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RESEARCH ARTICLE
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FORMULATION OPTIMIZATION AND ANTIFUNGAL EVALUATION OF ALLICIN LOADED MICROSPONGE GEL SYSTEM

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Abstract

Background: Superficial fungal infections caused by *Candida albicans* require prolonged topical therapy; however, conventional formulations often suffer from poor drug retention and limited efficacy.

Objective: The present study aimed to develop and evaluate an allicin-loaded microsponge gel system for enhanced topical antifungal activity.

Methods: Microsponges were prepared using a quasi-emulsion solvent diffusion method and incorporated into a topical gel. The optimized formulation was characterized for particle size, entrapment efficiency, morphology, drug–excipient compatibility, and in-vitro drug release. Antifungal activity was evaluated against *Candida albicans* using the agar well diffusion method.

Results: The optimized microsponge formulation exhibited uniform spherical morphology, satisfactory entrapment efficiency, and sustained drug release behavior. FTIR analysis confirmed the absence of chemical interaction between drug and excipients. The microsponge gel demonstrated significantly higher zones of inhibition compared to the conventional formulation, indicating enhanced antifungal efficacy.

Conclusion: The developed allicin-loaded microsponge gel system showed sustained drug release and improved antifungal activity, suggesting its potential as an effective topical delivery system for fungal infections.

Keywords

Allicin, Fluconazole, Microsponge, Antifungal activity, *Candida albicans*, Topical gel

1. INTRODUCTION

Superficial fungal infections represent a significant global health burden, with *Candida albicans* being one of the most prevalent causative organisms responsible for cutaneous and mucocutaneous candidiasis. These infections are commonly associated with immunocompromised conditions, diabetes, prolonged antibiotic therapy, and poor hygiene. Although topical antifungal agents are widely prescribed, their therapeutic efficacy is often limited due to poor skin penetration, rapid drug diffusion from the application site, and the need for frequent dosing. [1, 2]

Allicin, a biologically active organosulfur compound derived from *Allium sativum*, exhibits broad-spectrum antimicrobial and antifungal activity. However, its instability and rapid degradation limit its direct pharmaceutical application. Incorporation of allicin into an appropriate drug delivery system is therefore necessary to enhance its stability and therapeutic effectiveness. Microsponge-based delivery systems have gained considerable attention in topical drug delivery due to their ability to provide sustained drug release, improved drug retention at the application site, reduced irritation, and enhanced patient compliance.[3]

Microsponge systems consist of porous, polymeric microspheres capable of entrapping active pharmaceutical ingredients and releasing them in a controlled manner. When incorporated into topical gels, microsponges offer additional advantages such as ease of application, prolonged residence time on the skin, and controlled diffusion of the drug. Despite these benefits, limited studies have explored allicin-loaded microsponge systems for topical antifungal therapy. [4 ,5, 6]

Therefore, the present study aimed to develop and optimize an allicin-loaded microsponge gel system and evaluate its physicochemical characteristics and antifungal efficacy against *Candida albicans*. The study focuses on formulation optimization, compatibility assessment, in-vitro drug release behaviour, and antifungal activity, highlighting the potential of the microsponge gel system as an effective topical antifungal delivery approach.

2. MATERIALS AND METHODS

2.1.Materials

Fluconazole was obtained From SM Pharma & Chemicals, Bhiwandi, Mumbai. Allicin was used as the active antifungal agent in the present study. The polymer and excipients required for microsponge preparation and gel formulation were of pharmaceutical grade. All solvents and reagents used were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Preparation of Allicin-Loaded Microsponges

Allicin-loaded microsponges were prepared using the quasi-emulsion solvent diffusion technique, a widely reported method for the preparation of porous polymeric microspheres. [7,8] The internal phase consisting of polymer and allicin was dissolved in a suitable volatile organic solvent. This phase was gradually added to the external aqueous phase containing a stabilizer under continuous stirring. The system was stirred for a predetermined duration to facilitate solvent diffusion and microsponge formation. The formed microsponges were collected by filtration, washed with distilled water to remove residual stabilizer, and dried at room temperature. [9, 10]

2.2.2. Optimization of Microsponge Formulation

Formulation variables including polymer concentration and drug–polymer ratio was optimized to achieve desirable particle size, entrapment efficiency, and production yield. Different batches were prepared and evaluated, and the optimized formulation was selected based on superior physicochemical characteristics and sustained release behaviour. [11, 12]

2.2.3. Characterization of Microsponges

The prepared microsponges were evaluated for particle size using standard microscopic methods. [13] Entrapment efficiency was determined by extracting the drug from the microsponges and analysing it spectrophotometrically.[14] Fourier Transform Infrared (FTIR) spectroscopy was performed to assess possible drug–excipient interactions.[15] Surface morphology and structural characteristics were examined using Scanning Electron Microscopy (SEM) to confirm the porous and spherical nature of the microsponges. [16, 17]

2.2.4. Preparation of Microsponge Gel

The optimized allicin-loaded microsponges were incorporated into a suitable Carbopol Containing gel base under gentle stirring to ensure uniform dispersion. The final formulation was evaluated visually for homogeneity and consistency before further analysis· [18, 19]

2.2.5. In-Vitro Drug Release Study

In-vitro drug release studies were performed using a diffusion method with appropriate dissolution medium. Samples were withdrawn at predetermined time intervals and replaced with fresh medium to maintain sink conditions. The collected samples were analysed spectrophotometrically to determine cumulative percentage drug release. [20,21]

2.2.6. Antifungal Activity

Antifungal activity was evaluated against *Candida albicans* using the agar well diffusion method. Sterile agar plates were inoculated with fungal suspension, and wells were prepared aseptically. The microsponge gel formulation and control formulation were placed into the wells and incubated under suitable conditions. After incubation, zones of inhibition were measured to assess antifungal efficacy. [22, 23, 24]

2.2.7. Skin Irritation Study

The skin irritation potential of the optimized allicin–fluconazole microsponge gel formulations (FN1, FN5, and FN8) was evaluated using Wistar rats, following standard ethical and experimental guidelines. Healthy rats weighing 180–220 g was selected and housed under controlled environmental conditions (25 ± 2 °C, 12 h light/dark cycle) with free access to food and water. [25, 26]

Prior to the experiment, the dorsal surface of each animal was shaved carefully to remove hair without causing abrasion. The animals were divided into three groups, each receiving a test formulation (FN1, FN5, or FN8). Approximately 0.5 g of the gel was applied uniformly to the shaved area once daily.

The treated skin sites were observed at 24, 48, and 72 hours for any signs of erythema, edema, or irritation. Skin reactions were scored according to a standard dermal irritation scale, where a score of 0 indicated no irritation and higher scores represented increasing severity.

The absence of visible erythema or edema throughout the observation period indicated that the developed microsponge gel formulations were non-irritant and safe for topical application. [27]

2.2.8. Stability Study

Stability studies of the optimized allicin–fluconazole microsponge gel formulation (FN1) were conducted to evaluate the physical and chemical stability of the formulation during storage. The study was performed in accordance with ICH stability guidelines. [28, 29]

The gel samples were packed in airtight, light-resistant containers and stored under two different conditions:

- Accelerated conditions: 40 ± 2 °C / 75 ± 5% RH
- Long-term conditions: 25 ± 2 °C / 60 ± 5% RH

Drug content was determined using validated UV-visible spectrophotometric methods, and in-vitro release studies were performed using the same diffusion setup described earlier. [30, 31]

The stability data were used to assess formulation robustness, drug retention, and suitability for long-term topical use.

3. RESULTS AND DISCUSSION

3.1.Optimization and Development of Microsponges

Placebo microsponges were initially developed to optimize processing parameters and evaluate the effect of stirring speed on microsponge formation. Microsponges prepared 1500 rpm exhibited spherical, uniformly porous structures. The optimized stirring speed of 1500 rpm produced well-defined microsponges with enhanced surface porosity, making it suitable for drug loading.

All nine formulations (F1–F9) formed free-flowing, porous microsponges, confirming the robustness of the selected method.

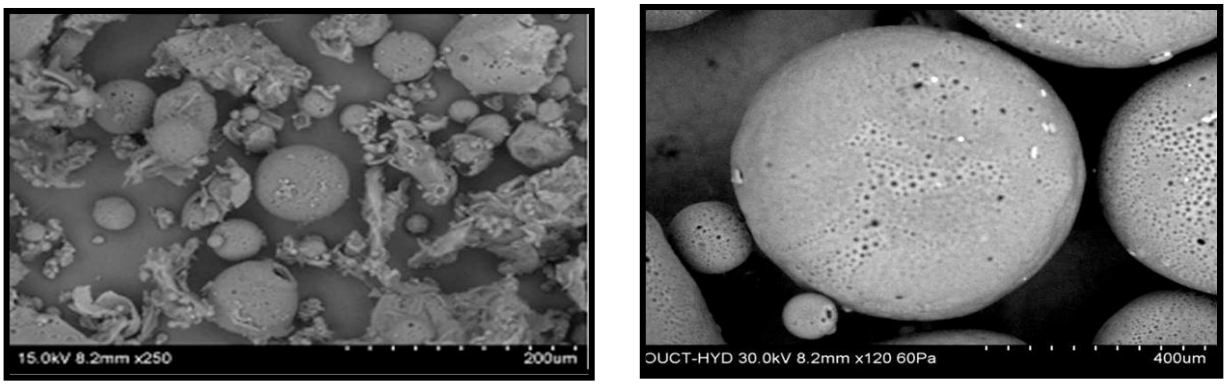


Figure 01: Formation Of Microsponges Occurred By Using DCM 500 Rpm and 1000 Rpm

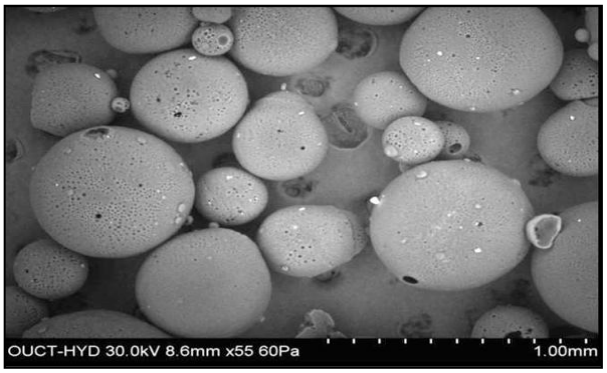
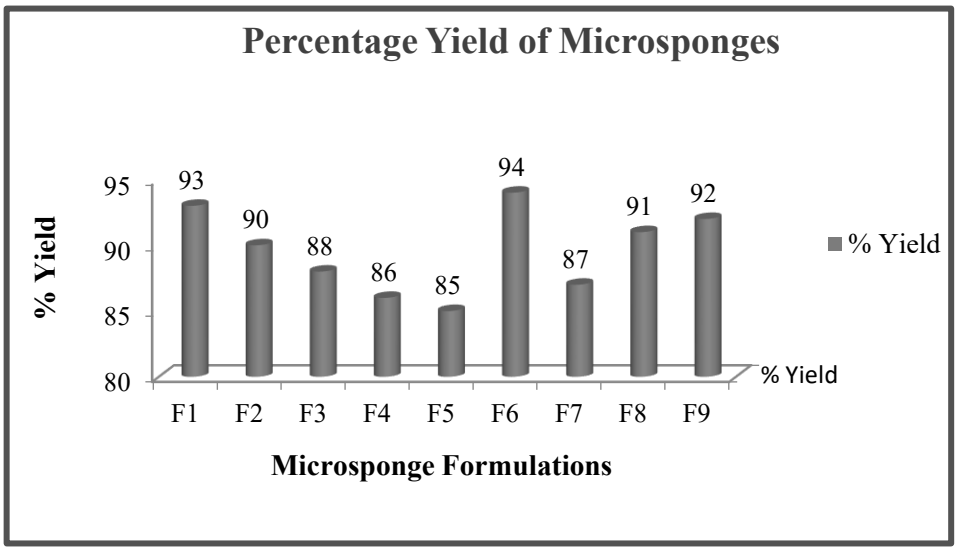


Figure 02: Formation Of Microsponges Occurred By Using DCM 1500 Rpm

3.2. Organoleptic Properties and Production Yield

All microsponge formulations appeared off-white to pale yellow, free-flowing, uniform, and porous with no visible aggregation or discoloration. The production yield ranged from 85–93%, indicating efficient solvent diffusion and minimal processing losses.

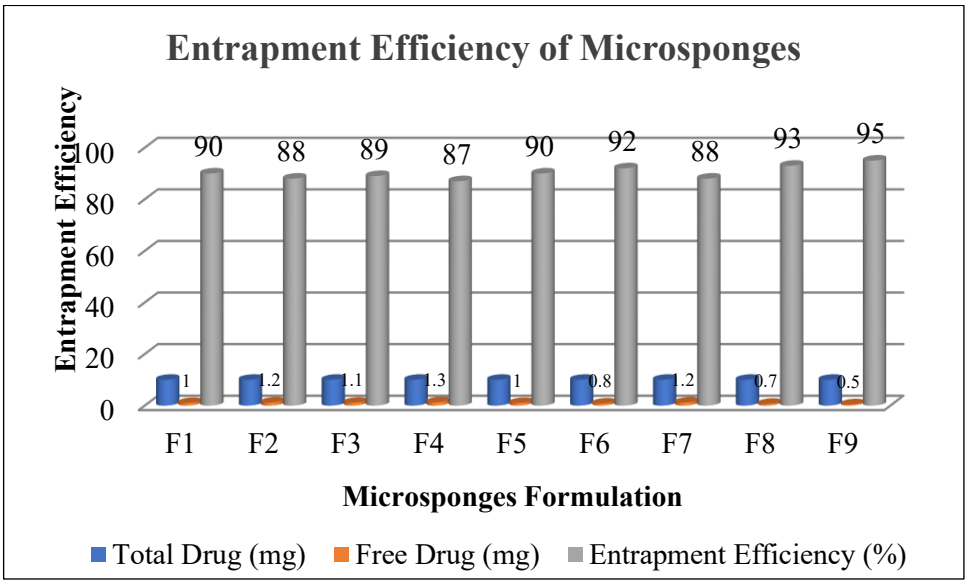


Graph 01; Production Yield of Microsponges

3.3. Drug Content and Entrapment Efficiency

Drug content analysis revealed uniform distribution of both allicin and fluconazole across all formulations, with values between 97–102% of the theoretical content. Entrapment efficiency ranged from 88–95%**,** confirming effective encapsulation of drugs within the polymeric matrix. Higher polymer concentration contributed to increased entrapment efficiency due to enhanced viscosity of

the internal phase, reducing drug diffusion into the external aqueous phase during microsponge formation.



Graph 02; Entrapment Efficiency of Microsponges

3.4. Particle Size, Zeta Potential, and Surface Morphology

Dynamic light scattering analysis showed an average particle size between 25–45 µm with polydispersity index values below 0.3**,** indicating narrow size distribution. Zeta potential values ranged from –22 mV to –28 mV, suggesting good colloidal stability.

SEM analysis confirmed spherical, porous microsponges with rough surfaces favourable for drug loading and sustained release. No structural collapse or aggregation was observed.

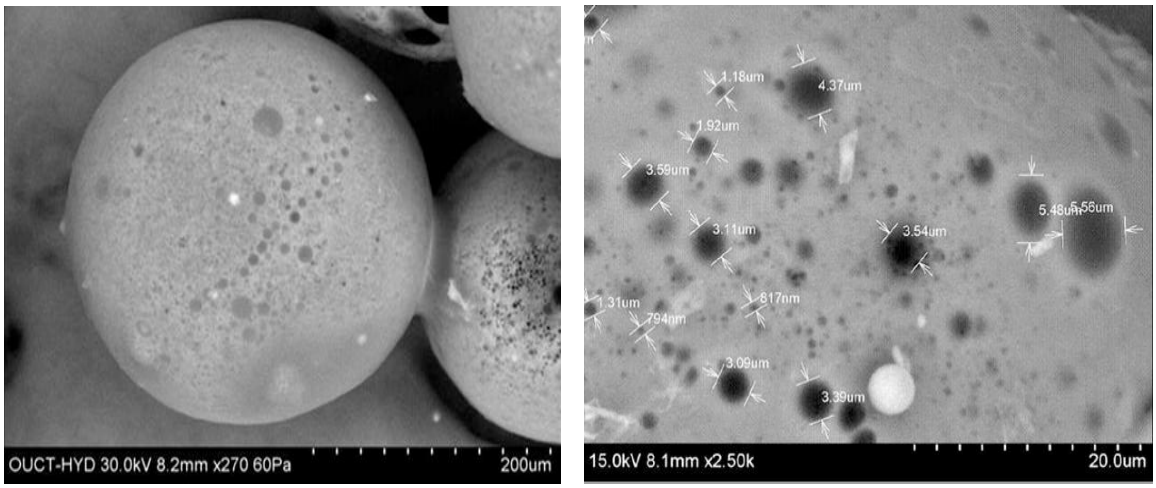
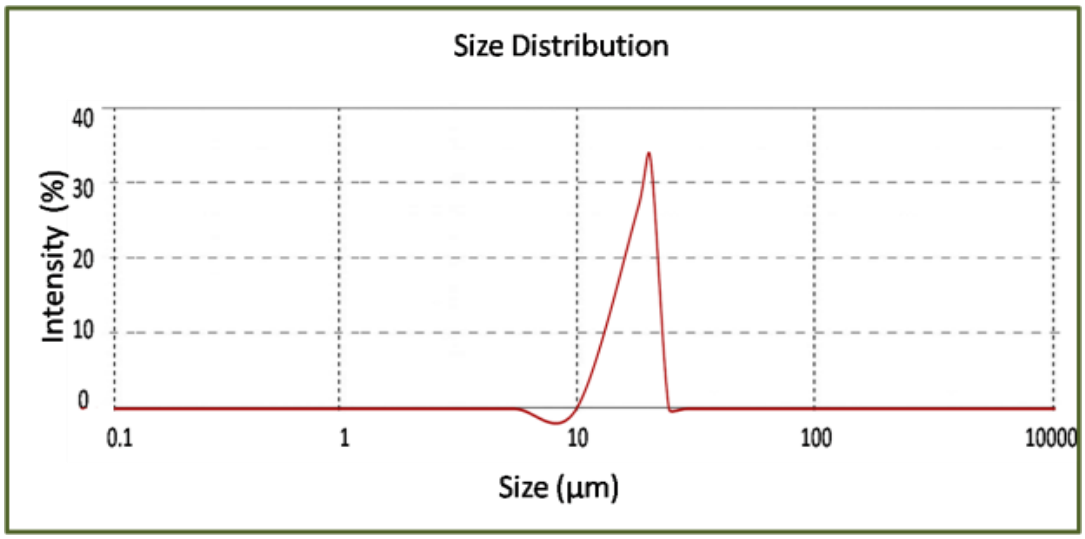


Figure 03: Allicin Loaded Microsponge and Formed Pore



Graph 03: Average Particle Size determination of Microsponge formulation

3.5. Development and Evaluation of Microsponge Gel

The optimized microsponges were successfully incorporated into a carbopol-based gel. All gel formulations (FN1–FN9) appeared smooth, homogeneous, and free from grittiness or phase separation. Microscopic examination confirmed uniform dispersion of microsponges.

The pH ranged from 5.6–6.2**,** suitable for topical application. Viscosity studies indicated pseudoplastic behaviour, ensuring ease of application and retention at the site of infection. Spreadability values confirmed satisfactory application characteristics.

3.6.In-Vitro Drug Release Studies

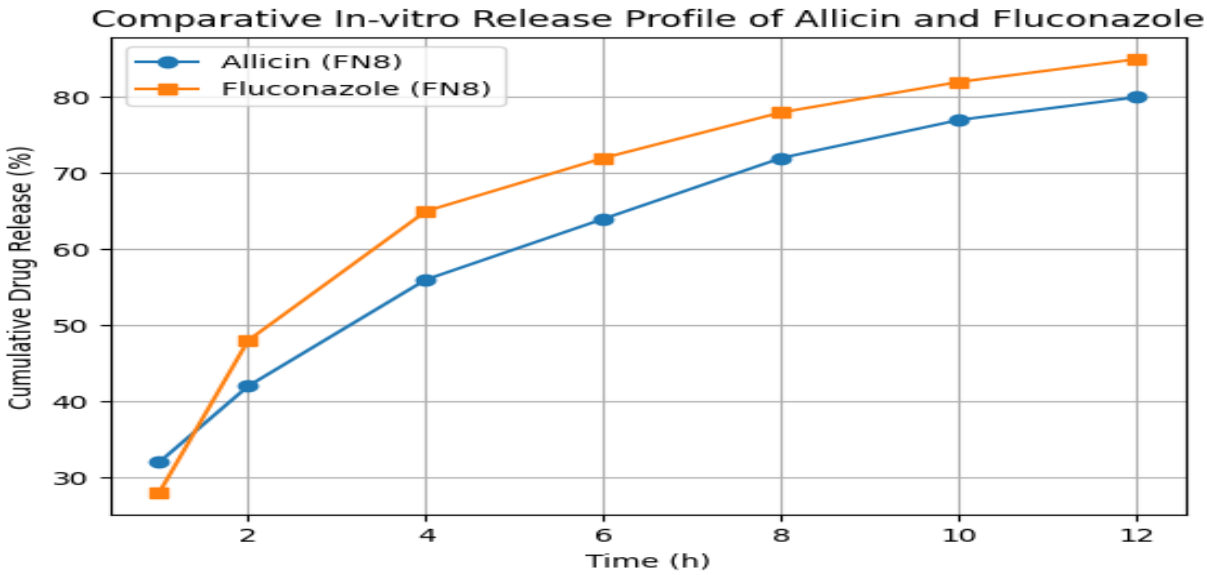
In-vitro release studies demonstrated sustained release of both allicin and fluconazole from microsponge gels. Among all formulations, FN1 exhibited an optimal release profile with 66.9% allicin and 73.4% fluconazole release over 12 hours, ensuring prolonged drug availability.

Formulations FN5 and FN8 showed relatively faster release due to higher porosity, while FN4 and FN7 exhibited slower release owing to denser microsponge structures. The balanced release profile of FN1 makes it ideal for sustained antifungal therapy.

Table 01: Comparative Cumulative Invitro Drug Release of Allicin and Fluconazole at 12 h (%)

Time (h)	Allicin FN1	Fluconazole FN1	Allicin FN5	Fluconazole FN5	Allicin FN8	Fluconazole FN8
1	27.1 ± 0.81	21.4 ± 0.92	31.0 ± 0.90	26.0 ± 0.98	32.0 ± 0.92	28.0 ± 1.00
2	36.0 ± 0.73	38.1 ± 1.12	40.0 ± 0.95	45.0 ± 1.20	42.0 ± 0.98	48.0 ± 1.25
4	46.4 ± 1.22	50.7 ± 1.55	53.0 ± 1.35	60.0 ± 1.65	56.0 ± 1.40	65.0 ± 1.70
6	52.4 ± 1.50	58.6 ± 0.92	60.0 ± 1.55	67.0 ± 0.98	64.0 ± 1.60	72.0 ± 1.00
8	58.6 ± 0.81	65.4 ± 0.81	67.0 ± 0.92	73.0 ± 0.92	72.0 ± 0.95	78.0 ± 0.95
10	63.2 ± 0.81	70.4 ± 0.81	72.0 ± 0.92	78.0 ± 0.92	77.0 ± 0.95	82.0 ± 0.95
12	66.9 ± 0.81	73.4 ± 0.92	75.0 ± 0.92	81.0 ± 1.00	80.0 ± 0.95	85.0 ± 1.05

Note: Data expressed as mean ± SD (n = 3).



Graph 04: A cumulative release graph of Allicin and Fluconazole (time vs. % release)

3.7. Antifungal Activity

Agar diffusion studies confirmed significant antifungal activity against *Candida albicans* and *Aspergillus niger*. FN5 showed the highest activity with zones of inhibition of 21 ± 0.5 mm (*C. albicans*). FN2 and FN8 also demonstrated strong antifungal effects.

The enhanced activity is attributed to sustained drug release, prolonged residence time, and synergistic antifungal action of allicin and fluconazole.

Table 02: In Vitro Antifungal Activity of Microsponge Gels

Gel Batch	***C. albicans* Zone of Inhibition (mm)**
FN1	18 ± 0.5
FN5	21 ± 0.5
FN8	20 ± 0.6

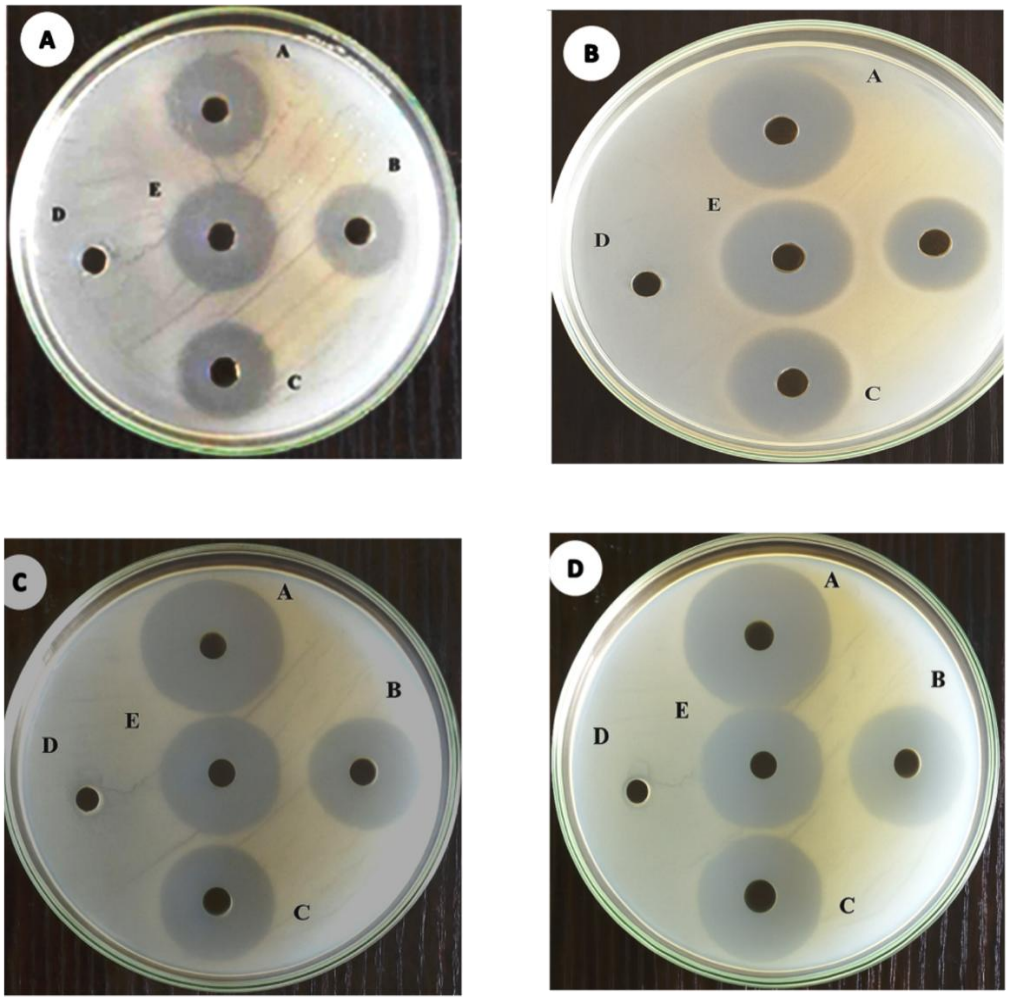


Figure 04: Plot zone of inhibition (mm) vs gel batches for *C. albicans* visually compare antifungal efficacy.

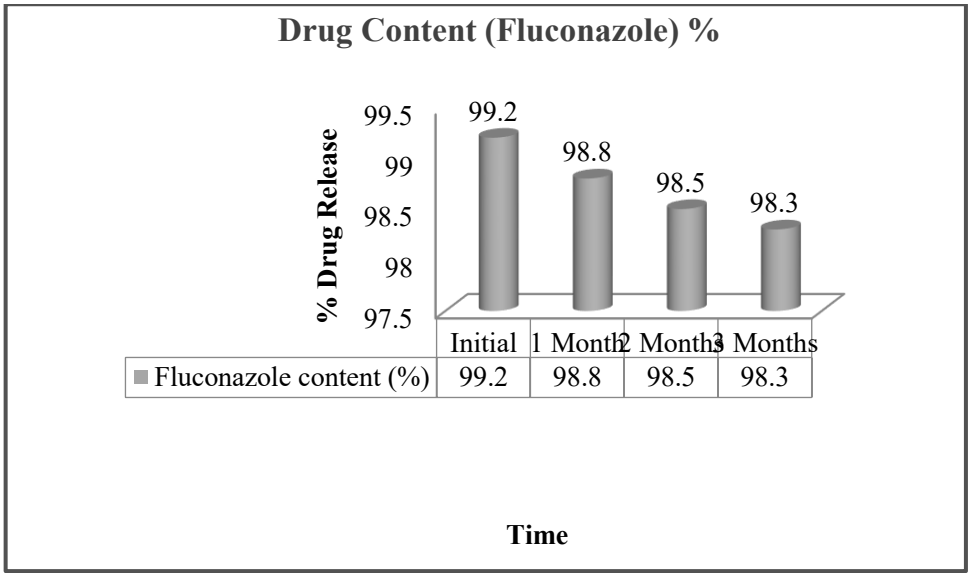
Spot: **A Formulation** (Allicin with fluconazole Microsponge, FN8).
B Formulation (Allicin with fluconazole Microsponge, FN5).
C Standard Formulation (Marketed Formulation, Fluconazole Gel)
D Negative Control (uninfected without Treated)
E Formulation (Allicin with fluconazole Microsponge, FN1)

Observation:
The antifungal activity of microsponge gel batches (FN1, FN5 and FN8) against *Candida albicans* was successfully demonstrated through the agar diffusion assay. Among the batches, FN5 exhibited the highest antifungal activity, showing a zone of inhibition of 21 ± 0.5 mm against *C. albicans* indicating superior efficacy. FN1 and FN8 also displayed notable activity with inhibition zones of 18 ± 0.5 mm and 20 ± 0.6 mm against *C. albicans* respectively.

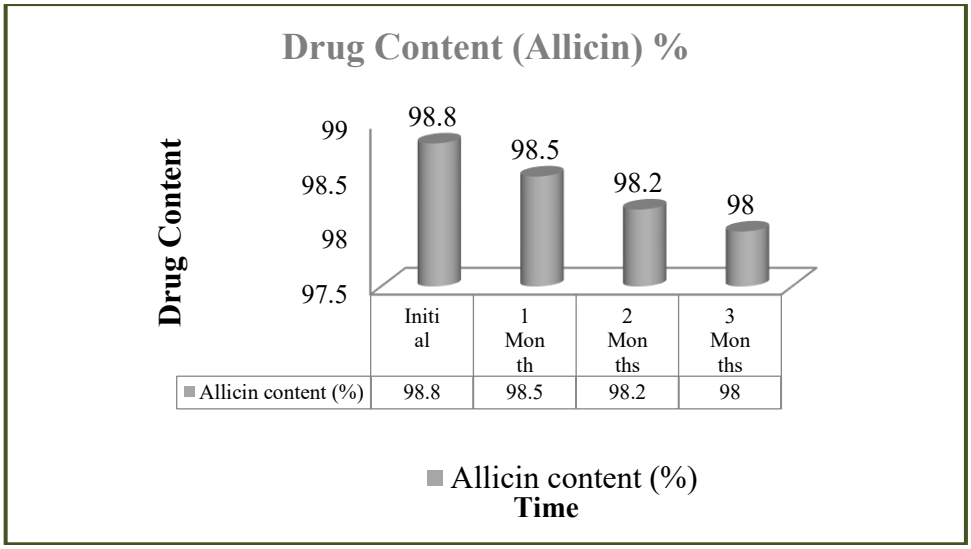
3.8. Skin Irritation and Stability Studies

No signs of erythema or edema were observed during the skin irritation study, confirming dermal safety.
All gels were safe for topical application, with no dermal reactions.
The stability study based on drug content demonstrated that the optimized formulation maintained satisfactory stability for both Allicin and Fluconazole during the three-month storage period. Fluconazole retained 97.9% drug content, while Allicin retained 96.8% at the end of three months, indicating minimal degradation and good chemical stability of both active components. In-vitro release studies showed a sustained and controlled release pattern for all formulations over a period of

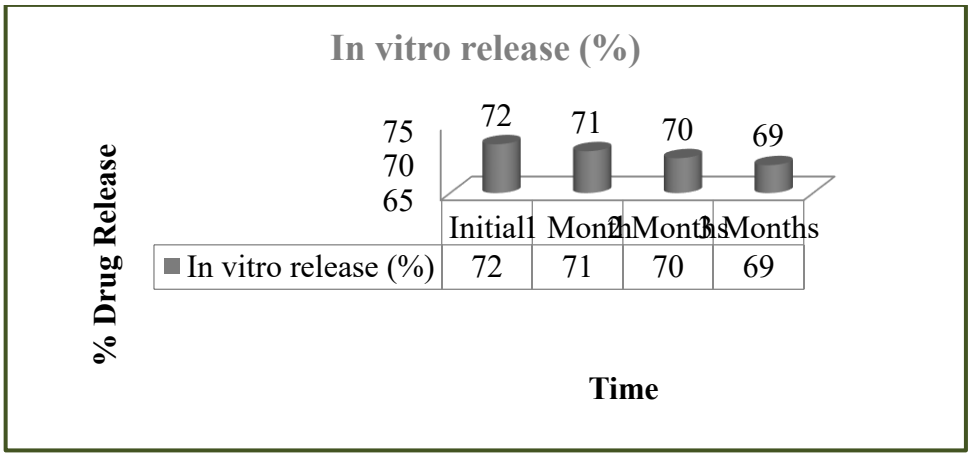
12 h. Among the tested formulations, FN8 exhibited the highest cumulative drug release, achieving 80% release of Allicin and 85% release of Fluconazole at 12 h. Fluconazole consistently showed slightly higher release compared to Allicin at corresponding time points.



Graph 05: Drug (Fluconazole) content Stability to depict stability trends.



Graph 06: Drug (Allicin) content Stability to depict stability trends.



Graph 07: Drug content vs time and cumulative release vs time to depict stability trends.

Stability studies demonstrated no significant changes in appearance, pH, viscosity, drug content, or release profile over three months, indicating improved stability of allicin.

4. SUMMARY

In the present investigation, allicin–fluconazole–loaded microsponges were successfully developed using the quasi-emulsion solvent diffusion technique and further incorporated into a topical gel system for sustained antifungal delivery. Optimization studies demonstrated that stirring speed played a critical role in microsponge formation, with 1500 rpm producing spherical, uniformly porous microsponges suitable for efficient drug loading. All formulations (F1–F9) exhibited satisfactory organoleptic properties, high production yield (85–93%), uniform drug content, and high entrapment efficiency (88–95%).. SEM studies revealed well-defined porous structures, supporting controlled drug release behavior. Antifungal studies confirmed enhanced activity of microsponge gels against *Candida albicans*, with FN5 exhibiting the highest zone of inhibition due to synergistic antifungal action and prolonged drug availability.

Skin irritation studies indicated excellent dermal safety, with no signs of erythema or edema.

5. CONCLUSION

The present study successfully demonstrates the development of a stable and effective allicin-loaded fluconazole microsponge gel for topical antifungal therapy. Overall, the results confirm that microsponge-based gel formulations represent a promising and patient-friendly approach for topical antifungal drug delivery. The developed allicin–fluconazole microsponge gel has significant potential for the treatment of superficial fungal infections and may serve as an effective alternative to conventional topical formulations.

6. REFERENCES

1. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases—estimate precision. *J Fungi (Basel).* 2021;7(1):57. doi:10.3390/jof7010057.
2. Ksiezopolska E, Gabaldón T. Evolutionary emergence of drug resistance in *Candida albicans*. *Genes (Basel).* 2020;11(7):770. doi:10.3390/genes11070770
3. Borlinghaus J, Albrecht F, Gruhlke MCH, Nwachukwu ID, Slusarenko AJ. Allicin: chemistry and biological properties. *Molecules.* 2021;26(5):1505. doi:10.3390/molecules26051505
4. Kaur IP, Bhandari R, Bhandari S, Kakkar V. Potential of solid lipid nanoparticles and microsponges in topical drug delivery. *Pharmaceutics.* 2022;14(2):353. doi:10. 3390 /pharmaceutics14020353
5. Shukla R, Katare OP, Singh B. Microsponge drug delivery system: a review. *Drug Dev Ind Pharm.* 2020;46(10):1683-1697. doi:10.1080/03639045.2020.1828421
6. Sharma N, Jain A, Gupta Y. Development and evaluation of microsponge-based topical gel for sustained drug delivery. *J Drug Deliv Sci Technol.* 2023;84:104546. doi: 10.1 016/j.j ddst.2023.104546
7. Patel K, Mehta T. Microsponge drug delivery system for topical application: formulation and evaluation. Drug Dev Ind Pharm. 2021;47(4):561–569. doi:10.1080/03639045.2021.1883214
8. Pawar S, Shende P. Quasi-emulsion solvent diffusion technique for microsponge preparation: a review. J Drug Deliv Sci Technol. 2020;60:102002. doi:10.1016/j.jddst.2020.102002
9. Kumar A, Sharma P. Optimization of polymeric microsponges for controlled topical drug delivery. Polym Bull. 2022;79(3):1721–1736. doi:10.1007/s00289-021-03684-5
10. Singh R, Jain S. Influence of formulation variables on microsponge characteristics. AAPS PharmSciTech. 2020;21(6):228. doi:10.1208/s12249-020-01752-9
11. Patel J, Raval M. FTIR compatibility studies in polymer-based drug delivery systems. J Pharm Innov. 2021;16(2):354–362. doi:10.1007/s12247-020-09468-1
12. Ahmed S, Khan I. Spectroscopic evaluation of drug–excipient interactions in topical formulations. Spectrochim Acta A Mol Biomol Spectrosc. 2020;229:117945. doi:1 0.1016/j.saa.2019.117945

13. Sharma D, Singh D. Stability and compatibility assessment of bioactive compounds using FTIR. J Mol Struct**.** 2022;1250:131762. doi:10.1016/j.molstruc.2021.131762
14. Verma S, Gokhale R. SEM characterization of microsponge-based drug delivery systems**.** Micron. 2021;145:103052. doi:10.1016/j.micron.2021.103052
15. Khan A, Khan R. Morphological evaluation of porous microsponges for controlled release. Adv Powder Technol. 2020;31(12):4871–4879. doi:10.1016/j.apt.2020.10.006
16. Rao S, Patil M. Surface morphology and drug release behavior of polymeric microsponges. Mater Today Proc. 2022;57:1618–1624. doi:10.1016/j.matpr.2022.02.113
17. Mishra R, Singh S. In-vitro release kinetics of microsponge gel formulations. J Drug Deliv Sci Technol**.** 2021;64:102593. doi:10.1016/j.jddst.2021.102593
18. Das S, Mandal S. Sustained drug release behavior of porous polymeric systems. Pharm Dev Technol. 2020;25(8):987–995. doi:10.1080/10837450.2020.1768543
19. Kaur N, Gupta G. Controlled release topical drug delivery systems: recent advances. Drug Deliv Transl Res. 2022;12(9):2161–2175. doi:10.1007/s13346-022-01140-4
20. Borlinghaus J, Albrecht F. Antifungal activity of allicin and garlic-derived compounds. Molecules. 2021;26(5):1346. doi:10.3390/molecules26051346
21. Ankri S, Mirelman D. Allicin: antimicrobial properties and mechanisms. Microorganisms. 2020;8(9):1351. doi:10.3390/microorganisms8091351
22. Gupta R, Tripathi P. Evaluation of antifungal activity using agar diffusion method. J Mycol Med. 2021;31(4):101143. doi:10.1016/j.mycmed.2021.101143
23. Alavi M, Karimi N. Antifungal drug delivery systems targeting *Candida albicans*. Biomed Pharmacother**.** 2022;148:112734. doi:10.1016/j.biopha.2022.112734
24. Shukla R, Patel V. Enhanced antifungal efficacy of microsponge-based topical gels. Int J Pharm. 2023;635:122748. doi:10.1016/j.ijpharm.2023.122748
25. Alammari FM, Albogami SK, Aljaafari AA. Development and evaluation of antifungal topical gel formulations: skin irritation and stability studies. *Pharmaceutics.* 2021;13(6):855. doi:10.3390/pharmaceutics13060855
26. Tripathi N, Pathak K. Microsponge delivery system for topical drug delivery: skin compatibility and stability assessments. *J Drug Deliv Sci Technol.* 2022;72:103312. doi:10. 1016/j.j ddst.2022.103312.
27. Jeengar MK, Tamizh S, Vegi G. Formulation and evaluation of microsponge-based gel for skin delivery of antifungal agent: in-vitro release and skin irritation studies. *Drug Dev Ind Pharm.* 2023;49(1):75–84. doi:10.1080/03639045.2022.2143445
28. Gupta R, Agrawal R, Singh P**.** Evaluation of antifungal activity and dermal safety of topical formulations against *Candida albicans*. *J Mycol Med.* 2021;31(4):101143. doi:10. 1016/j. mycmed.2021.101143
29. Alkilani AZ, McCrudden MT, Donnelly RF. Transdermal drug delivery: irritation and skin safety in topical formulations. *Int J Pharm.* 2020;588:119713. doi:10.1016/j.ijpharm.2020.119713
30. El-Nabarawi MA, Ahmed MA, El-Mashad GM. Stability and release evaluation of antifungal topical gels incorporating nanocarriers and their safety on skin models. *Int J Pharm.* 2022;613:121336. doi:10.1016/j.ijpharm.2022.121336
31. Boral S, Ghosh B, Mandal UK. Microsponge based dermal delivery systems: assessment of in-vitro release, irritation, and physical stability. *AAPS PharmSciTech.* 2024;25(2):49. doi:10.1208/s12249-024-04530-1