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Formulation, Evaluation and Optimization of Isoconazole Loaded Microsponge Using Box Behnken Design

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ABSTRACT
The aim of present work is to formulate, evaluate and optimize microsponges loaded with isoconazole and the release of proposed formulation is controlled, which is further loaded in the form of gel.Using the quasi-emulsion solvent diffusion method, the microsponges were successfully synthesized with varying ratios of PVA as the surfactant and Eudragit RL100 as the polymer by using Box behnken design. The study looked at how the drug-to-polymer ratio, surfactant concentration, inner phase volume, and stirring speed affected the microsponges' physical characteristics and drug entrapment effectiveness. Furthermore, FT-IR methods were used to examine the drug's compatibility with different additives. The microsponges had a spherical, spongy shape, according to SEM examination. The particle sizes of the improved formulations ranged from 20.11 to 29.4 µm, and their drug entrapment efficiencies were between 73% and 97.6%. These formulations for microsponge gels were made with carbopol 934, and their pH, viscosity, and in-vitro drug release were examined.
Keywords: microsponges, isoconazole, gel, Box behnken design.

1. Introduction

Topical drug delivery is growing in popularity as a way to administer medications directly to specific areas of the body or even throughout the body. Unlike traditional oral methods, topical formulations have several benefits. They can decrease the likelihood of stomach irritation caused by enzyme and lessen the potential for medications to interact with food. Additionally, they provide an option when oral delivery is not feasible, such as during episodes of vomiting or diarrhea.

The development of topical dosage forms aims to optimize drug delivery by enhancing local effects and minimizing systemic absorption. This approach ensures both safety and efficacy in medication administration through improved targeting or effective skin penetration.

Isoconazole nitrate (ISN) is categorized under class II of the Biopharmaceutics Classification System (BCS), indicating low solubility but high permeability . Consequently, there is a growing interest in developing nanoemulsions (NE) for topical and transdermal drug delivery, as the skin offers optimal conditions for medication administration. ISN has been widely employed to treat various skin conditions, including microbial and fungal infections.

The development of microsponge formulations for topical and transdermal drug delivery is particularly promising. The skin serves as an advantageous site for drug administration due to its accessibility and potential for targeted therapy. ISN has demonstrated efficacy in treating a spectrum of skin disorders, including fungal and microbial infections. [1]

First discovered by Won in 1987, microsponges are polymeric microparticles with porous surfaces. They offer numerous advantages for medical applications. Microsponges exhibits stability across a broad pH range and can withstand temperatures as high as 131°C. They are compatible with various pharmaceutical ingredients and delivery systems.

By controlling drug release, topically applied microsponges can enhance safety and reduce irritation associated with medications. When used orally, they mitigate side effects and improve the bioavailability and solubility of poorly absorbed drugs. Their ability to self-sterilize is attributed to their small pore size, approximately 0.25 μm in diameter, which prevents bacteria and other contaminants from entering. Furthermore, microsponges are non-mutagenic and non-irritating, making them highly suitable for pharmaceutical formulations.[2]

A novel and effective method to enhance drug delivery is through the use of skin-applied microsponge gel compositions. These formulations mitigate local skin reactions caused by active ingredients and effectively minimize systemic exposure. The active ingredient is encapsulated within macroporous beads, designed to release the medication slowly in response to external factors such as friction, temperature variations, or pH changes. [3]

2. Materials and methods

2.1. Materials

Isoconazole Nitrate purchase from Yarrow Chem Products. Eudragit RL100, Ethanol, Dichloromethane, Dibutyl phthalate, Poly vinyl alcohol, Carbopol 934 and Triethanolamine from CDH Private Limited.

2.2. Preparation of Isoconazole nitrate microsponges

Isoconazole nitrate microsponges were prepared using the quasi-emulsion solvent diffusion method. In this method, there are two phases: the external phase and the internal phase. The external phase was prepared by mixing PVA in 75 ml of distilled water using a magnetic stirrer. The internal phase contained isoconazole nitrate and Eudragit RL100 mixed with 6 ml of ethanol:DCM(1:1) using a sonicator. After that, the internal phase was added dropwise to the external phase at 1500 rpm for 90 minutes at 35°C to remove DCM. The prepared microsponges were filtered through Whatman filter paper and then washed with distilled water. They were dried at 45°C for 12 hours and weighed.[4]

2.3. Optimization of Isoconazole nitrate loaded microsponges by design expert (version 12.0.3.0)

A total of 17 runs were produced when the design was applied using the Design-Expert program (trial version 12, Stat-Ease). The ultimate optimization of isoconazole nitrate-loaded microsponges was carried out using a three-factor, three-level Box-Behnken design, along with a response surface technique. We considered the medication to polymer ratio to be independent factors. Particle size, yield percentage, and entrapment efficiency, on the other hand, were regarded as dependent reactions.

Table 1.Factors and their used levels used in Box-Behnken Design

Factor	Name	Lower level (g)	Upper level (g)
A	Isoconazole nitrate	30	50
B	Eudragit RL100	130	150
C	PVA	36	40

Table 2.Summary by Design Expert (version 12.0.3.0)

Study type	Response surface
Design type	Box-Behnken
Sub type	Randomized
Runs	17
Design Model	Quadratic and Linear

The design of expert software was used to optimize the microsponges. There were fifteen runs total using the Box-Bahnken design, where a randomized subtype and a quadratic design model were produced. wherein three factors were employed to optimize the microsponges: Isoconazole nitrate (A), Eudragit RL100 (B), and PVA (C).

2.4. Evaluation of microsponges

2.4.1. Particle size analysis

The particle size distribution of the supplied materials was determined using a Zetasizer. A graphical representation was generated by analyzing the intensity distribution data of particles across various sizes using Malvern software. The peak in the graph corresponds to the predominant particle size within the sample.[7]

2.4.2. Zeta potential

Particle stability and surface charge have been assessed using zeta potential. If the zeta potential exceeds +30 mV or drops below -30 mV, particles are unable to adhere to each other.[7]

2.4.3.Determination of production yield

Production yield is determined by measuring the initial mass of raw material and final weight of microsponges. [5]

Production yield = final weight of microsponges /Initial weight (polymer + drug) × 100

2.4.4.Determination of entrapment efficiency

At a wavelength of 272 nm, the drug content of the microsponge was measured using spectrophotometry. A freshly prepared 100-ml phosphate buffer (pH 7.4) was applied to dissolve

a 10 mg sample of isoconazole nitrate microsponges. A calibration curve was used to determine the drug content, which was then listed as loading efficiency. [4]

Drug entrapment = Amount of drug present in the microsponges/ Initial weight × 100

2.4.5.In vitro dissolution studies

USP type II dissolution apparatus was used in this study. Where 900 ml of 7.4 phosphate buffer dissolution media was agitated at 50 rpm while maintained at 37±1 °C. Samples (5 ml) were taken out in aliquots at durations of up to 6 hours, filtered through Whatmann filter paper, and then added 7.4 phosphate buffer to achieve a volume of 10 ml. The samples' luteconazole content was determined using a UV-visible spectrophotometer at 272 nm. [6]

2.4.6. Scanning electron microscopy

The morphology ofIsoconazole nitrate microsponge formulation was evaluated using scanning electron microscopy (SEM). The sample was mounted onto the SEM sample holder using double-sided sticky tape. Images were captured at various magnifications with an acceleration voltage of 10 kV.[6]

2.4.7. FTIR studies

Fourier-transformed infrared spectra were acquired using a FTIR spectrophotometer (Thermo Scientific) employing the KBr disk method. Approximately 1–2 mg of the sample was carefully triturated with KBr powder and compressed into a disc under pressure for 10 minutes using a hydraulic press. The resulting disc was then positioned in the sample holder and scanned across the range of 4000 to 400 cm^-1.

3. Formulation of microsponge entrapped isoconazole nitrate gel

The gel-forming polymer Carbopol 934 was mixed with 3 ml of glycerol using a magnetic stirrer at 500 rpm. Subsequently, a small amount of distilled water was added and allowed to keep it for 2 hours. The mixture was then dispersed with agitation at approximately 500 rpm to achieve a smooth dispersion. After standing for 15 minutes to remove entrapped air, methyl paraben and EDTA were uniformly added. The resulting viscous solution was neutralized to pH 6.8 with triethanolamine under slow agitation. At this stage, isoconazole nitrate microsponges (1%, equivalent to 1% w/w of Carbopol 934) were incorporated to produce homogeneous gel-based topical delivery systems termed isoconazole-loaded gels and isoconazole nitrate microsponge-loaded gels.

Table 3. Formulation of microsponge entrapped isoconazole nitrate gel

Ingredients (g)	**G1**	**G2**
Microsponges	100mg	100mg
Carbopol 934	0.3	0.3
Methyl paraben	0.18	0.18
Propyl paraben	0.02	0.02
Glycerine	3	3
Triethanolamine	q.s	q.s
EDTA	0.05	0.05

Distilled water	q.s-100	q.s-100

Where G1 is gel of formulation4 and G2 is gel of formulation14

3.1. Characterization of microsponges loaded gel

3.1.1. Visual inspection

Reviewing the finished compositions against a black and white background allowed us to evaluate their clarity and appearance.[8]

3.1.2.pH measurement

Using a pH meter, the pH of each formulation (1 gram) was measured after that dissolved in 50 mL of distilled water.[11]

3.1.3.Spreadability studies

Using a wooden block and glass slide equipment, the prepared isoconazole nitrate gel's conduct the spreadability study. After adding about 1 g of gel to the wooden pan, the period of time it took for the top slide to fully separate from the fixed slide was observed. The following formula was used to calculate the gel's spreadability: [9]

$$S = ML/T$$

Where M = weight fixed with glass slideS = spreadability, , L = length of slide and T = time taken by the slide to separate from

3.1.4.Viscosity measurement

The viscosity of gel is measured by Brookfield viscometer with spindle number 64. It was set to operate at 100 rpm and 25 °C. Using Brookfield Viscometer, the viscosity of the optimized prepration was determined without dilution. This device consists of a spinning spindle and a stationary cup. To determine the viscosity of the test material, different-sized spindles are selected; larger spindles with a larger surface area and diameter are used for low-viscosity liquids, and smaller spindles are used for higher-viscosity liquids.

The selected spindle is placed in the microsponge gel and rotated to measure viscosity until the viscometer display shows an identical dial reading. This process was carried out three times to guarantee that the outcomes were repeatable. [12]

3.1.5.Drug content

Take a freshly prepared phosphate buffer pH 7.4 and dissolved one gram of gel formulation and it was mixed by magnetic stirrer for duration of 12 hours. After filtering the resulting dispersion, the drug content was determined using spectrophotometry at a wavelength of 272 nm.[10]

3.1.6.In vitro release study

Using a cellophane membrane with the membrane diffusion technique, the in vitro release of isoconazole nitrate from gel formulations loaded with microsponges was studied. The freshly prepared pH 7.4 phosphate buffer was used as the dissolve medium. A specially made open-ended glass cylinder has one end of the cellophane membrane tightly attached to it after it has been pre-soaked in the dissolution medium for an entire night. An exact 1 gram of the ingredients was carefully put into this assembly. The stand-mounted cylinder was submerged in 200 ml of 37°C dissolution medium, which touch the receptor media surface..

A Teflon-coated magnetic bead was used for stirring the dissolving fluid at 100 rpm. To ensure proper sink conditions, aliquots of 5 ml each were taken out at fixed intervals for 4hours and replaced with an equivalent volume of fresh receptor media. Using neutralized phasphate buffer as the blank, the obtained aliquots were properly diluted with the receptor medium and subjected to UV-spectrophotometer assessment at 272 nm.[13]

4.RESULTS AND DISCUSSION

4.1. Particle size analysis

The average particles size of the microsponges was ascertained. These microsponges minimum and maximum mean diameters were reported to be 20.11 µm and 31 µm respectively. (Table 7.)

4.2. Zeta Potential

The majority range of the microsponge particles in the formulation had this charge, as seen by the plot peak's from-24.04 to -27.2 mV, indicating a strong affinity between the particles..

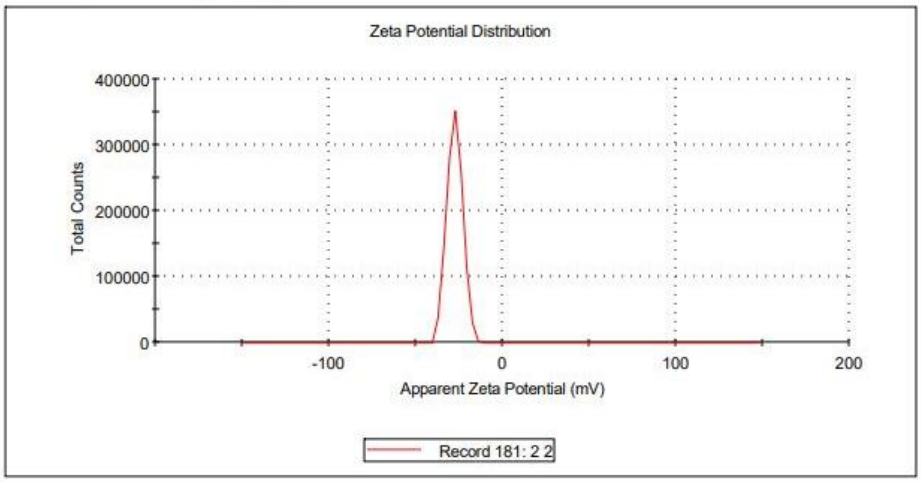


Fig 3. Zeta potential

4.3.Determination of Percentage yield and entrapment efficiency

All batches had production yields that ranged from 67% to 88.8%. the concentration of PVA and drug:polymer ratio significant impact on the production yield.

It was shown that while the production yield fell with increasing polyvinyl alcohol concentration, the production yield increased with increasing polymer concentration. The dispersed phase's viscosity was positively raised when larger PVA concentrations were utilized to create microsponges at higher drug-polymer ratios. Almost all of the scattered phase changed into solid microsponges when the inner phase's solvents diffused out and separated particles emerged. These formulations' maximum drug loading efficiency may be explained by the fact that the quantity of polymer in each unit of drugs (Table 7.)

Table 7.

Run	Drug (mg)	Eudragit RL100 (mg)	PVA (mg)	Particle size (nm)	Drug Entrapment (%)	% Yield (%)	% Drug Release(%)
1	40	140	38	25.16	90.2	84	72
2	30	130	38	20.11	83	77.6	82
3	40	140	38	26.13	92	78.8	70
4	30	150	38	29.4	97.2	79.3	89.5
5	40	150	36	28.9	94.2	67	87.2
6	40	140	38	25.4	93.31	89	79.6
7	40	150	40	31	97.6	78	76
8	30	140	36	24.5	89	89	74
9	50	140	40	27.2	87	78	75
10	40	140	38	25.9	89.6	88.8	71.2
11	50	140	36	24.3	94.2	86	72
12	50	130	38	21.33	73	71.4	82.5
13	30	140	40	27.23	86	85.9	82.8
14	50	150	38	28.3	95.6	71.5	90
15	40	130	36	20.13	79	74	62
16	40	130	40	21.22	81	71.5	72.8
17	40	140	38	26.14	88.2	88.3	70.5

6.4.In vitro drug release

In an in vitro dissolution test, isoconazole nitrate-loaded microsponges released the medication for up to eight hours, and their performance was compared with that of the commercially available preparation of isoconazole nitrate. The drug release increased in proportion to time, indicating that the medication will remain in the topical drug delivery system for a longer period of time and release continuously over time. (table 7.)

4.5.SEM analysis of microsponge

Using scanning electron microscope surface morphology of the isoconazole nitrate microsponge formulation was assessed. Using double-sided sticky tape, the sample was immediately placed onto the SEM sample holder, and scanning electron microscopy pictures were captured at 11.3 mm x 100 SE magnifications at an acceleration voltage of 30 kV. The microsponge's SEM picture is displayed in fig 4.

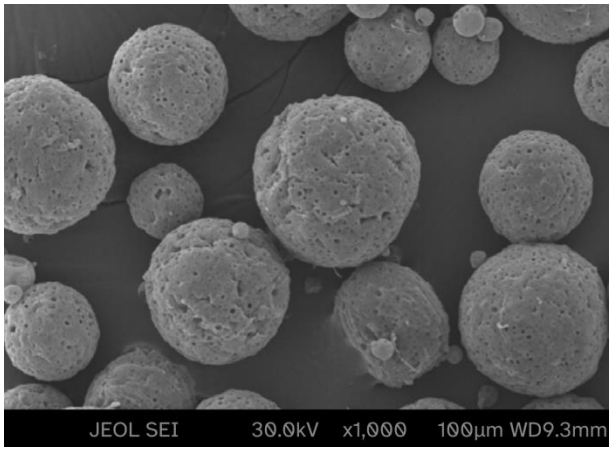


Fig. 4. SEM view of microsponge

4.6. FTIR study

According to an FTIR spectroscopic analysis, no new peaks appeared, and those that already existed vanished, indicating that the medication and the polymer did not interact chemically. C-N stretching produced unique peaks in the IR spectra at 1274.54 (cm-1). C=C stretching is responsible for the peaks at 1579.33 (cm-1) and C-Cl stretching is responsible for the peaks at 742.94 (cm-1). The C-H methylene is responsible for the stretching peak at 2957.51. N-O stretching is the cause of the peak at 1384.58. During the compatibility research inquiry, the IR spectra of the physical combination and isoconazole nitrate-loaded microsponges showed all of the typical peaks of isoconazole nitrate. Therefore, the medication was compatible with certain polymers and excipients, according to IR spectroscopy data. This suggested that isoconazole nitrate was stable in the microsponges and compatible with certain polymers.

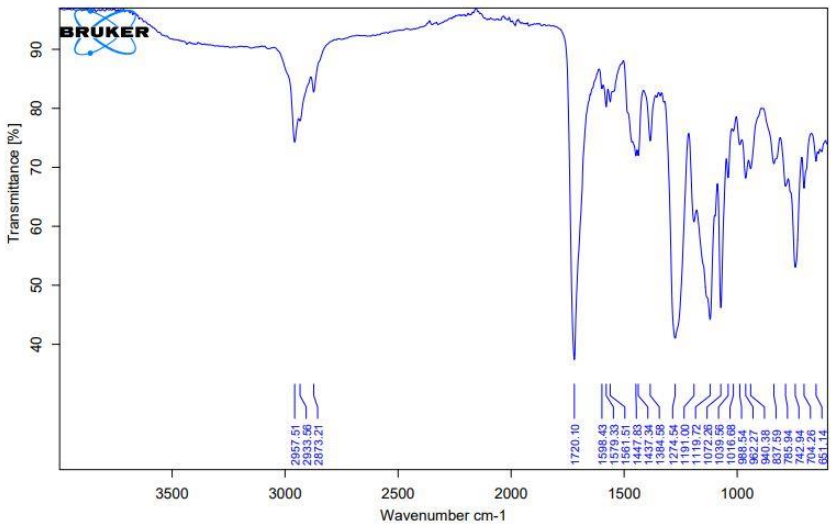
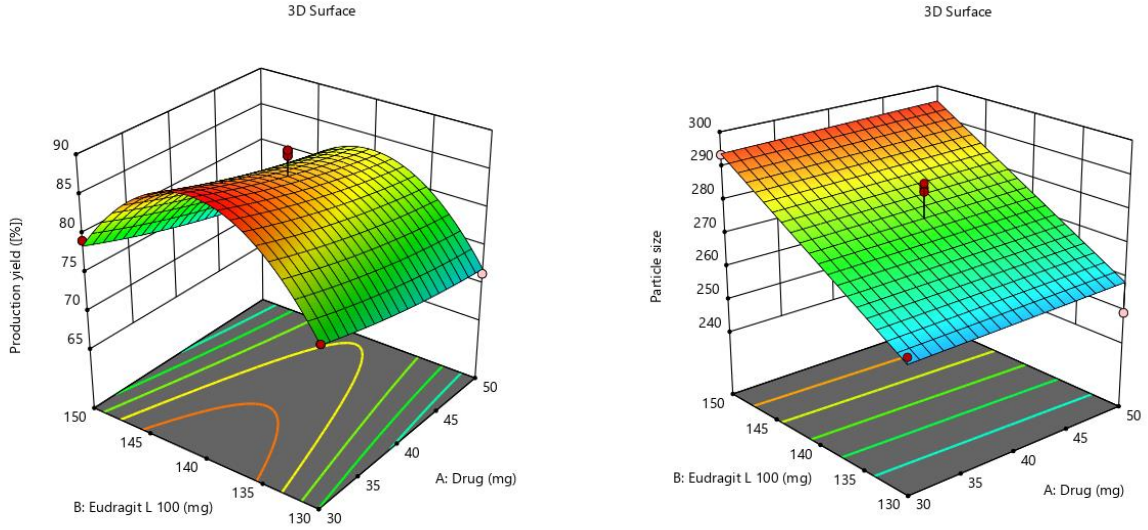


Fig 5. . FTIR spectra of isoconazole loaded microsponges.



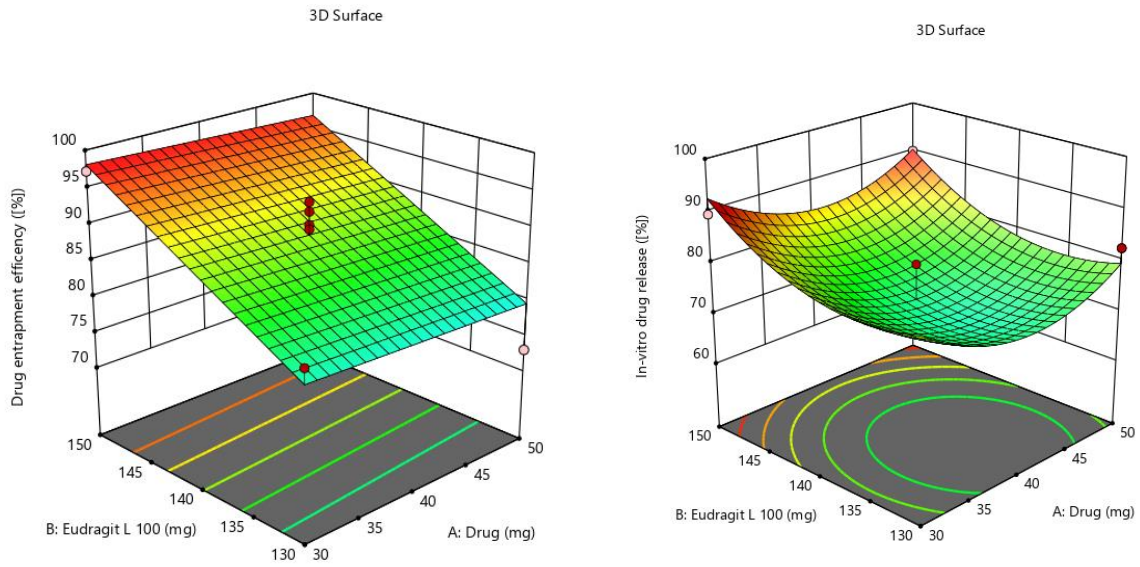


Fig.6 Model analysis of Response surface plots of (A)%yield, (B)particle size,(C) EE, (D) %drug release

Isoconazole nitrate microsponge loaded gel

4.7. Visual inspection

The visual characteristics including color, texture, and appearance of the prepared gel formulations containing isoconazole nitrate microsponges were evaluated. Each formulation displayed a uniform glossy white appearance with a thick consistency, indicating good homogeneity and a smooth texture. Furthermore, there were no lumps observed, although some formulations exhibited syneresis.

4.8.pH measurement

All developed formulations had pH values between 6.7 and 6.8, which was deemed appropriate to reduce the possibility of skin irritation after application.

4.9.Spreadability studies

The spreadability data showed that a tiny amount of shear may readily spread the gel. The spreadability of the drug-loaded microsponge gel was found to be good, with a spreadability of 7.3g cm/sec.

4.10.Viscosity studies

Studies on viscosities for formulations of microsponges were conducted. Table 8 shows the viscosities of each formulation. This indicates that the required viscosity for microsponge gel is present.

4.11.Drug content study

Studies on the drug content of microsponge formulations were done. Table 8 lists each formulation's drug content. The formulations' drug contents demonstrated that the medication was evenly dispersed throughout the gels. The results above indicate that microsponge gel contains additional drugs**.**

Table 8.

S.No.	Formulation code	pH	Drug content (%)	Viscosity (cps)	Spreadability studies (gm-cm/s)
1	G1	6.7	84.2	1004	7.3
2	G2	6.8	88.37	1010	6.9

4.12.In vitro diffusion study

For every formulation, in vitro diffusion test were conducted using phosphate buffer (pH 7.4). Table 9displays the in vitro diffusion of formulations G1 and G2.

Table 9.

S.No.	Time (h)	G1	G2
1	00	00	00
2	01	24.14	17.54
3	02	32.98	22.11
4	03	38.02	33
5	04	46	39.64
6	05	51.99	43.76
7	06	68.34	60.21
8	07	75	69.57

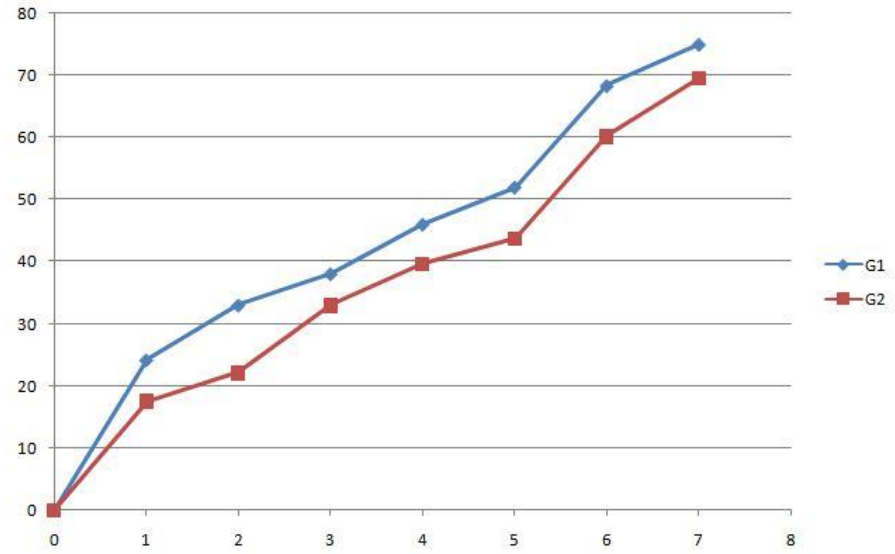


Fig 4.In vitro diffusion study

CONCLUSION

A microsponge-based delivery system was developed using the quasi-emulsion solvent diffusion method to achieve sustained release of isoconazole nitrate for topical applications. The drug-to-

polymer ratio significantly influenced both the drug entrapment efficiency and the size of the microsponges produced. The microsponge formulation exhibiting favorable characteristics was incorporated into carbopol 934 gels at same concentrations. Among the two gel formulations tested, G1 demonstrated superior antifungal activity. The drug release mechanism from the carbopol 934 gel loaded with the selected microsponge formulation follows Fickian diffusion, influenced by the porosity of the microsponges.

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