"MICROSPONGE DELIVERY SYSTEM FOR VAGINAL CANDIDIASIS: FORMULATION AND EVALUATION OF SYNTHETIC AND HERBAL DRUG-LOADED MICROSPONGES"

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Abstract:
This paper aimed to prepare and evaluate capsules containing novel microsponges loaded with Clotrimazole and Allicin to provide antifungal activity for vaginal candidiasis.
Methods: Capsules are prepared by HPMC, which are incorporated with clotrimazole and allicin. Microsponges are prepared by Quasi emulsion technique using EC, PVA, and BCD for clotrimazole microsponges and Eudragit RS100, PVA for Allicin Microsponges. These microsponges are characterized by SEM, FTIR, DSC, and Particle size analysis and evaluated for drug loading capacity, percent yield, encapsulation efficiency, also in-vitro drug release studies.
Result: FTIR & DSC studies show that no chemical interaction occurred between drug and polymer, and the batch shows good drug release and % yield
Conclusion: The created microsponge formulation has the potential of a vaginal candidiasis treatment. The microsponges are a viable remedy substitute due to their better physicochemical characteristics, prolonged drug release, and sustained action. It is necessary to conduct additional clinical studies to confirm their safety and efficacy.

Keywords: Microsponges, Vaginal Candidiasis, Clotrimazole, Allicin, NDDS, antifungal activity

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DOI: - 10.48047/ecb/2023.12.si10.00141

Introduction:

Many transdermal medication delivery systems have been created to follow the controlled and preset drug release by employing the skin as a doorway for administration. Despite improved medication delivery efficiency, the active ingredient still penetrates the skin layers and enters the bloodstream even if the skin itself is the final recipient of the drug. Microsponges are made to effectively distribute a pharmaceutically active substance at a low dose while improving stability, elegance, formulation pliability, minimising adverse effects, and changing the drug release pattern. (1)

The microsponges function as an active ingredient reservoir. These may be used to deliver a wide range of compounds under control, including perfumes, emollients, sunscreens, anti-inflammatory, antifungal drugs, and antibacterial agents. A microsponge system can retain active substances in a safe environment and release them to the skin in a controlled manner over time. (2)

Microsponges are highly cross-linked, non-collapsible, pores polymeric microspheres with particle sizes that vary from 5 to 300 m that can entrap a variety of active ingredients and release them gradually. Due to their sponge-like structure, microsponges exhibit special compression and dissolving characteristics. They have enhanced patient compliance and are extremely effective, stable, non-irritating, non-toxic, non-allergic, and non-mutagenic. (3) These microsponge composites are made safer, given the fact that the microsponge particles themselves are too big to be absorbed through the dermis. The possibility of bacterial contamination of the components trapped in the microsponge is another safety issue. Bacteria between 0.007 and 0.2 m cannot enter the tunnel structure of the microsponges because of the lower pore diameter. (4)

The technique for designing microsponges that is most frequently utilized is the quasi-emulsion solvent diffusion method. It is a two-step procedure in which a plasticizer and a diffusible material (porogen) are present while an internal phase made of a suitable polymer dissolves in solvents like dichloromethane (DCM), acetone, or ethanol.Then, this internal phase is distributed into an external aqueous phase that contains stabilizer-containing polyvinyl alcohol. (5)

Topical application helps to prevent pre-systemic metabolism and lower systemic toxicity. Different medications, including ketoconazole, itraconazole, and clotrimazole, are applied topically to the skin by rubbing or spreading. Antifungal medications applied topically have the potential to irritate and itch the skin and trigger allergic reactions. (6) Additionally, conventional formulations require frequent administration at high doses, which increases the risk associated with both local and systemic toxicity. To decrease local side effects and improve therapeutic efficacy, innovative drug delivery systems are therefore being considered. The current study discusses cutting-edge topical nanocarrier methods for the vaginal delivery of anti-fungal medications. One of the topics of topical formulation that has received considerable attention in pharmaceutical research is novel drug delivery systems (NDDS). (7)

As an infectious agent, Candida albicans is the most prevalent. This commensal dimorphic yeast colonises the skin, gastrointestinal system, and reproductive systems. Emerging pathogens that can colonise human mucocutaneous surfaces include non-C. albicans species. (8)

In the treatment of uncomplicated (acute) symptomatic vulvovaginal candidosis, topical imidazole was superior to placebo. In the majority of side-by-side comparisons, intravaginal clotrimazole and oral imidazole/triazole antifungal medications displayed similar levels of efficacy. Particularly, single-dose treatments using topical clotrimazole and oral fluconazole were used. (9,10)

A common medicinal plant used for health reasons is garlic (Allium sativum). Studies have demonstrated the anticancer, antibacterial, antiviral, and antifungal properties of garlic. Because it contains allicin sulphur (di-allyl-thio-sulfurnate), garlic has antifungal properties. This sulphur component makes it possible to employ different denture cleaning products. (11)

for the preparation of antifungal formulation HPMC capsule is used as its pH and dissolution rate are compatible with the vaginal environment. Prepared microsponges of clotrimazole and Allicin are added to it and evaluated this capsule for vaginal candidiasis. (12)

Material and Methods:

Clotrimazole is a gift sample by Amoli Pharmaceuticals. Allicin is extracted in the laboratory of D Y Patil College of Pharmacy, Akurdi, Pune. Ethyl Cellulose, PVA, and BCD are From Research Lab fine chem, Mumbai.

List of instruments

Sr No	Instrument	Manufacturer
1	Single-pan electronic balance	LC-GC precision, Mumbai
2	UV spectrophotometer	Shimadu UV 1700, Japan
3	Infrared spectrophotometer	JASCO 4100, Japan
4	Ultrasonicator	LAG Entrench Electronics Pvt Ltd Mumbai
5	Mechanical stirrer	Remi equipment pvt ltd Mumbai
6	Magnetic stirrer	Remi equipment pvt ltd Mumbai
7	Hot air oven	Pathak Electrical Work, Mumbai
8	Scanning electron microscope	JEOL JSM-6360 A

Methods:

Allicin Extraction method:

10 g of freshly peeled Garlic was taken mashed with a high-speed tissue mixture and enzymatically hydrolyzed at 250C for 10 min. add 40 ml extraction solvent, i.e., diethyl ether extracted for 350c for 1 hr after centrifugation at 12000 rpm for 10 min. filtration of supernatant was done. (13)

Identification test for Allicin:

Preliminary phytochemical analysis:

A preliminary phytochemical test was carried out to determine the chemical components. Because garlic is primarily composed of sulphur compounds, two sulphur tests were conducted.

1. Sulphur test/ lead acetate test: In a test tube, 2 mL of extract was added along with 3–4 drops of lead acetate plus 2–3 drops of 40% sodium hydroxide. The mixture was then stirred until the precipitate was gone. The test tube was heated for two minutes before cooling. A precipitate that was brownish-black appeared. This demonstrates that evaluated garlic extract contains sulphur. (14)
2. Sodium nitroprusside test: Thiosulfinate substance is generated by liquid allicin. Three to four drops of sodium nitroprusside were added to 2 mL of extract in the test tube. Two to three drops of 40% sodium hydroxide were added to the medium to make it alkaline. The presence of sulphur was indicated by the emergence of a deep violet colour in the provided extract. (14)

Preparation of microsponges by Quasi emulsion solvent diffusion technique:

For developing microsponges, the quasi-emulsion solvent diffusion approach appeared to hold promise due to its simplicity, reproducibility, and speed, as well as its ability to prevent solvent toxicity.

1. Preparation of Clotrimazole microsponges by Quasi emulsion solvent diffusion technique:

Microsponges with different compositions were designed using polymers like BCD, EC, and Eudragit. Batches were created with varying polymer ratios and polyvinyl alcohol concentrations for each medication at a speed of 1500 rpm. And finally, microsponges of clotrimazole prepared by using Dichloromethane as a solvent for the inner phase with BCD and EC as polymer and PVA as a stabilizer for the external phase polymer solution and drug solution are sonicated at 35^{o}C in the inner phase, and this inner phase poured into external phase containing PVA and water and followed by continues stirring at 1500 rpm for 2 hrs on a magnetic stirrer. After that, the mixture is filtered to separate microsponges and dried them using a hot air oven at 40^{o}C for 6 hr.

2. Preparation of Allicin microsponges by Quasi emulsion solvent diffusion technique:

For developing Allicin Microsponge with respective compositions, different compositions were designed using polymers like BCD, EC, and Eudragit. Batches were created with varying polymer ratios and polyvinyl alcohol concentrations for each medication at a speed of 1500 rpm. And microsponges of clotrimazole were prepared by using Dimethylformamide as a solvent for the inner phase with Eudragit S100 as polymer and PVA as a stabilizer for the external phase. Polymer solution and drug solution are sonicated at 35^{o}C in the inner phase, and this inner phase is poured into the external phase containing PVA and water and followed by continuous stirring at 1500 rpm for 2 hrs on a magnetic stirrer. After that, the mixture is filtered to separate microsponges and dried them using a hot air oven at 40^{o}C for 6 hr.

Preliminary studies

The following preliminary research was conducted to create a microsponge with a sustained release mechanism; ethyl cellulose was used as the polymer. Polyvinyl alcohol is employed as a cross-linking agent, while DMSO is used as a solvent. Initial research on the creation of microsponges was done using varied concentrations.

Table 1: Preliminary studies of clotrimazole and allicin microsponges

Ingredients	PT1	PT2	PT3	PT4	PT5	PT6
Clotrimazole IP (mg)	250	250	250	250	250	250
Allicin (mg)	10	10	10	10	10	10
Ethyl Cellulose (mg)	200	200	-	-	-	200
Beta cyclodextrin(mg)	150	150	-	-	150	150
Eudragit L 100 (mg)	-	100	-	-	-	-
Eudragit S 100(mg)	100	-	-	-	100	100
Eudragit RS 100 (mg)	-	-	100	100	-	-
Polyvinyl Alcohol (ml)	250	250	250	250	250	250
Water (ml)	100	100	100	100	100	100
Dichloromethane	10	10	10	10	10	10

Selection of drug-polymer ratio for preparation of Clotrimazole and Allicin:

Table 2: Clotrimazole microsponges formulations were prepared with different weight ratios of drug to ethyl cellulose (2:1, 1.5:1, 1:1, 1:1.5)

Formulation	Drug (mg)	Ethyl Cellulose (mg)	BCD	PVA(mg)	DCM(ml)	Water(ml)
CLTZMS1	250	100	100	100	10	100
CLTZMS2	250	150	150	150	10	100
CLTZMS3	250	250	250	250	10	100
CLTZMS4	250	300	300	300	10	100
CLTZMS5	250	75	75	75	10	100
CLTZMS6	250	200	100	250	10	100

Table 3: Allicin microsponges formulations were prepared with different weight ratios of drug to ethyl cellulose (2:1, 1.5:1, 1:1, 1:1.5)

Formulation	Drug (mg)	Eudragit RS 100(mg)	PVA (mg)	DMF (ml)	Water (ml)
ALCNMS1	250	300	300	10	100
ALCNMS2	250	150	200	10	100
ALCNMS3	250	250	250	10	100
ALCNMS4	250	200	150	10	100
ALCNMS5	250	125	75	10	100
ALCNMS6	250	75	125	10	100

- **Evaluation of Clotrimazole and Allicin Microsponges:**

1. Drug- Excipient interaction study:

FT-IR and DSC experiments were used to examine the interaction between the drug and the excipient. IR spectra were analysed to determine whether the drug and the excipients were compatible. The physical characteristics of the sample form (crystalline or amorphous) are revealed by DSC, as well as any potential interactions between the drug and excipients.

Thermal Analysis: A popular method for analyzing the behaviour of drug molecules during melting and recrystallization is differential scanning calorimetry (DSC). It is a thermoanalytical method that identifies the materials' thermodynamic properties by supplying details on the polymorphic transformations that occur when they are exposed to a regulated heat flux. thermogram Differential scanning calorimetry was used to create the formulation for the Clotrimazole drug, microsponges, and allicin microsponges. Microsponge samples were heated at a steady rate of 10°C/min across a temperature that varied from 40-200°C while being hermetically sealed in an aluminium pan. The inert atmosphere was kept by purging nitrogen at a flow rate of 10 ml/min.

Fourier transform-infrared spectroscopy: The wavelength range of 4000-400 cm-1 was used to record the spectra of Clotrimazole and Allicin, physical mixtures of Clotrimazole and BCD, and microsponge formulation using an FT-IR spectrophotometer using the Kbr pellet method.

2. Production Yeild: Calculating properly the initial weight of the raw materials and the final weight of the microsponges obtained facilitated the determination of the production yield of the microsponges.

% production yield = [Practical mass of Microsponge/ Theoretical mass (polymer + Drug)] X100

3. Encapsulation Efficiency and actual drug content:

In a mortar and pestle, a sample of dried microsponges weighing 10 mg was placed. A small quantity of phosphate buffer with a pH of 7.4 was then added, and the mixture was allowed to stand for 24 hours. Then pour the contents into a 100 ml volumetric flask and add pH 7.4 phosphate buffer to get the volume to 100 ml. The Whatman filter paper (No. 41) was used to filter the solution. Take 1 ml of the final solution into a 10-ml volumetric flask, then add pH-7.2 phosphate buffer to bring the volume up to 10. A UV spectrophotometer adjusted at 261 nm was used to measure the drug content.

% Encapsulation Efficiency = [Actual drug content in Microsponge/ Theoretical drug content] X100
%Actual drug content = [Act D / MS] X100

4. Particle size:

With the help of pixel pro software, particle size was determined by using an optical microscope. The average particle size was expressed in terms of µm. microsponges were mounted on the Slide and placed over the micrometer stage. The software pixel pro for image analysis of microparticles was used. Each determination was carried out on a minimum of 100 particles, and their mean was reported.

5. Scanning electron microscopy:

The surface morphology of particles was studied with scanning electron microscopy (SEM). Microsponges were mounted on double-faced adhesive tape and coated with a thin gold-palladium layer by a sputter-coated unit and analyzed with a scanning electron microscope (JEOL JSM- 6360).

6. In vitro Dissolution Study:

From Capsules Containing Microsponges of Clotrimazole and Allicin

A new simple technique that mimics the vaginal environment was developed (by Esra Bolglu et al.) to investigate the release behaviour of vaginal tablet formulations. The apparatus mainly consisted of a perfuser and syringe, which were connected with a thin latex connector and a sample collection vessel. The vaginal physiology was simulated with a syringe that has an inner diameter of 20 mm and a total length of 75 mm. Capsules were placed at the bottom of the syringe without a needle, and the assembly dipped into a water bath at 37 °C. The daily production of vaginal fluid is approximately 6 ml/d, and 0.5—0.75 ml is continually present in the vagina. Therefore, a perfusor was connected with the upper side of the syringe to give 6 ml of vaginal fluid to tablets in 24 h. (15)

The same amount of samples were collected concurrently from the bottom and then diluted with methanol 1:1 and check the absorbance under UV spectroscopy to check the absorbance of the Clotrimazole and allicin drug release from Microsponges via the perfusion method. (15)

Result and discussion

In this research, various polymers were used for the preparation of microsponges BCD, EC, Carbapol 940, Eudragit L100, S100, RS 100, and chitosan. As CTZ and Allicin are both water-insoluble, they are dissolved in organic solvents with polymers for the internal phase of the quasi-emulsion solvent diffusion method. BCD, EC & PVA are used for the preparation of clotrimazole microsponges, and Eudragit S 100 and PVA are used in the preparation of Allicin microsponges

Table 4: Particle size Entrapment efficiency and product yield of Clotrimazole Microsponges

	Run	Factor 1 BCD	Factor 2 EC	Factor 3 PVA	Response 1 Vescicle size	Response 2 Drug entrapment	Response 3 % Yield
14	1	125	100	251.134	6	60.79	63.45
8	2	200	200	200	10.11	62.89	63.28
5	3	50	0	200	9	75.67	65
1	4	50	0	50	7	70.9	70.11
15	5	125	100	125	9.7	80.2	80.27
9	6	1.13446	100	125	3.11	75.12	80.64
10	7	251.134	100	125	7.55	76.09	69.23
4	8	200	200	50	19.11	82.46	65.44
16	9	125	100	125	9.7	80.2	80.27
3	10	50	200	50	16	58.78	62.56
11	11	125	-68.1793	125	22	63.14	58.21
17	12	125	100	125	9.7	80.2	80.27
2	13	200	0	50	14.78	71.25	74.23
20	14	125	100	125	9.7	80.2	80.27
7	15	50	200	200	12.95	65.14	79.15
12	16	125	268.179	125	29	62.13	61.48

19	17	125	100	125	9.7	80.2	80.27
18	18	125	100	125	9.7	80.2	80.27
13	19	125	100	-1.13446	9.8	73.54	67.59
6	20	200	0	200	12	53	55.38

Table 5: Determination of Product yield and encapsulation efficacy And Particle size of allicin microsponges

Run	Component 1 A:Edragit s100	Component 2B: PVA	Response 1 Product yield	Response 2 DE	Response-3 PS
1	150	200	66.93	96.81	9
2	187.5	162.5	34.75	47.31	11
3	200	150	49.42	73.48	18
4	166.667	183.333	67.81	76.38	14.72
5	183.333	166.667	55.73	65.28	12.6
6	200	150	49.42	73.48	18
7	162.5	187.5	54.22	64.77	13.46
8	200	150	49.42	73.48	18
9	175	175	73.8	91.8	14
10	150	200	66.93	96.81	9
11	150	200	66.93	96.81	9
12	175	175	73.8	91.8	14
13	175	175	73.8	91.8	14

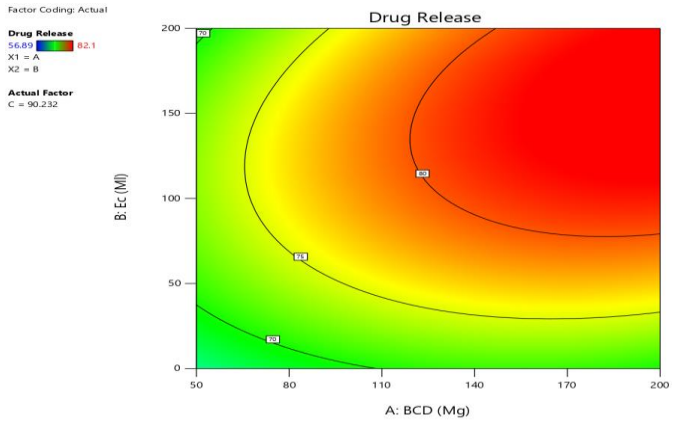
Invitro drug release study:

Drug release is dependent on the dissolution of the capsule medium inside the simulated vaginal fluid and its thickness. The microsponges are going to adsorb on the inner lining of the vagina, so the thickness can create a barrier, and the drug release from the microsponges takes a bit extra time.

The drug release by the ethyl cellulose microsponges is 78% slow as compared to Eudragit S 100 microsponge, which gives 89.32% more release capacity.

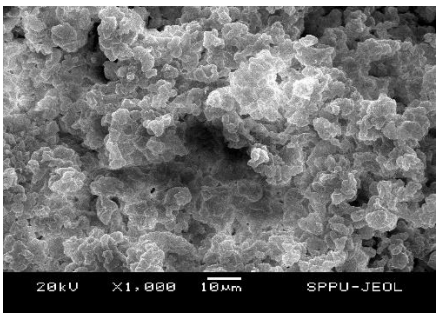
Capsule gets dissolved within 7 minutes after insertion. First, the allicin from its microsponges is released and gives immediate effect, and after that, the clotrimazole Ms gives adsorptive action to the vaginal membrane and gives a sustained release effect for 3-4 hrs.

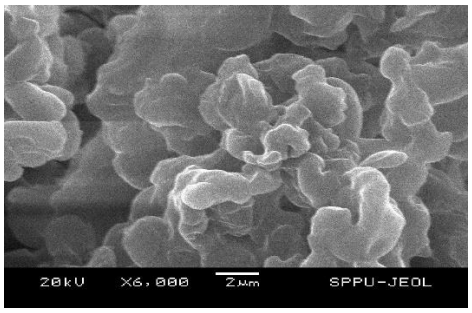
Optimized Capsules are HPMC capsules because of their compatibility with vaginal fluid.



Scanning electron microscopy:

SEM analysis was used to examine prepared microsponges to evaluate their surface topography and morphology. Microsponges were created, and SEM photomicrographs revealed that they were highly porous and primarily spherical. Pores were created by the solvent diffusing from the surface of the microsponges. Additionally, it became clear that the unique interior design consisted of a spherical chamber around a rigid shell made of medicine and polymer.



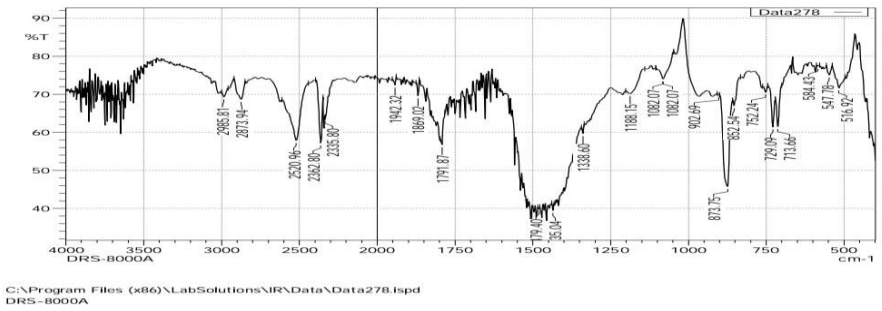


Drug excipient interaction study by FTIR:

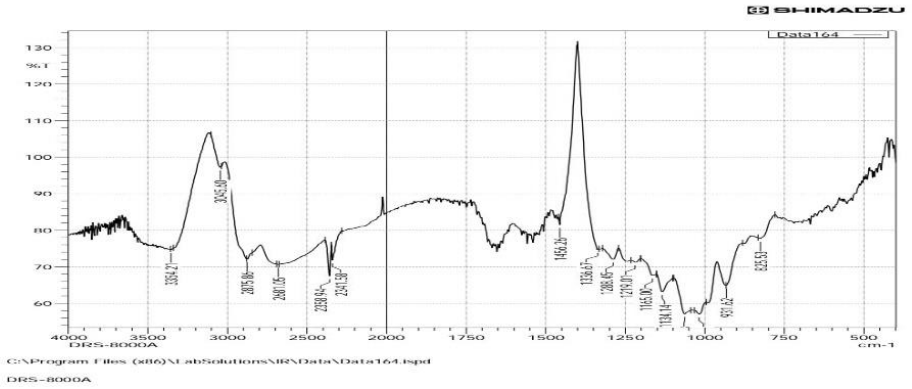
The results of an FTIR spectroscopy examination showed that no new peaks appeared and that no existing peaks vanished, showing that the medication and the utilized polymer did not interact chemically.

During the analysis of the compatibility study, all of the Clotrimazole and Allicin characteristic peaks were seen in the IR spectra of the physical mixture and Clotrimazole, and Allicin loaded microsponges.

Therefore, the results of the IR spectroscopy demonstrated that the drug was compatible with particular polymers and excipients. It was inferred from this that Clotrimazole and Allicin were compatible with given polymers and appeared to be stable in microsponges.



Sr No	IR Signal (cm-1)	Functional group
1	2985.81	C-H Stretching
2	1338	C-N Stretching
3	729	C-CL Stretching

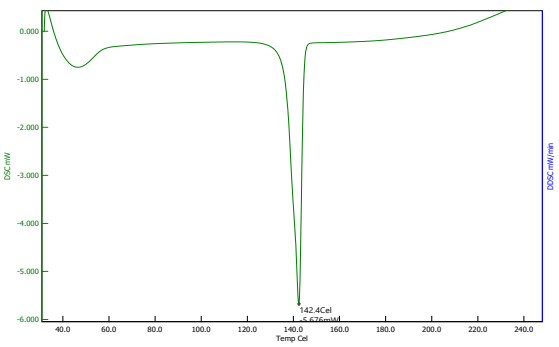


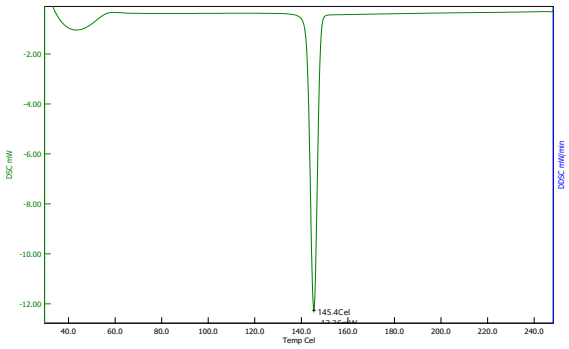
Sr No	IR Signal (cm-1)	Functional group
1	3354	C-H Streching
2	540	S-S streching
3	1219	S=O Streching

Drug-excipient interaction study by DSC thermogram

Dispersed in polymer demonstrated the same thermal behaviour as a pure molecule in DSC experiments. The medication was entrapped inside the microsponges during microsponges' formation and was not available to display any exothermic peak. As a result, no endothermic peak close to the

drug's melting point was seen, which supported the drug's entrapment in microsponges. This suggests that while making microsponges using eudragit S-100, the physical properties of Clotrimazole and Allicin are changed.





Conclusion:

Microsponge formulation holds great promise for the treatment of vaginal candidiasis, offering improved physicochemical characteristics, extended drug release, and enhanced antifungal activity. These microsponges provide a viable alternative to traditional treatments by allowing precise drug delivery with optimized size, shape, and surface properties. Their sustained release capabilities ensure prolonged drug presence at the infection site, potentially reducing treatment frequency and improving therapeutic outcomes. Furthermore, microsponges exhibit superior antifungal activity, demonstrated by their ability to inhibit Candida species growth. However, additional clinical studies are needed to confirm the safety, efficacy, and patient acceptance of microsponges as a treatment for vaginal candidiasis.thereby this research shows capsules containing clotrimazole and allicin microsponges shows significant antifungal activity in Candida albicans species which is responsible for vulvovaginitis.

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