Available online at http://jddtonline.info

RESEARCH ARTICLE

ETHYL CELLULOSE BASED MICROSPONGE DELIVERY SYSTEM FOR ANTI-FUNGAL VAGINAL GELS OF TIOCONAZOLE

Katkade Mayur*[1], Kalkotwar Ramesh[2], Jain Nitin [1], Patil Prashant[1], Gadakh Rajendra[2], Naikwade Jeevan[1]

[1]S.N.D. College of Pharmacy, Yeola, Nashik, India

[2]Matoshri College of Pharmacy, Eklahare, Nashik, India

**Corresponding Author's Email Address: mayukatkade@gmail.com, Contact No.: +91 9421091092*

ABSTRACT:

Valvovaginal Candidiasis is a fungal infection of the vagina and causes are itching, burning, soreness, dysparunia and phenohypical signs such as vaginal and vulvar erythema and edema caused by various species of the genus Candida. Tioconazole is an antifungal medication of the Imidazole class used to treat infections caused by a fungus or yeast. Negative aspects associated with oral systemic antifungal therapy for Vulvovaginal Candidiasis include its limited success rate, toxicity, contraindications, drug interactions, high cost of medication and increased microbial resistance. Orally administered tioconazole is extensively metabolized, major metabolites are glucuronide conjugates. Topical therapy does not lead to systemic side effects or drug interactions. The aim of the study was to produce Ethyl Cellulose microsponge loaded with Tioconazole gel which was able to control the release of Tioconazole to the vaginal tissue. Drug content, Encapsulation efficiency and Percentage yield as such 73.97±0.01, 92.15±0.02 and 81.57±2.87 were determined in the prepared microsponges. The Scanning electron microscopy (SEM) of microsponges showed that they were spherical in shape and contained pores. Tioconazole microsponges were then incorporated into gel for release studies. It was found that the 12 hrs in-vitro drug release study of microsponge was best studied by Korsmeyer Peppas model.

Key Words: Ethyl Cellulose, Microsponge Delivery System, Tioconazole, Vulvovaginal Candidiasis.

INTRODUCTION

Microsponges are polymeric delivery systems composed of porous microspheres of an inert polymer that can entrap active ingredients and control their delivery rate. They are tiny true sponge like spherical particles that consists of myriad of interconnecting voids within a non-collapsible structure with large porous surface. The size of these microsponges can be varied, usually from 5 to 300µm in diameter depending on the degree of smoothness[1].

However by optimizing formulation parameters such as drug : polymer ratio and agitation/ stirring rate it might be possible to manufacture nanosponge bead is of 25µm sized spheres which can have up to 2,50,000 pores and an average internal pore structure equivalent to 10 feet in length and average pore volume of about 1ml/g. The surface can be varied from 0.1 to 500m^2/g and pore volume range from 0.1 to 0.3cm^2/g. Since bacteria cannot penetrate a pore of this size, the beads once manufactured, remain sterile and do not require a preservative, once made they are very hard. This results in a large reservoir within each microsponge, which can be loaded with active agent up to its own weight[2,3,4,5].

Tioconazole interacts with 14-α-demethylase, a cytochrome P-450 enzyme that converts Lanosterol to Ergosterol, an essential component of the yeast membrane. In this way, Tioconazole inhibits ergosterol synthesis, resulting in increased cellular permeability. Tioconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms and the uptake of purine, impair triglyceride and/or phospholipid biosynthesis, and inhibit the movement of calcium and potassium ions across the cell membrane by blocking the ion transport pathway known as the Gardos channel[6,7].

The proposed work involves formulation and evaluation of Tioconazole microsponge formulation by using Ethyl Cellulose as polymer. And finally the optimized tioconazole microsponges formulation are incorporated into gel to apply on vaginal tissue for the treatment of vaginal candidiasis and reduced the side effects. Hence, in the present work an attempt was to develop controlled release microsponges using synthetic polymer to minimize frequent dosing, prolong the pharmacological effect and thus improve patient compliance.

MATERIALS AND METHODS:

Tioconazole is gift sample from Apollo Life Sciences pvt. ltd. Mumbai. Ethyl Cellulose, PVA and Carbopol 940 were purchased from S. D. fine chemicals, Mumbai.

Method of Preparation of Microsponge:

The microsponges were prepared by a quasi-emulsion solvent diffusion method. The Method two steps, In first step inner phase was prepared and in second step outer phase prepared outer phase was prepared. Inner phase was prepared by dissolving a Ethyl Cellulose polymer in Dichloromethane and then after Tioconazole drug added in to solution. Then after Outer phase was prepared by dissolving PVA in distilled water and the process is carried out at room temperature. Then inner phase poured into the outer phase at room temperature.

After emulsification, the mixture was continuously stirred for 5-6 hours at 2000 rpm. Then the mixture was filtered to separate the microsponges. The product was dried by vacuum oven at 40°C for 24 hours [8-10]. The microsponges were prepared by 3^2 factorial designs using volume of internal phase (X1) and stirring rate (X2) as independent variables table no. 1 gives detailed of the formulation batches. Once the formulation was prepared characterization were done by determining percent yield, drug loading and encapsulation efficiency, Fourier Transform Infrared (FTIR), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM) and *In-vitro* study.

Preparation of Tioconazole Microsponge Gel:

Gel of tioconazole is prepared by using following formula given in table no. 2. A clear dispersion of Carbopol was prepared in water using moderate agitation. Tioconazole microsponge was dissolved in propylene glycol methanol. Various ingredients viz. parabens, sodium metabisulphite and disodium edetate were dissolved in water and added to the drug solvent system. Triethanolamine is used to neutralize and volume made up with water. Gels prepared were degassed by ultrasonication[11].

Table 1: Batches of Microsponge

Batches	Drug (Tioconazole) (in gm)	Ethyl Cellulose (in gm)	Polyvinyl Alcohol (in gm)	DCM (in ml)	Water (in ml)
F1	1.0	0.4	0.05	20	100
F2	1.5	0.4	0.05	20	100
F3	2.0	0.4	0.05	20	100
F4	1.0	0.5	0.05	20	100
F5	1.5	0.5	0.05	20	100
F6	2.0	0.5	0.05	20	100
F7	1.0	0.6	0.05	20	100
F8	1.5	0.6	0.05	20	100
F9	2.0	0.6	0.05	20	100

Table 2: Formulation of Tioconazole gels containing free and microsponge-entrapped drug

S. No.	Ingredients	Formulations (% w/w)
1	Tioconazole (free or entrapped, equivalent to)	0.02
2	Propylene glycol	40.0
3	Methanol	8.0
4	Menthol	0.04
5	Methyl Paraben	0.18
6	Propyl Paraben	0.02
7	Sodium Metabisulphite	0.1
8	Disodium edetate	0.1
9	Carbopol 940	1.0
11	Triethanolamine	Q.S.
12	Lavender	Q.S.
13	Water Q.S.	100

A. Microsponge Evaluation[12,13]

Compatibility Studies

FTIR helps to confirm the identity of the drug and to detect the interaction of the drug with carriers. FTIR of pure drug and physical mixture of drug with polymer were obtained on FTIR instrument. The spectrum was scanned over the wave number range of 4000-400 cm^{-1}.

Particle Size and Shape

Scanning Electron Microscopy (JSM 840A) was used to study the surface morphology of the microsponge formulation. The samples were analyzed after they had been gold sputtered (coated) using 25nm gold film thickness.

Encapsulation Efficiency:

10 mg of microsponge were accurately weighed and microsponges were dissolved in 10 ml in Chloroform. Then it was filtered through Whatmann filter paper no 44 and 1 ml of this solution diluted to 10 ml with solution and absorbance was measured at 248 nm. The drug content was determined from the standard curve.

Percent Yield:

The yields of production of microsponge of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of solid dispersions and percent production yields.

DSC

The DSC study was performed on a Shimadzu (DSC 60 A) Differential Scanning Calorimeter with thermal analyzer. Accurately weighed samples (about 3 mg of samples) were placed in sealed aluminum pan, before heating under nitrogen flow (20 ml/min) at a scanning rate of 10^0C per min from 20^0C to 300^0C. An empty aluminum pan was used as reference.

B. Gel Evaluation[14,15]

Appearance:

The physical appearance showed that all the systems were gelled. This observation was made by visual inspection of the formulated gels.

Fragrance Efficiency

The various prepared formulation was observed for their smell due to the presence of the fragrance compound added in the formulation.

Determination of pH:

The pH of gel formulation was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of formulation was done.

Determination of Homogeneity:

The developed gel was tested for homogeneity by visual inspection after the gel has been set in the container. The gel was tested for its appearance and presence of any aggregates.

Determination of Viscosity:

Rheology of gel formulation containing Tioconazole was studied by Brookfield digital viscometer (Model no: DV-2P L- Model) using spindle S96, at 20 rpm and torque 60% to 100%, at 25±1^{0}C.

Determination of Extrudability:

The formulation was filled in the collapsible tube after the gel was set in the container. The Extrudability of the formulation was determined in terms of weights in grams required to extrude a 0.5 cm. ribbon of gel in 10 seconds.

Determination of Drug Content:

Weighed accurately 2.5gm of Tioconazole gel, (100 mg equivalent) and transferred into a 100ml of volumetric flask. The volume was made up to 100ml using Phosphate Buffer pH 4.0 and filtered it, 5ml of this solution was pipette out and diluted up to 50ml using Phosphate Buffer pH 4.0. The absorbance of the solution was measured using UV spectrophotometer at 248nm.

***In-vitro* Diffusion Study:**

The *in-vitro* release of Tioconazole from the gel formulation was studied through Sheep Vaginal tissue using Diffusion Cell apparatus. The diffusion medium used was freshly prepared PBS 4.0. Finally the separated membrane was washed with distilled water for several times. Sheep vaginal tissue was soaked overnight in the diffusion medium and cut into required size as of the diameter of the donor chamber and the membrane was mounted between the two compartment of the diffusion cell and 5gm of Tioconazole gel equivalent to 200 mg of Tioconazole was applied on the drug releasing surface. The diffusion profile of the drug was obtained by sampling 1ml of an aliquot from the receptor solution at predetermined intervals until 12hours (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12). The sampled aliquot was every time replaced by fresh diffusion medium of the same quantity. Throughout the study temperature of 37+ 0.5°C and speed of 100 rpm was maintained[16,17].

Stability Studies:

The developed microsponge formulation found to be stable upon storage of 12 weeks. No change was observed in their physical appearance, pH, rheological properties, Drug content and drug release profiles.

RESULTS:

Compatibility studies

The peak at 3090.25 cm^{-1} indicate ring C-H Streching, 1688.03 cm^{-1} for the Aromatic C=C Stretching, 1262.10 cm^{-1} for the C-N Stretching, 657.83 cm^{-1} for the C-S Stretching, 1223.37 cm^{-1} for the C-O Stretching and 732.76 cm^{-1} for the C-Cl Stretching. These are the major peaks of the spectra of the drug. All these peaks were present in the spectra of formulation and thus this confirms that the drug did not interact with the excipients shown in fig. 1, 2 and 3.

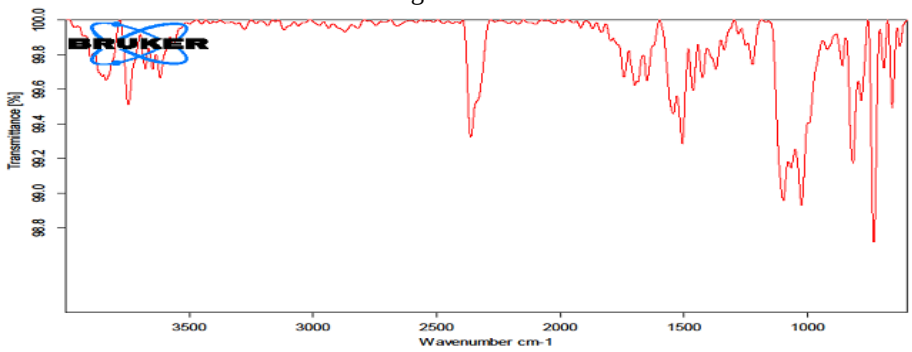

Figure1: FTIR Spectrum of Tioconazole drug

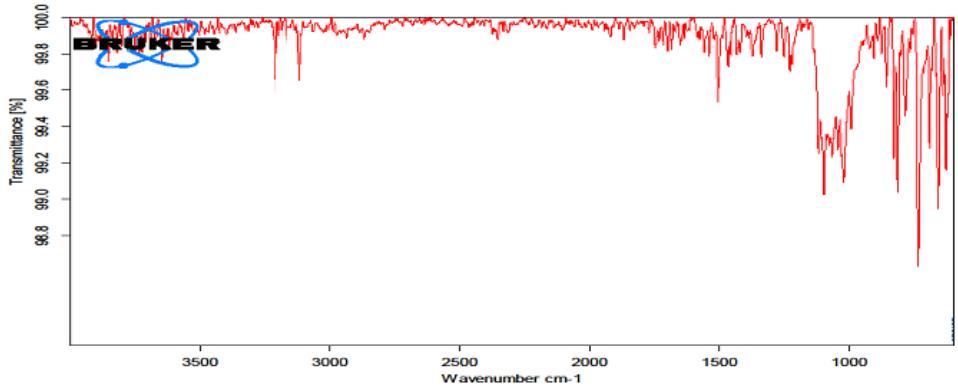

Figure 2: FTIR Spectrum of polymer

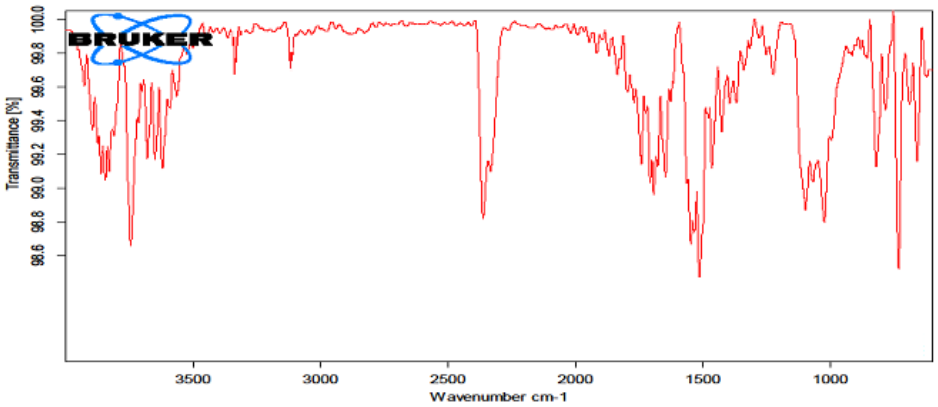

Figure 3: FTIR Spectrum of Microsponge

Evaluation of Microsponge

Particle Size and Shape

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 4 and 5. SEM images showed the microsponges porous and spherical in shape.

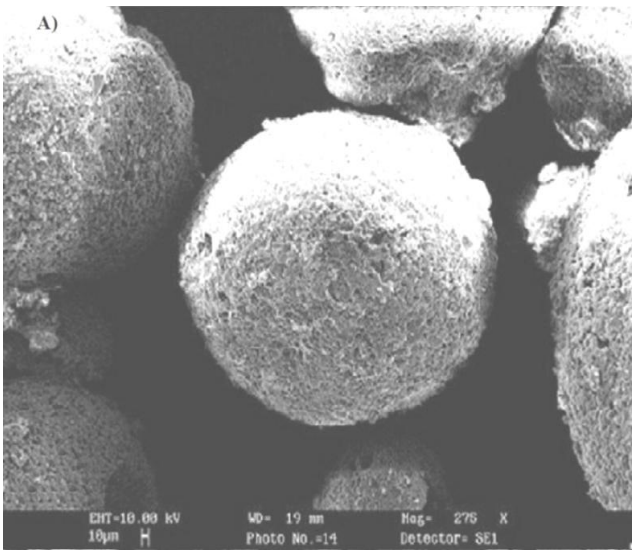

Figure 4: View Showing Smooth Surface of Microsponge

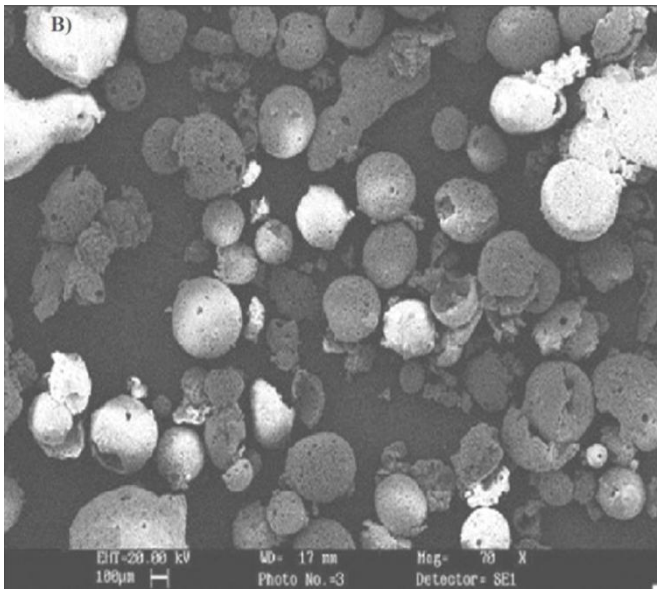

Figure 5: View Showing Microsponge

Percentage Yield and Encapsulation Efficiency

Percentage Yield and Encapsulation Efficiency of Tioconazole microsoponge formulation are given in Table 3. Batch F6 showed the production yield of more than 80% and Encapsulation efficiency is 92 % so only these batches were further evaluated for in vitro drug release.

Table 3: Evaluation of Microsponge: Encapsulation Efficiency and Percentage Yield

Sr. No.	Batch Code	Encapsulation Efficiency	Percentage Yield
1	F1	84.21±0.01	70.55±1.68
2	F2	86.43±0.03	71.50±2.45
3	F3	89.13±0.02	73.68±2.38
4	F4	84.51±0.01	72.11±1.97
5	F5	90.82±0.01	78.08±2.07
6	**F6**	**92.15±0.02**	**81.57±2.87**
7	F7	84.02±0.01	71.40±1.34
8	F8	88.68±0.02	77.00±2.54
9	F9	89.19±0.02	76.57±1.89

 ISSN: 2250-1177 CODEN (USA): JDDTAO

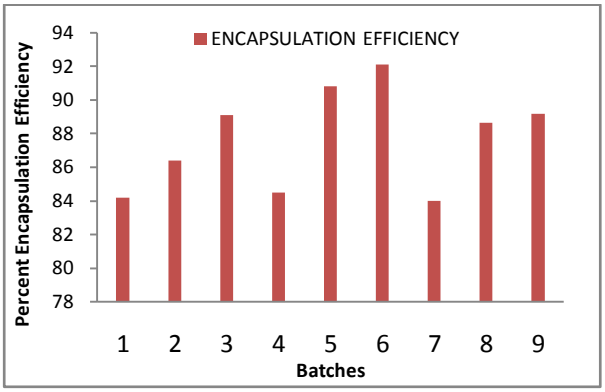

Figure 6: Graph Showing Percent Encapsulation Efficiency

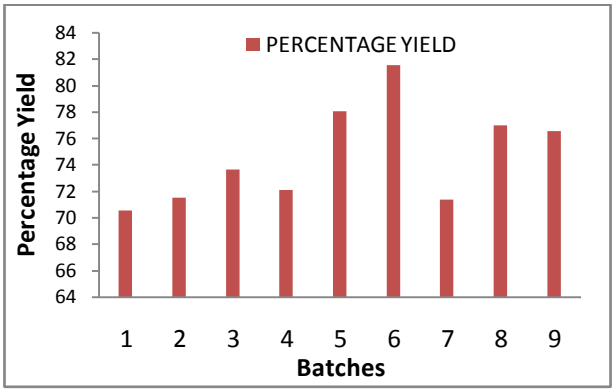

Figure 7: Graph Showing Percentage Yield

DSC

The results were illustrated in fig. no.8, 9 and 10.

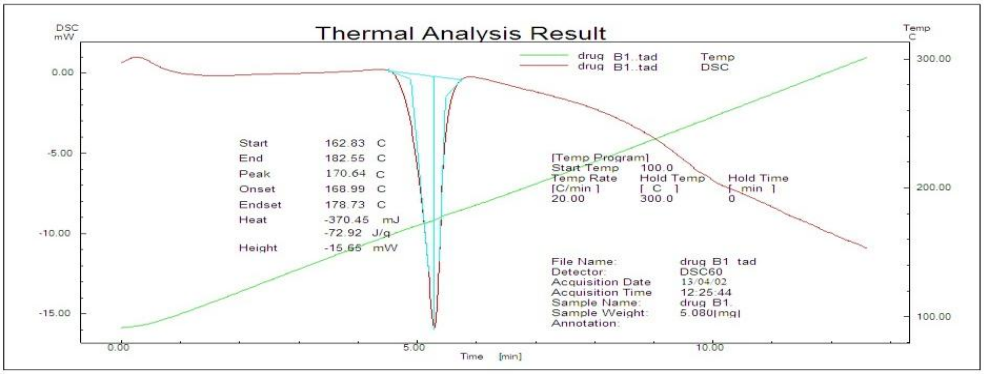

Figure 8: DSC spectrum of Tioconazole Drug

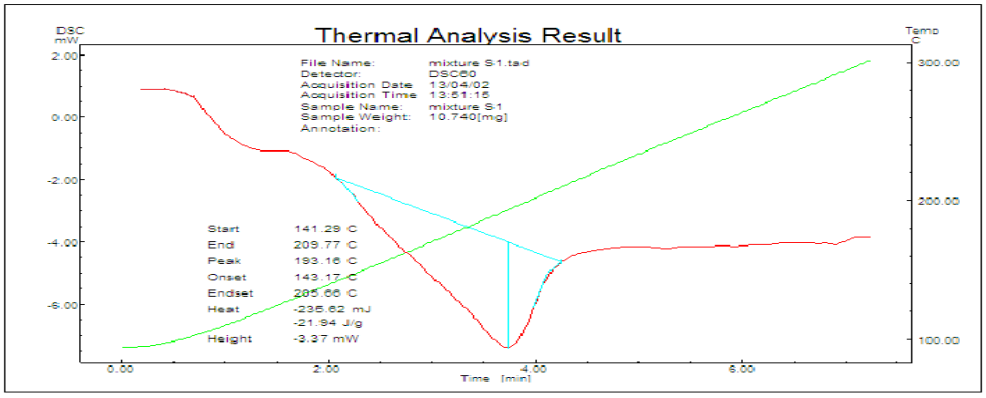

Figure 9: DSC spectrum of Polymer

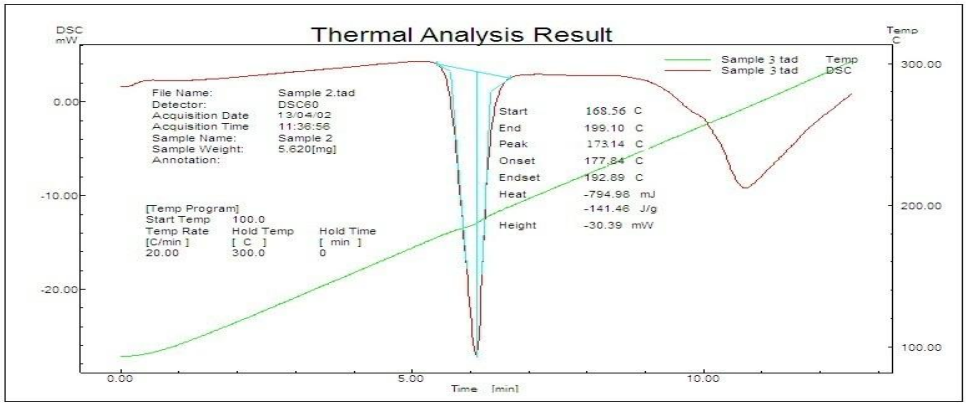

Figure 10: DSC spectrum of Microsponge

 ISSN: 2250-1177 CODEN (USA): JDDTAO

The thermogram of Tioconazole Microsponge showed peak at 173.14^{0}C indicating the melting point of the drug is 171^{0}C which is identical to the melting point determined by open capillary method. The DSC thermogram of Tioconazole showed a sharp endothermal peak (168^{0}C-170 $\pm$3^{0}C).

Gel Evaluation

Table 4: Evaluation Parameters and its Observation/ Results

Sr. No.	Evaluation Parameters	Observation / Results
1	Appearance	Whitish in Colour
2	Fragrance Efficiency	Pleasant Odour
3	pH of Gel	5.6-6.0
4	Homogeneity	Good
5	Viscosity	48,528±145 cP.
6	Extrudability	15.15±0.27gm.
7	Drug Content	93.76±2.35%.

From the Table no. 4 Evaluation of Microsponge Gel follow all the parameters or results.

***In-Vitro* Diffusion Studies**

The *in-vitro* diffusion data obtained for formulation plane drug gel and Microsponge gel are shown in fig no. 11 and Table no. 5.

The formulations showed Percent Cumulative Drug Release (%CDR) of Plane Drug Gel was 91.326%. And the formulation of Microsponge Gel which controlled the % release of drug shows the %CDR was 95.467%.

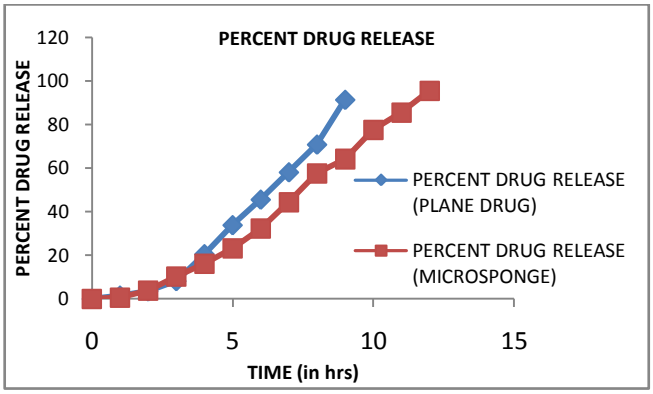

Figure 11: Graph Showing the Comparison between Percent Drug Release of Microsponge Gel and Plane drug gel

Table 5: Release kinetics of microsponge in 4.0 pH Phosphate buffer

Sr. No.	Model	Correlation Coefficient (r^2)	
		Plane Drug Gel	Microsponge Gel
1	Zero Order Kinetics	0.9430	0.9663
2	First Order Kinetics	0.8168	0.8376
3	Matrix T- Test	0.7995	0.8328
4	Korsmeyer Peppas model	0.9921	0.9929
5	Hix. Crow T- Test	0.8735	0.9006

The mode of drug release from microsponge was evaluated using Korsmeyer Peppas model. The correlation coefficient (r^2) was

A. Plane Drug Formulation = 0.9921
B. Microsponge Formulation = 0.9929

Stability Study

The result table of stability study is tabulated in Table No. 6

Table 6: Stability study data for Optimum formulation of Microsponge

Sr. No.	Weeks	% Drug Release at 40±2 ^{0}C and RH 75±5%
1	4	95.549±0.18
2	8	94.670±0.11
3	12	94.163±0.16

The formulation was evaluated for various parameters such as Appearance, Fragrance, efficiency, pH, Spreadability, Viscosity, Drug Content and *In-vitro* Diffusion study after 4 weeks interval upto 12 weeks. The results of stability studies revealed that there was no change at all in Appearance, Fragrance efficiency and pH while Spreadability, Viscosity, Drug Content and *In-vitro* Diffusion study changed negligibly.

DISCUSSION:

Factorial design (3^2) approach was used to optimize formulation and process variables in preparation of microsponges. 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 increased, particle size 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. The smaller size of microsponges obtained at higher stirring rate may be attributed to better dispersion at higher stirring rates. Increase in the amount of emulsifying agent resulted in decreased production yield. This may be due to the development of some hydrophobic regions which probably dissolved some of the drug and polymer. Furthermore, the increase in microsponge particle size as the amount of emulsifying agent increased could be due to increased viscosity leading to larger droplets which, in turn, resulted in larger microsponges.

Characterization Tioconazole microsponge were done by

FTIR spectroscopy, DSC and SEM for pure drug and polymer or formation of any decomposition product in final formulation.

From the thermogram of DSC study obtained, it was observed that there was no interaction between pure drug and polymer as well as crystalline nature of drug remains thermally stable upto the final formulation which was also supported by the SEM study revealed that the microsponge observed of the discrete and spherical. On analysing the *in vitro* diffusion data with various release models, the highest regression coefficient showing the best fit was found for the Korsmeyer Peppas model.

With this kind of formulation, the undesirable side effects and presystematic metabolism of the drug can be eliminated and controlled effect can be obtained. Finally it can be concluded that the objective of this study is achieved.

CONCLUSION:

Microsponge systems are made up 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 represents an approach for the production of Tioconazole containing microsponge gel with prolonged release characteristics.

Microsponge formulation **F6** showed a good physical parameter and was used for formulating into gel. Microsponges incorporated gel showed a good physical parameter study and *in vitro* drug release.

The Microsponge formulations showed enhanced retention of drug in skin, indicating better potential of delivery system as compared with plane formulation of tioconazole gel. On the grounds of efficacy and improved patient compliance due to reduction in frequency of application, causes reduction in the severity of the side effects.

FUTURE PROSPECTUS:

In future Microsponges can be used to prepare suitable dosage form and its in-vivo absorption studies in animals/human can be carried out to know the bioavailability of sustained release formulation.

ACKNOWLEDGEMENTS:

Authors wish to thank Pune University, Principal, Professors and Colleague of SND College of Pharmacy, Yeola, Nashik, and Matoshri College of Pharmacy, Eklahare, Nashik,MS, India for providing necessary facilities.

DECLARATION OF INTEREST STATEMENT: No conflict of interest.

REFERENCES:

1. Vyas L.K., Tapar K.K., Laddha B.H., Lahoti A.O. and Nema R.K., Formulation and development of anti-blemish preparation using microsponge technology, J. Chem. Pharm. Res., 2(5), 2010:562-573.
2. Mahajan A.G., Jagtap L.S., Chaudhari A.L., Swami S.P. and Mah P.R., Formulation and Evaluation of Microsponge Drug Delivery System using Indomethacin, International Research Journal of Pharmacy, 2(10), 2011: 64-69.
3. Jelvehgari M., Siahi-Shadbad M.R., Azarmi S., The microsponge delivery system of benzoyl peroxide: Preparation, characterization and release studies. Int J Pharm 2006; 308:124-132.
4. Orlu M., Cevher E. and Araman A., Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges. Int J Pharm 2006; 318: 103-117.
5. Nikam V.K., Somwanshi S.B., Dolas R.T. and Kashid V.A., The Microspoge Drug Delivery System: A Overview, Pharmacologyonline, 2011: 965-977.
6. FDA Draft guidance of Tioconazole containing Nonbinding Recommendation, Recommanded March 2011: 1-8
7. British Pharmacopoeia, Medicinal and Pharmaceutical Substances, Volume I & II Monographs, 2009: 6070-74.
8. Osborne D.W. and Aman A.H., Topical drug delivery formulations, New York: Marcel Dekker; 1990: 299-325.
9. Parthiban K.G., Manivannan R., Krishnarajan D., Chandra S. and Nidhin R., Microsponge Role In Novel Drug Delivery System, IJPRD, 3(4), 2011:117-125.
10. Jain V. and Singh R., Development and characterization of eudragit RS 100 loaded microsponges and its colonic delivery using natural polysaccharides. Nature Precedings: hdl:10101/npre.2009.3676.1: Posted 22 Aug 2009:67-72.
11. John I D'souza and Harinath N More, Topical Anti-Inflammatory Gels of Fluocinolone Acetonide Entrapped in Eudragit Based Microsponge Delivery System, Research J. Pharm. and Tech., 1(4), 2008: 502-506.
12. Nacht V. and Katz M., The microsponge: a novel topical programmable delivery system, in: Osborne D.W. and Amman A.H., (Eds.), Topical Drug Delivery Formulations, Marcel Dekker: New York, Basel, 1990: 299-325.
13. Kawashima Y., Niwa T., Handa T., Takeuchi H., Iwamoto T. and Itoh K., Preparation of controlled-release microspheres of ibuprofen with acrylic polymer by a novel quasi-emulsion solvent diffusion method, J Pharm Sci, 78, 1989: 68-72.
14. Setty C.M., Babubhai S.R. and Pathan I.B., Development of valdecoxib topical gel, effect of formulation variables on the release of valdecoxib, International Journal of Pharmacy and Pharmaceutical Science, 2(1), 2010: 70-73.
15. Penzes T., Blazso G., Aigner Z., Falkay G. and Eros I., Topical absorption of piroxicam from organogels- invitro and invivo correlations, International Journal of Pharmaceutics, 298, 2005:47-54.
16. Yellanki S.K., Nerella N.K., Goranti S. and Deb S.K., Development of Metronidazole Intravaginal gel for the treatment of bacterial vaginosis: Effect of Mucoadhesive Natural Polymers on the Release of Metronidazole, International Journal of Pharm Tech Research, 2(3):1746-1750.
17. ICH harmonized tripartite Guidelines, Stability testing of New Drug Substances and Products. QIA (R2): 2003.

