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Research Article

"Enhancing Antifungal Treatment Using Microsponges Loaded Emulgel to Improve Topical Delivery System"

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ABSTRACT

Objective: The objective of this research is to investigate the effectiveness of microsponge-based delivery system for enhancing antifungal treatment of Bifonazole. This study aims to evaluate the potential of microsponges technology in improving drug delivery, increasing drug bioavailability and enhancing therapeutic outcomes of Bifonazole in treatment of fungal infections.

Background of the Study: In recent years, the development of novel drug delivery systems has revolutionized the field of pharmaceuticals, offering solutions to improve drug efficacy and patient compliance.

Methodology: This experimental study was conducted in Shree Pushpasen Sawant College of Pharmacy, Jaywant Nagar, Humarmala. Study duration was 12 weeks.

Procedure: All chemicals and solvents were procured from commercial sources were purified and sterilized using standard procedures from literature whenever required.

Results: The microsponges prepared using Eudragit S100 polymer was found to be suitable for the sustained release formulation and also Bifonazole microsponges containing gel also showed the sustained release action.

Keywords: Microsponges, Antifungal Treatment, Bioavailability, Bifonazole, Eudragit S 100.

INTRODUCTION

Topical application has been used for centuries, predominantly in the treatment of localized skin diseases. Local treatment requires only that, the drug permeate the outer layers of the skin to treat the diseased state, with the hope that this occurs with little or no systemic accumulation. The past decade has witnessed a significant increase in the prevalence of resistance to antibacterial and antifungal agents.[1] When compared with antibacterial research, little progress has been made in the development of new antifungal agents, which has been justified by the low occurrence of fungal infections. However, the current increase in incidence of fungal infections has led to aggressive research on new antifungal agents as evidenced by the rise in the number of publications since the 1960s.[2-4] Fungal biofilms have caused several medical problems, resulting in significant morbidity and mortality as well as poor response to antifungal drugs.[5] Many factors have been reported relating to this increased incidence of fungal disease, many factors have been reported, but it is widely agreed that perhaps the enhanced and extensive use of such medical methods is required. Thus, immunosuppressive treatments, invasive diagnostic interventions and, the use of broad-spectrum antibiotics, are critical.[6]

Bifonazole is a substituted imidazole antifungal agent structurally related to other drugs in this group. It possesses a broad spectrum of activity in vitro against dermatophytes, moulds, yeasts, dimorphic fungi and some Gram-positive bacteria. Both non-comparative and comparative clinical trials have clearly demonstrated the efficacy and safety of various formulations of bifonazole 1% (cream, gel, solution and powder) applied once daily in the treatment of superficial fungal infections ofthe skin such as dermatophytoses, cutaneous candidia sis and pityriasis versicolor.[7] Bifonazole has also been used in the treatment of a number of miscellaneous infections such as

onychomycoses, otomycoses, erythrasma, psoriasis, sebopsoriasis, seborrhoeic dermatitis and rosacea. Excellent results have been reported in many of these conditions but only small numbers of patients have been evaluated and the findings must be considered preliminary. Microsponges is recent novel technique for control release and target specific drug delivery system.[8] Microsponges are polymeric delivery systems composed of porous microspheres. They are tiny sponge-like spherical particles with a large porous surface.[9]

Microsponges are patented polymeric delivery systems consisting of porous microspheres that can entrap a wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens, and anti-infective, anti-fungal, and anti-inflammatory agents.[10] Like a true sponge, each microsphere consists of a myriad of interconnecting voids within a non-collapsible structure, with a large porous surface. The microsponge technology was developed by Won in 1987, and the original patents were assigned to Advanced Polymer Systems, Inc.[11] This company developed a large number of variations of the technique and applied those to the cosmetic as well as over-the-counter (OTC) and prescription pharmaceutical products. At the present time, this interesting technology has been licensed to Cardinal Health, Inc., for use in topical products. The size of the microsponges can be varied, usually from 5 – 300 µm in diameter, depending upon the degree of smoothness or after-feel required for the end formula. Although the microsponge size may vary, a typical 25 µm sphere can have up to 250000 pores and an internal pore structure equivalent to 10 ft in length, providing a total pore volume of about 1 ml/g. This results in a large reservoir within each microsponge, which can be loaded with up to its own weight of active agent. The microsponge particles themselves are too large to be absorbed into the skin and this adds a measure of safety to these microsponge materials. Another safety concern is the potential bacterial contamination of the materials entrapped in the microsponge.[12-13]

MATERIAL AND METHOD

Drug Characterization:

Bifonazole Drug Authentication: Preformulation studies were performed to determine the physicochemical properties of drug that affect the development and efficacy of new drug formulations. Polymer Characterization (Eudragit S 100): The sample of drug was visually observed for the colour, odour and appearance.

Formulation of Microsponges Containing Bifonazole:

Optimization of Microsponges:

A. Rational for Selection of Ingredients and Process:

Eudragit S 100 is a rate controlling element; it is soluble in acetone but insoluble in water. So, it is porous in nature and allows diffusion. Hence, it is selected for preparation of microsponges. Modified multiple emulsion techniques method was chosen since, it yields more uniform particles. The method is reffered as O/W (oil in water) since a polymeric solution in organic solvent is considered as a oil in microencapsulation terminology.

B. Selection of Independent Variables:

Following are two independent variables, which were selected in this study

1) Polymer concentration (i.e.Eudragit S 100)
2) Stirring speed

Polymer concentration (i.e.Eudragit S 100) affects the particle size, % entrapment efficiency and drug release characteristics of drug. Stirring speed affect also particle size, production yield etc. A significant decrease in the rate and extent of drug release was observed with increase in polymer concentration in microsponges could be attributed to increase on density of polymer matrix and also increases in the diffusion path length which the drug molecules have to traverse. Therefore, these parameters were chosen for optimization of microsponges characteristics.

C. Optimization of Process Parameters:

During optimization of various parameters, the process parameters whose effect was measured are varied while other process parameters are maintained constant during preparation of microsponges. The final product was evaluated for their morphology, physical characteristics, production yield, actual drug content, entrapment efficiency and % drug entrapment.

Effect of Drug to Polymer Ratio:

The drug and polymer in the ratios 1:1, 1:2, 1:3, 1:4 and 1:5 were taken to prepare different microsponge formulations. In each formulation, the amounts of drug (200 mg), dichloromethane (100 ml), was kept constant. The microsponge formulations were prepared

using mechanical stirrer (Remi RQT127-D) at a stirring rate of 1300 rpm for 3 hours. The prepared batches MSC-1, MSC-2, MSC-3, MSC-4 and MSC-5 were analyzed for physical properties, production yield, entrapment efficiency, and % drug entrapment.

Table No.1: Formulation Table of Batches to Study the Effect of Polymer Concentration

Components	Formulation Code and Amount				
	MSC-1	**MSA-2**	**MSA-3**	**MSA-4**	**MSA-5**
Bifonazole (mg)	200mg	200mg	200mg	200mg	200mg
1. External phase- Eudragit–S100 - dichloromethane 1%solution	100mg	200mg	300mg	500mg	600mg
Span 80 (0.5)%	0.5%	0.5%	0.5%	0.5%	0.5%
Tween 80 (0.6%)	60ml	60ml	60ml	60ml	60ml
2. Internal phase- Xanthun gum 0.2% solution	10ml	10ml	10ml	10ml	10ml
Acetone: Doubly distilled Water (2:8)	40ml	40ml	40ml	40ml	40ml

Formulation of Microsponges Containing Bifonazole:

Microsponge was prepared by method in which two immiscible phases (internal and external phases) are emulsified with the aid of surfactant by reducing the interfacial tension.

Method of preparation of Bifonazole Microsponges:

Bifonazole microsponges were prepared by the modified multiple emulsion technique. The microsponges were prepared by the xanthan gum facilitated W/O/W emulsion solvent evaporation technique. 100mg of Bifonazole was dissolved in 8 ml of double distilled water and then 2 ml of acetone was added to the solution. Xanthan gum was dispersed slowly into the acetone/water mixture to obtain a concentration of 0.2 % (m/v). Up to 40–50 % of acetone can be added to an aqueous solution of xanthan gum without precipitation of the gum. For this reason, xanthan gum was used. This internal aqueous phase was emulsified into a 25 ml 1 % (m/v) solution of Eudragit S 100 in dichloromethane containing 0.5 % (m/v) Span 80 using a rotor-stator homogenizer (Model RQ-127A, Remi Motors Ltd., India) for 5 min at 2000 rpm. The resulting water-in-oil (W/O) emulsion was then transferred into 60 ml of water containing 0.6 % (m/v) Tween 80 under continuous mechanical stirring at 1300 rpm to form a W/O/W type emulsion. The stirring was continued with a three-blade propeller for a period of 1.5 h to allow evaporation of the organic solvent. The resulting microsponges were separated by filtration and finally air-dried.

Formulation of Bifonazole Drug Containing Emulgel:

Method of Preparation

A. Different formulations were prepared using varying amount of gelling agent. The method only differed in process of making gel in different formulation. The preparation of emulsion was same in all the formulations. The gel bases were prepared by dispersing Carbopol 934 and Viscopol 934 in distilled water separately with constant stirring at a moderate speed using mechanical shaker, then, pH was adjusted to 6-6.5 using triethanolamine and soaked for 24h.

B. The oil phase of the emulsion was prepared by dissolving Span 80 in light liquid paraffin, dimethyl sulfoxide and clove oil, while the aqueous phase was prepared by dissolving Tween 80 in purified water. Methyl and propyl paraben were dissolved in propylene glycol and mixed with aqueous phase. Bifonazole, being hydrophobic was dissolved in oil phase. Oleic acid was also mixed in oil phase.

C. Both the oily and aqueous phases were separately heated to 70° to 80°C, then, the oily phase was added to the aqueous phase with continuous stirring until it got cooled to room temperature. The obtained emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtain the emulgel. The composition of different formulations has been discussed in further studied for their release kinetics by using Franz diffusion cell and other evaluation parameters.

Table No.2: Formulation Table for Emulgel

Sr. No.	Ingredients	F1	F2	F3	F4	F5	F6
1	Microsponges of Bifonazole	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g
2	Carbapol934	1.5g	1.3g	1.4g	1.2g	1g	1.3g
3	Viscopol934	0.2g	0.1g	0.3g	0.4g	0.6g	0.3g
4	Liquidparaffin	6ml	6ml	6ml	6ml	6ml	6ml
5	Tween 80	2ml	2ml	2ml	2ml	2ml	2ml
6	Span80	1ml	1ml	1ml	1ml	1ml	1ml
7	Oleicacid	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
8	Propyleneglycol	4.8ml	4.8ml	4.8ml	4.8ml	4.8ml	4.8ml
9	Dimethylsufoxide	3ml	3.4ml	3.6ml	3.8ml	3.2ml	3.3ml
10	Methylparaben	0.03g	0.03g	0.03g	0.03g	0.03g	0.03g
11	Propylparaben	0.04g	0.04g	0.04g	0.04g	0.04g	0.04g
12	Triethanolamine	qs	qs	qs	qs	qs	qs
13	Cloveoil	6ml	6ml	6ml	6ml	6ml	6ml
14	Water	qs	qs	qs	qs	qs	qs

Characterization of Emulgel

Physical Examination: Emulgel formulations were inspected visually for their color, homogeneity, and apparent phase separation.

Rheological Studies: Brookfeld viscometer attached with spindle no. 6 (50 rpm) was used to measure the viscosity of prepared emulgel formulations at 25 °C. Thirty grams emulgel was placed in a beaker and allowed to equilibrate for 15 min; after that, the dial reading was measured. The viscosity was measured in triplicate for each emulgel formulation, and an average of three readings was considered as final viscosity.

Drug Content: One gram microsponge-loaded emulgel was added in 100 ml phosphate bufer pH 5.5 and sonicated for 1 h. The resulting solution was fltered through 0.45 membrane flter, and absorbance was measured at 251.5 nm using a UV-visible spectrophotometer.

Antifungal Drug Tests:

Antifungal drug sensitivity tests have been carried out by using Cup-plate method performed in Sabouraud's agar medium.

a. Preparation of Culture Media For Antifungal Test:

Sabouraud's agar was prepared by weighing 10 gms of peptone, 40 gm of dextrose, and 20 gm of agar in 1000 ml of distilled water. Then it was sterilized in an autoclave at 15 lbs pressure (121^0C) for 15mins. After sterilization the media was cooled up to 45^0C and then it was poured in sterile petri plates in aseptic condition. Then the media from the plate was allowed to get solidified.[15]

b. Inoculation of Suspension of Fungi on Culture Media:

Sterile, non-toxic cotton swab were dipped in to the standardized inoculums of Candida albicans (turbidity as adjusted as to obtained confluent growth on the Petri plate) and then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60º angle between streaking. Then the streaked inoculum was allowed to dry for 5-15mins with lid in place. Prepared well in a seeded plate by using cork borer that was sterilized by burning with absolute ethanol. Prepared formulation of gel was dissolved in dichloromethane making concentration. of 50 µg/ml and 100 µg/ml and then it is introduced in to the wells, and other two wells, kept as a control and standard, then these plates are incubated at 25-30º C for 96 hr. The sensitivity of Candida albicans was indicated by zones of inhibition around the well and the diameter of the zone of inhibition was measured.

RESULT

Mechanism of Forming Microsponges

The formation of microsponges by the quasi-emulsion method is proven to be easy, reproducible, rapid, and without any solvent-related toxicity issues.

Formulation, Optimization, Selection of Internal Phases of Microsponges Containing Bifonazole

The effect of 4 different solvent systems which were prior selected depending upon solubility of drug as well as polymer; namely ethanol, dichloromethane, acetone and 1:1 combination of ethanol and dichloromethane. In case of ethanol and acetone; there is no formation of small, discrete, spherical, polymeric particles. The product obtained was in the form of lump or irregular in shape. In case of combination system of ethanol and dichloromethane; the yield was very low and it is not consistent.

Table No. 3: Effect of Volume of the Internal Phase Internal Phase

FormulationCode	Internal Phase Volume	% Productionn Yield	Actual Drug Content(%)	Theoretical Content (%)	% Entrapment Efficiency	% Encapsulation Efficiency	% loading Efficiency
MS1	22ml	78.66	52.56	50	84.86	82.56	78.84
MS2	29ml	76.46	48.68	50	78.76	80.98	72.68
MS3	36ml	70.45	44.68	50	72.78	78.65	67.56
MS4	43ml	40.34	36.66	50	67.48	74.66	60.78
MS5	50ml	Very low	-	-	-	-	-

Study the Effect of Surfactant Concentration

Table No.4: Effect of Surfactant Concentration

Formulation Code	Surfactant Concentration	% ProductionYield	Actual Drug Content (%)	TheoreticalContent (%)	% Entrapment Efficiency	% Encapsu Lation Efficiency
MS1	12ml	86.46	46.56	50	84.68	76.78
MS2	24ml	78.65	43.89	50	80.64	65.68
MS3	36ml	74.86	40.56	50	60.64	44.78
MS4	48ml	54.78	38.78	50	58.85	54.88
MS5	60ml	No yield	-	-	-	-

When the amount of surfactant (combination of Span 80 and Tween80) was increased, the production yield and drug content and encapsulation efficiency of microsponges decreased.

Effect of Drug to Polymer Ratio

Table No.5: Effect of Drug to Polymer Ratio

Formulation Code	D:Prati o (Mg)	% Production Yield	Actual Drug Content (%)	Theoretical Content (%)	% Entrapment Efficiency	% Encapsulation Efficiency
MS1	2:1	74.46	26.45	50.46	46.68	56.78

MS2	2:2	76.64	28.56	48.46	50.46	64.58
MS3	2:3	80.78	22.68	36.48	58.68	72.78
MS4	2:5	84.68	18.45	34.56	74.56	78.65
MS5	2:6	91.46	15.56	28.46	84.56	85.56

With increase in drug: polymer ratio leading to increase in production yield and encapsulation efficiency.

Increased production yield and entrapment efficiency and entrapment encapsulation is due to the amount of polymer is increased with ratio of drug to polymer, this is probably due to that at higher relative drug content; the amount of polymer available per microsponges to encapsulate the drug become more thus increases thickness of the polymer wall and hence larger the size of microsponges.

Surface Morphology (Scanning Electron Microscopy)

The SEM data obtained for drug loaded microsponges are shown in figure(a), (b), (c), (d), (e) and (f) In general; the SEM micrographs of microsponges exhibited spherical shape (Fig.no.1 a, b and c) indicated that spherical solid microsponges could be prepared by this method. Also, it showed the porous nature on this surface in (Fig no 1 .d, e and f).

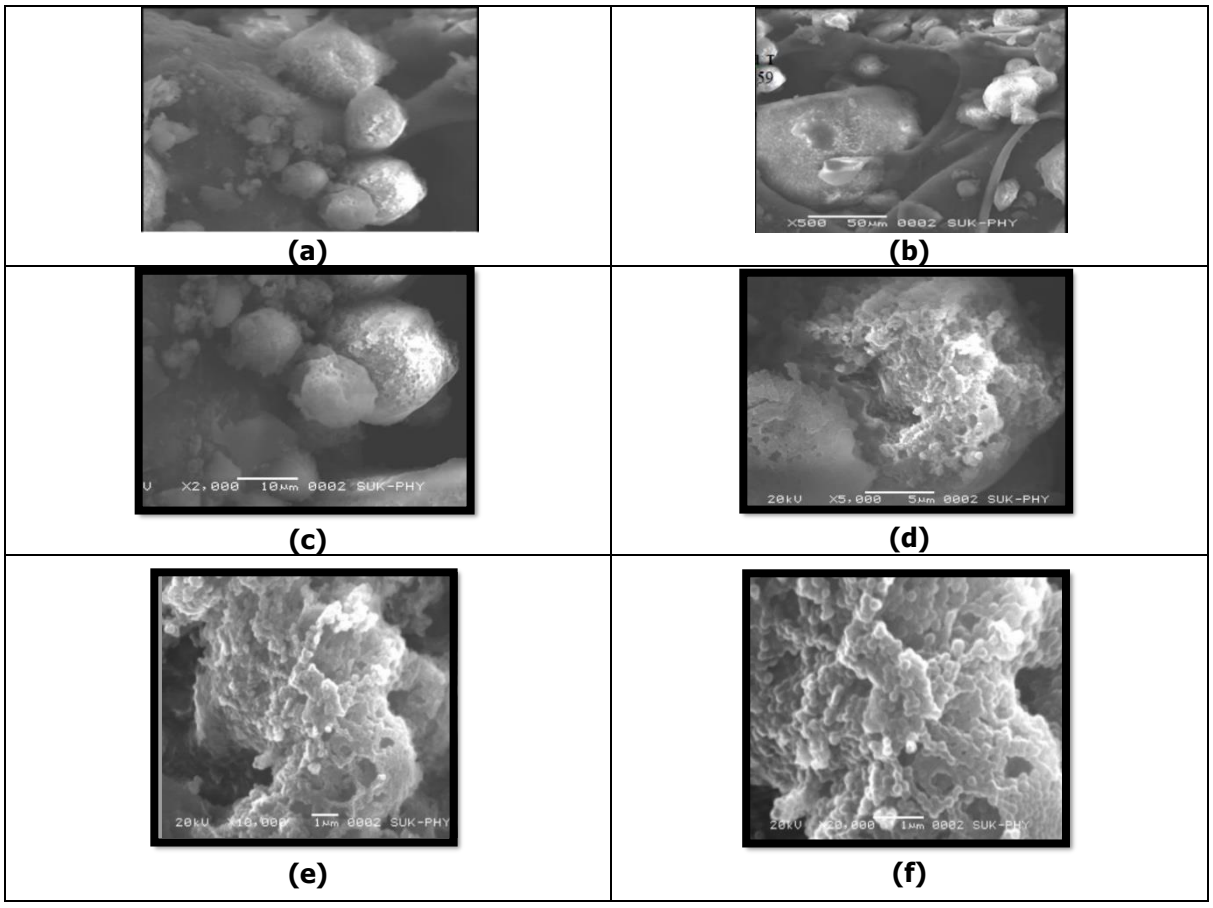

Fig No.1: SEM Images of Bifonazole Microsponges

Frequency Distribution Analysis

Table No.6: Particle Size Determination of Different Formulations

Formulation No.	Mean Particle Size(µm)
F1	45.68

F2	68.64
F3	72.42
F4	30.46
F5	38.12
F6	48.42
F7	32.66
F8	35.44

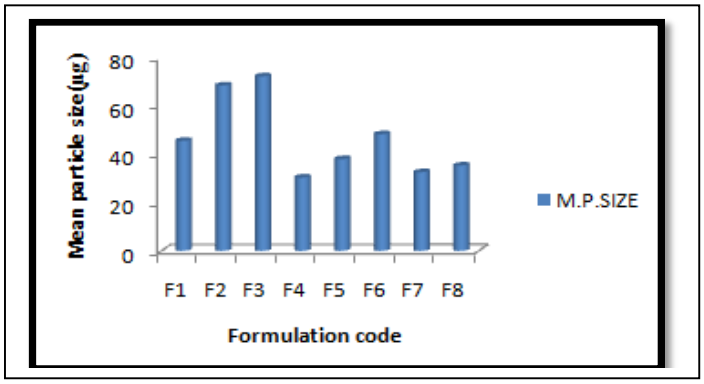

Fig.No.2: A Plot of Particle Size Determination of Different Formlations

As **Drug : Polymer** ratio increased the % production yield also increased due to the reduceddiffusion rate of dichloromethane from concentrated solution in to aqueous phase this providemore time for the droplet formation and improve yield of microsponges.

Effect of Stirring Speed: It was observed that at higher stirring rates due to the turbulence created within the external phase, polymer adhered to the paddle and production yield was found to be decreased.

% Production Yield

Table No. 7: %Production Yield of Different Formulations

Formulation No.	% Production Yield
F1	60.27%
F2	66.78%
F3	74.78%
F4	82.68%
F5	88.68%
F6	71.45%
F7	75.34%
F8	80.56%

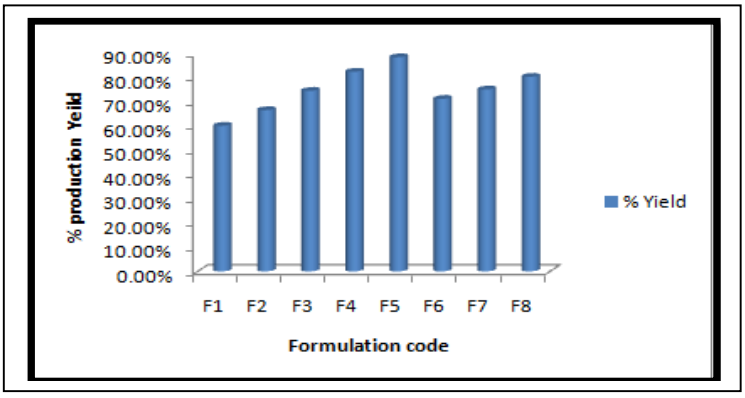

Fig. No.3: A Plot of % Yield vs. Formulation Code Effect of Polymer Concentration

%Entrapment Efficiency

Table no. 8: % Entrapment efficiency of Different formulations F1-F8

Formulationn Code	% Entrapment Efficiency
F1	82.45%
F2	84.68%
F3	78.85%
F4	86.56%
F5	94.68%
F6	80.68%
F7	74.76%
F8	88.24%

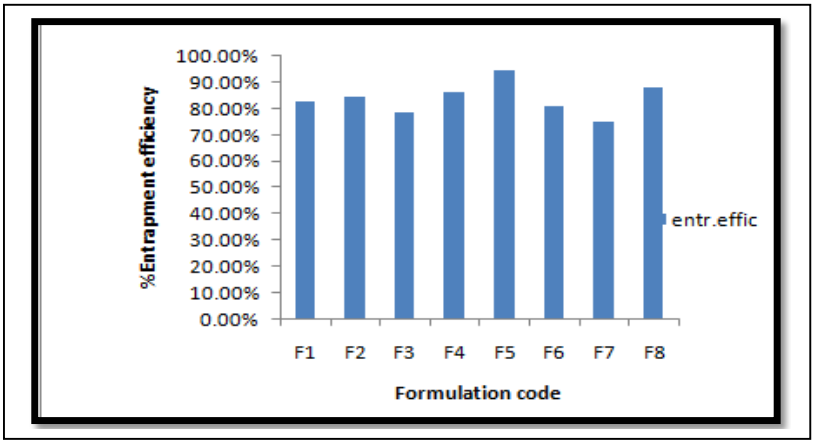

Fig. No.4: A Plot of % Entrapment Efficiency Vs Formulation Code

Entrapment efficiency of all formulation are shown in Table no 8. The entrapment efficiency was influenced by the Eudragit S 100 polymer concentration and stirring speed. Entrapment efficiency improved by greater proportion of polymer with respect to amount of drug available, hence, more polymer can entrap more drug particle, i.e. more amount of polymer present per unit.

Encapsulation Efficiency

Table No. 9:% Encapsulation Efficiency of Different Formulations F1-F6

Formulation Code	%Encapsulation Efficiency
F1	72.88%
F2	78.92%
F3	70.56%
F4	84.78%
F5	93.46%
F6	86.66%
F7	77.12%
F8	90.22%

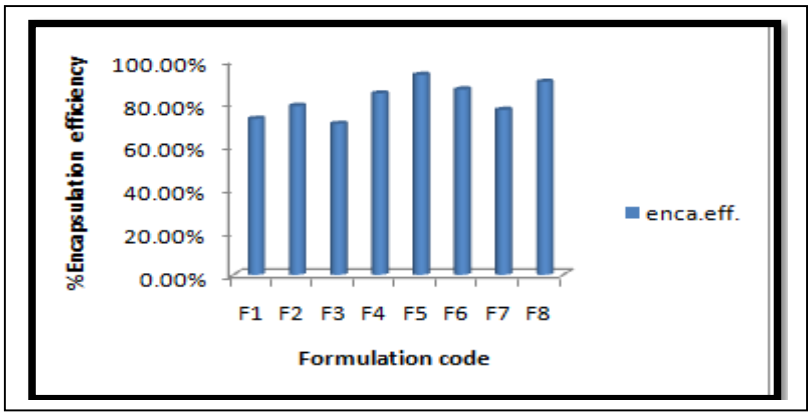

Fig.No.5: A Plot of % Encapsulation Efficiency Vs Formulation Code

Encapsulation efficiency of all the formulations is shown in Table no 9. The encapsulation efficiency was influenced by the Eudragit S 100 polymer concentration. And stirring speed. Encapsulation efficiency improved by greater proportion of polymer with respect to amount of drug available, hence more polymer can entrap more drug particle, i.e. more amount of polymer present per unit.

% Drug Entrapment

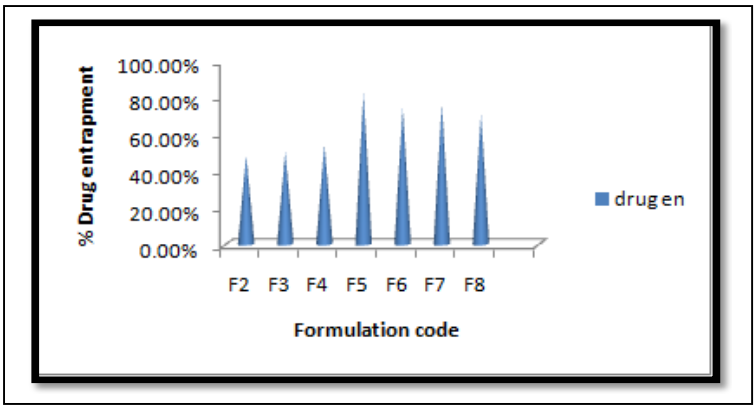

Fig.no.6: % Drug entrapment of Different Formulations F1-F8

Table No.10: % Drug Entrapment of Different Formulations F1-F8

Formulation Code	%Drug Entrapment
F1	42.46%
F2	48.52%
F3	50.44%
F4	54.46%
F5	82.77%
F6	74.88%
F7	76.54%
F8	70.88%

% Drug entrapment of all formulation is shown in Table no 10. The % drug entrapment was influenced by the Eudragit S 100 polymer concentration and stirring speed. % drug entrapment improved by greater proportion of polymer with respect to amount of drug available hence, more polymers can entrap more drug particle, i.e. more amount of polymer present per unit.

In Vitro Drug Release and Release Kinetic Study

Table No. 11: In Vitro Drug Release Profile for Bifonazole Microsponge Formulation

Time (Hrs)	F 1	F 2	F 3	F 4	F 5	F 6	F7	F 8
0.5	20.46± 0.4	22.34± 0.3	24.34± 0.4	8.56± 0.3	10.46± 0.2	14.54± 0.4	16.44±0 .2	18.45±0 .5
1	36.56± 0.4	38.45± 0.4	26.34± 0.4	12.6±0.4	14.86±0. 2	20.43±0 .4	24.68± 04	22.46± 0.9
2	42.66± 0.5	44.45± 0.3	28.45± 0.4	26.8±0.4	18.96±0. 2	28.67±0 .4	30.88±0 .4	30.68± 0.9
3	48.74± 0.4	50.54± 0.4	30.34± 0.4	32.6±0.3	28.94±0. 2	34.66±0 .4	40.8±0. 4	42.67± 0.9
4	52.42± 0.5	54.44± 0.5	34.36± 0.4	42.8±0.2	34.88±0. 2	44.68±0 .2	46.34±0 .3	48.8±0 .9
5	62.44± 0.4	56.43± 0.4	45.24± 0.5	50.6±0.4	48.04±0. 4	52.89±0 .4	50.67±0. 4	56.8±0 .4
6	64.65± 0.4	60.56± 0.5	52.34± 0.4	56.7±0.2	52.08±0. 2	64.98±0 .2	54.86±0 .4	64.24± 0.9
7	66.67± 0.8	62.56± 0.4	60.24±0. 2	76.45±0. 4	76.68± 0.5	74.88± 0.4	64.56±0 .6	74.87± 0.9
8	68.98± 0.6	65.56± 0.1	64.46±0. 4	80.34±0. 4	88.98± 0.2	86.76± 0.4	70.46±0 .9	76.67± 0.9
9	70.65± 0.9	68.66± 0.3	72.56±0. 4	82.45±0. 5	96.78± 0.2	90.65± 0.2	78.65±0 .6	82.56± 0.9

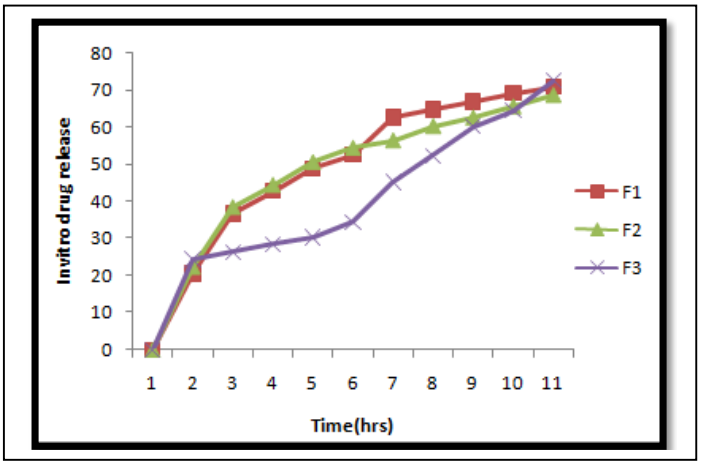

Fig. No.7: In Vitro Drug Release Profile of Bifonazole (Batch F1-F3)

Drug release from the formulations decreased with increase in the amount of polymer in the microsponges. The present study showed that increase in the ratio of drug: polymer resulted in decrease in release of Bifonazole from microsponges. While higher concentration of polymer decreases release of drug from microsponges; this could be due to formation of a thicker matrix wall in microsponges with smaller drug: polymer ratios lead to a longer diffusion path, and consequently slower drug release rate. Batch F5 showed 96.78 % drug release at 8 hour, it indicated that the formulation F5 was found to be optimized batch .Formulation F1, F4 and F7.

Table No:-12:% Drug Content of Formulated Emulgel

Sr. No.	Formulation Code	%Drug Content
1	F1	88.45%
2	F2	86.24%
3	F3	78.68%

4	F4	76.78%
5	F5	74.87%
6	F6	72.45%

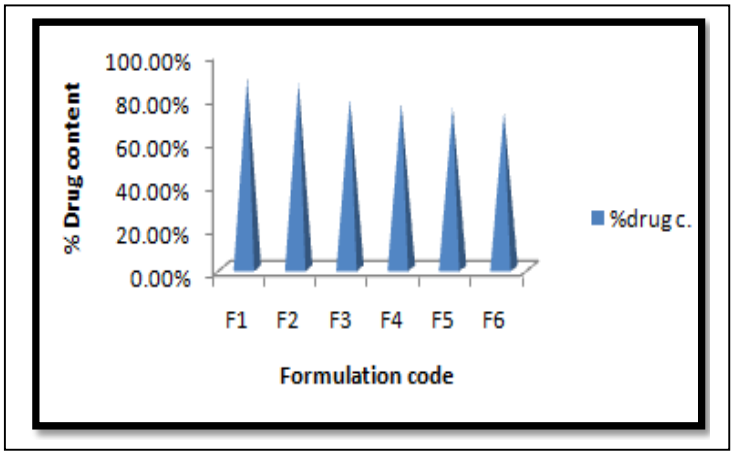

Fig.No.8: % Drug Content of Different Formulations F1-F6

Table No. 13: Antifungal Screening Results of Formulated Microsponge Containing Bifonazole Emulgel Measuring the Zone of Inhibition in Mm

Compound Name	Species	Concentration	
		50µg/ml	100µg/ml
Standard	Candida albicans	++	+++
F3	Candida albicans	+	++

Antifungal Activity Observations

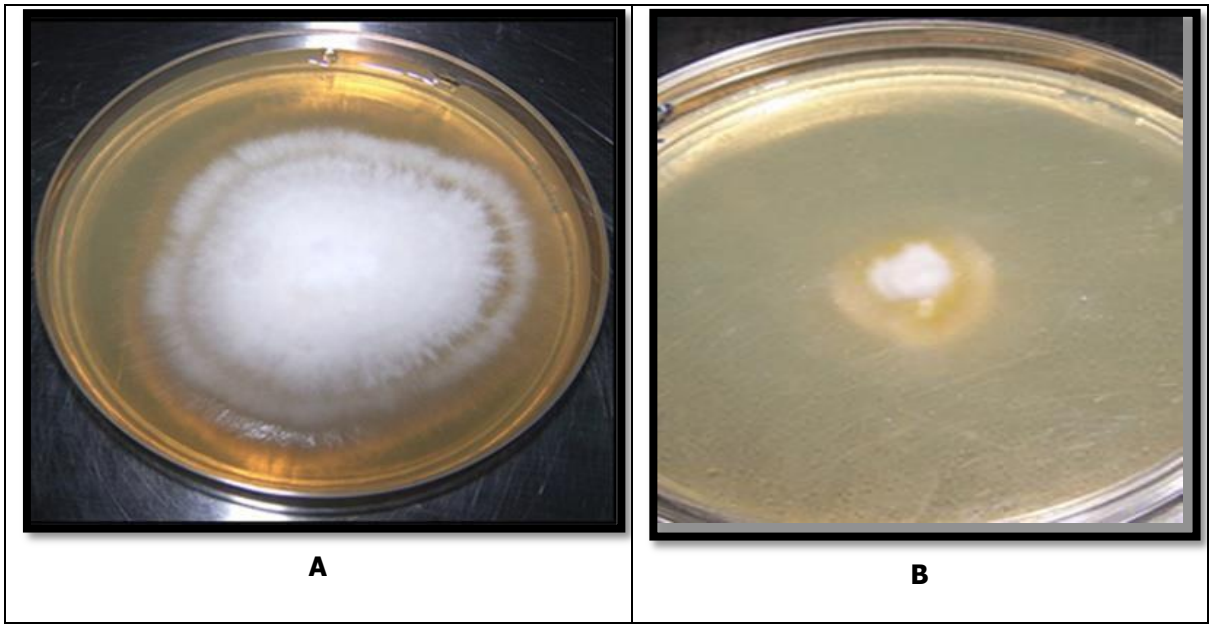

Fig. No.9: Antifungal Activity of Formulated Microsponge Containing (A) Emulgel of Bifonazole (B) Antifungal Activity of Bifonazole Drug Measuring the Zone of Inhibition

Key to Symbol
Highly active = +++ (inhibition zone > 9 mm) Moderately active = ++ (inhibition zone 6 - 8 mm) Slightly active = + (inhibition zone 3-5 mm) Inactive = - (inhibition zone < 3 mm).

CONCLUSION

It is possible to optimize the release of Bifonazole for better therapeutic efficacy. Bifonazole microsponges were prepared successfully using the modified multiple emulsion technique. The microsponges prepared using Eudragit S 100 polymer was found to be suitable for the sustained release formulation and also Bifonazole microsponges containing gel also showed the sustained release action. The result of in vitro drug release study proved that optimized microsponge-loaded emulgel was able to sustain the release of drug for 6 hours. Microsponge delivery systems that can precisely control the release rates or target drugs to a specific body site have a vast impact on the health care system.

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