28
60	46.98±1.20	44.32±1.35	41.28±1.50	41.74±1.19	44.54±1.73	41.68±1.43	45.92±1.63
120	58.68±1.51	50.91±1.18	49.86±1.88	48.92±1.52	48.92±1.58	49.63±1.52	50.45±0.28
180	64.21±1.44	58.54±1.40	54.64±1.31	52.63±1.85	51.36±1.97	52.36±1.52	56.69±0.37
240	67.85±1.55	64.86±1.42	61.24±1.59	59.87±2.15	57.62±1.52	57.85±1.58	60.36±0.78
300	71.52±1.51	72.52±1.54	68.87±1.09	66.32±1.52	65.21±1.52	62.96±1.52	64.12±1.16
360	75.62±1.48	77.25±1.29	73.65±1.09	71.96±0.75	74.85±1.53	68.32±1.53	75.85±1.18

*Each value is average of three separate determinations ±SD

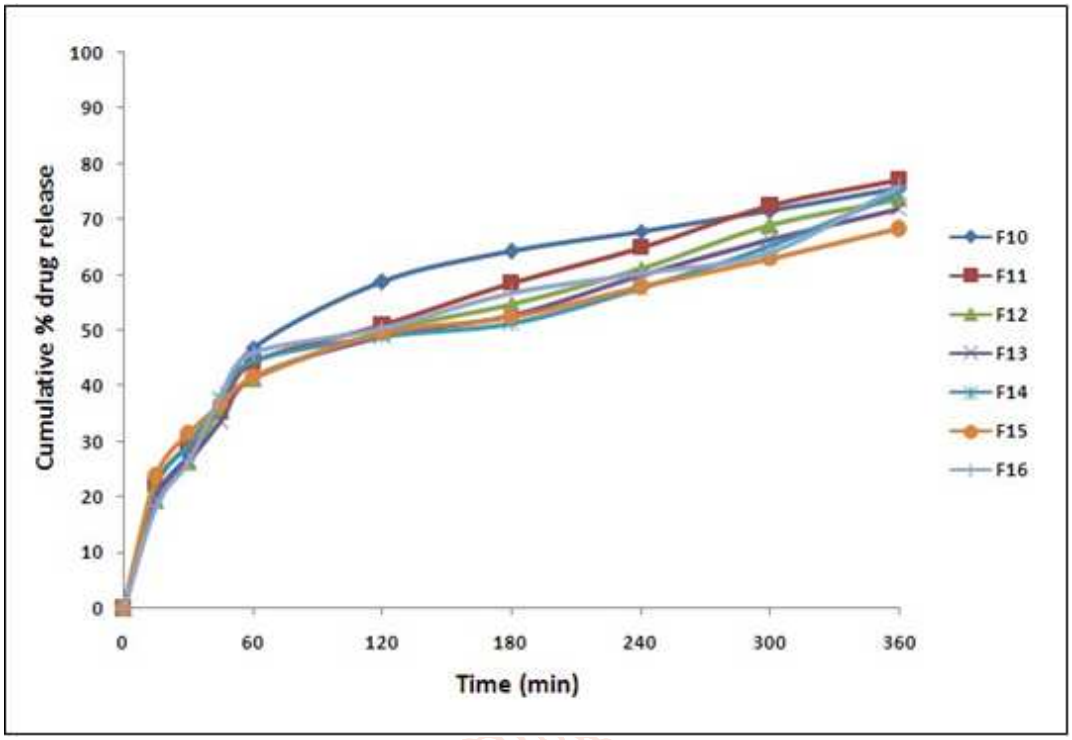

Figure 8: In-vitro drug release profiles of Ketoconazole microsponge formulations

The drug release profiles of the Ketoconazole microsponge formulations are illustrated in Table 7 and Figure 8. Drug release from Ketoconazole microsponge was found to range from 68.32 % to 77.25 % from all the formulations.

CONCLUSION

Quasi-emulsion solvent diffusion is now a days the preferred method to prepare porous micro particles. Eudragit RS100 microsponges containing Ketoconazole and Oxiconazole nitrate were successfully prepared by this method as these drugs were found incompatible with reaction conditions of liquid-liquid suspension polymerization. Seven, drug: polymer ratios were investigated (1:1, 3:1, 5:1, 7:1, 9:1, 11:1 and 13:1) for Eudragit based MDS. For Eudragit based microsponges, the mean particle size was found to increase with the decrease in the polymer amount. The microsponges showed homogenous particle size distribution with three blade centrifugal stirrer. Stirring speed and time has profound effect on particle size and size distribution of microsponges. The increase in the stirring rate resulted in a reduction in mean particle size. The results of loading efficiency showed that the higher drug loading efficiencies were obtained at the higher drug: polymerratios. The relatively high production yield and loading efficiency of ethanol entrapped styrene microsponges and Eudragit RS100 microsponges indicated that both the methods are suitable for preparing the microsponge formulations. Quasi-emulsion solvent diffusion method is simple, less time consuming and involves use of safer ingredients than free radical polymerization and hence more preferred. The microsponges differ from regular microspheres with their highly porous surface. This characteristic gives property to release the drug at a faster rate through the pores. Due to smaller pore diameter, the EudragitRs 100 microsponges showed less and slower drug release as compared with the styrene microsponge formulations, in the *in-vitro* release studies. Hence microsponge drug delivery system has great potential in improving therapeutic efficacy of topical delivery of Ketoconazole.

REFERENCES

[1] Vikas Jain., Ranjit Singh., Dicyclomine-loaded Eudragit-based Microsponge with Potential for Colonic Delivery: Preparation and Characterization, *Trop J Pharm Res.*, (2010); 9(1): 67-72

[2] Southwell D, Barry BW, Woodford R. Variations in permeability of human skin within and between specimens. *Int J Pharm.,* (1984); 18: 299 - 309.

[3] Wester R. C., Maibach H. I., Regional variation in percutaneous absorption. In: Bronaugh R L, Maibach H I, eds. *Percutaneous Absorption; Drugs-Cosmetics-Mechanisms-Methodology,* 3rd edn. Marcel Dekker: New York, (1999); 107-116.

[4] Purushotham Rao K., Khaliq K., Sagare P., Patil S. K., Kharat S. S., Alpana K., Formulation and Evaluation of Vanishing Cream for Scalp Psoriasis, *Int J Pharm Sci Tech.*, (2010); 4 (1): 32-41.

[5] Bhise S. B., More A. B., Malayandi R., Formulation and *In-vitro* Evaluation of Rifampicin Loaded Porous Microspheres, *ScientiaPharmaceutica.,* (2010); 78: 291-302.

[6] Ellaithy H. M., El-Shaboury K. M. F., The Development of CutinaLipogels and Gel Microemulsion for Topical Administration of Fluconazole, *AAPS Pharm Sci Tech.,* (2002); 3 (4): 1-9.

[7] Gordon L Flynn. Cutaneous and Transdermal Delivery – Process and Systems of Delivery, Chapter 8, In: Modern Pharmaceutics, Edited by Gilbert S. Banker and Christopher T Rhodes., (2002): 187- 233.

[8] Jain Ankur, Surya P Gautam P, Yashwant Gupta, Hemant Khambete, Sanjay Jain., Development and Characterization of Ketoconazole Emulgel for Topical Drug Delivery, *Der Pharmacia Sinica.,* (2010), 1 (3): 221-231.

[9] Amato M., Isenschmid M., Hippi P., Percutaneous Caffeine Application in the Treatment of Neonatal

Apnoea, *Eur J Pediatr.*, (1991); 150: 592-594.

[10] Draize J. H., Woodard G., Calvery H. O., Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes, *J PharmacolExpTher.,* (1944); 82: 377-390.

[11] John I. D'souza., More H. N., Topical Anti-Inflammatory Gels of FluocinoloneAcetonide Entrapped in Eudragit Based Microsponge Delivery System, *Research J. Pharm. and Tech.,* (2008); 1(4):502-506.

[12] Kawashima Y., Iwamoto T., Niwa T., Takeuchi H., Hino T. Control of Prolonged Drug Release and Compression Properties of Ibuprofen Microsponges with Acrylic Polymer, Eudragit RS, by Changing Their Interparticle Porosity, *Chem Pharm Bull.*, (1992); 40(1): 196-201.

[13] Lavrijsen A. P. M., Oestmann E., Hermans J., Bodde H., Vermeer B. J., Ponec M., Barrier Function Parameters in Various Keratinisation Disorders: Transepithelial Water Loss and Vascular Response to Hexyl Nicotinate, *Br J Dermatol.*, (1993); 129: 547-553.

[14] Ellaithy H. M., El-Shaboury K. M. F., The Development of CutinaLipogels and Gel Microemulsion for Topical Administration of Fluconazole, *AAPS Pharm Sci Tech.,* (2002); 3 (4): 1-9.

[15] Gupta M. M., Srivastava B., Sharma M., Arya V., Spherical Crystallization: A Tool of Particle Engineering for Making Drug Powder Suitable for Direct Compression, *Int J Pharma Res Develop.,* (2010); 1(12): 1-10.

[16] Joshi M. D., Patravale V. B., Formulation and Evaluation of Nanostructured Lipid Carrier (NLC) Based Gel of Valdecoxib, *Drug Dev Ind Pharm.,* (2006); 32: 911-918.

[17] Pradhan S. K., Microsponges as the Versatile Tool for Drug Delivery System, *Int J Res Pharm Chem.*, (2011); 1(2): 243-258.

[18] Southwell D, Barry BW, Woodford R. Variations in permeability of human skin within and between specimens. *Int J Pharm.,* (1984); 18: 299 - 309.