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FORMULATION AND EVALUATION NANOSPONGE BASED ANTIFUNGAL GEL CONTAINING FLUCONAZOLE

Bankar Ganesh Uttam[1], Gaikwad Aditya Bajrang[1], Jasud Anant Balkrushna[1], Jadhav Sahil Nitin[1], Kadam Aarti Sambhaji[1], Khamkar Akash Dipak[1], Giramkar Ankita Arjun[2]*, Shinde Pratiksha Jalindar[2], Khedkar Amol Navnath[3].

[1]Student, Shri Dattakrupa Foundations, Saikrupa Institute of Pharmacy, Ghargaon, Shrigonda, Ahilyanagar, Maharashtra, India 413728.

[2]Assistant Professor, Shri Dattakrupa Foundations, Saikrupa Institute of Pharmacy, Ghargaon, Shrigonda, Ahilyanagar, Maharashtra, India 413728.

[3]Principal, Shri Dattakrupa Foundations, Saikrupa Institute of Pharmacy, Ghargaon, Shrigonda, Ahilyanagar, Maharashtra, India 413728.

***Corresponding Author: *Giramkar Ankita Arjun,**
Email: pansareankita2019@gmail.com

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Abstract

The primary aim of this study is to develop nanosponges of fluconazole intended for topical application in gel form. The successful formulation of nanosponges was achieved using ethyl cellulose and eudragit RS100 as polymers, polyvinyl acetate as the surfactant, and dichloromethane as the solvent through the emulsion solvent diffusion technique. The resulting nanosponges were incorporated into a topical gel using agents such as Carbopol 934 and HPMC K4M. The prepared nanosponges and gel underwent various evaluations, including production yield, drug entrapment efficiency, FTIR, DSC, SEM, spreadability, pH, viscosity, and in-vitro drug release. Among the different formulations of nanosponges, those created with ethyl cellulose exhibited favorable properties and were subsequently transformed into gel form. The resulting gel demonstrated drug release for a duration of up to 12 hours. In summary, the emulsion solvent diffusion method proved to be an effective approach for the formulation of nanosponges.

INTRODUCTION:

To achieve either a topical or systemic effect, topical preparations are applied directly to the skin's surface. These dermal and transdermal delivery systems can potentially replace the needles traditionally used for administering various medications, offering several advantages such as the avoidance of first-pass hepatic metabolism, gastric degradation, and the need for frequent dosing (1). The Global Burden of Disease project has indicated that skin diseases remain the fourth leading cause of nonfatal disease burden globally (2).

This study focuses on the development of a nanosponge that contains a topical gel of the antifungal medication fluconazole. Nanosponge represents a novel class of nanoscale sponges, comparable in size to a

virus, which are filled with therapeutic agents linked by specific connectors (5). Nanosponges are polymeric delivery systems characterized by their spongy structure and small spherical particles with a highly porous surface. They enable passive targeting of drug molecules and cosmetics. The advantages of using nanosponges include reduced dosage requirements, avoidance of first-pass metabolism, prolonged retention of drugs on the skin, maintenance of the dosage form on the skin, and prevention of systemic absorption (6). Fluconazole is an antifungal medication classified as BCS class II. Its IUPAC name is 2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl) propan-2-ol. As a triazole derivative, it is utilized in treating various fungal infections, including candidiasis, blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, dermatophytosis, and pityriasis. Fluconazole effectively inhibits fungal cytochrome P-450 sterol C-14 alpha-demethylation, leading to the accumulation of fungal 14-alpha-methyl sterols. This disruption of normal fungal sterols results in its fungistatic activity.

Mechanism of action

Controlled Drug Release Fluconazole is encapsulated within nanosponges, which facilitate a gradual and continuous release upon application to the skin, thereby sustaining effective antifungal drug concentrations for an extended period.

Enhanced Skin Penetration: The nanosponge delivery system enhances the ability of Fluconazole to penetrate through the skin layers, enabling the drug to more effectively reach the site of fungal infection.

Antifungal Action: The released Fluconazole acts by inhibiting the fungal enzyme that is crucial for ergosterol synthesis, thereby disrupting the integrity of the fungal cell membrane and resulting in either the death or growth inhibition of fungal cells

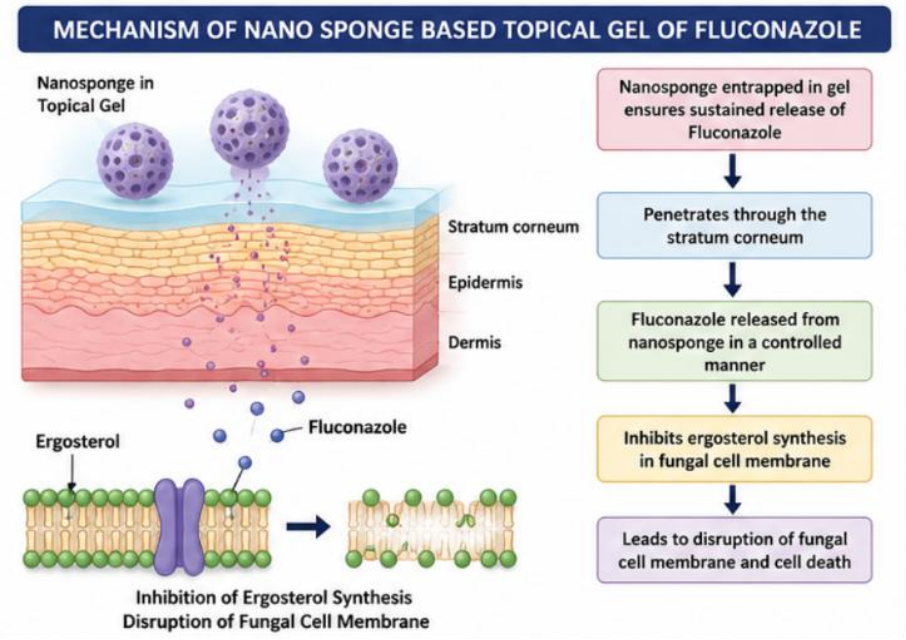


Figure No. 1: Mechanism of action of Nanosponge based Gel

Advantages of Nanosponge based Gel

1) Controlled Drug Release

Nanosponges facilitate a sustained and regulated release of fluconazole, ensuring therapeutic drug levels are maintained for an extended period.

2) Enhanced Skin Retention

The nanosponge system prolongs the residence time of fluconazole within the skin, thereby enhancing the local drug concentration at the site of infection.

3) Improved Drug Stability

Encapsulating fluconazole in nanosponges safeguards the drug from degradation due to environmental factors such as light and oxidation.

4) Reduced Dosing Frequency

Thanks to the extended drug release, the gel necessitates less frequent applications, thereby enhancing patient compliance.

5) Minimized Side Effects

Localized delivery limits systemic absorption and reduces the likelihood of adverse effects typically associated with oral antifungal treatments.

6) Enhanced Antifungal Activity

Improved penetration and retention of fluconazole in the layers of infected skin enhance its therapeutic efficacy against fungal infections.

7) Non-Greasy and Patient-Friendly Formulation

The topical gel is simple to apply, non-sticky, and cosmetically pleasing, which increases patient acceptance.

8) Improved Bioavailability at the Target Site

Nanosponges boost the solubility and availability of fluconazole at the infection site, resulting in a more effective treatment.

MATERIALS AND METHOD:

Materials:

Fluconazole was received as a complimentary sample from Virupaksha Organics Limited, located in Hyderabad, Telangana, India. Additional excipients such as ethyl cellulose, Eudragit RS 100, Dichloromethane (DCM), polyvinyl alcohol (PVA), Carbopol 934, Hydroxy Propyl Methyl Cellulose K4M, Methylparaben, Propylparaben, and Propylene glycol were sourced from Modern Science Lab in Nashik, Maharashtra, India. All chemicals utilized were of analytical grade and were employed in their received form.

Fuconazole

Fluconazole is a broad-spectrum antifungal drug belonging to the triazole class. It inhibits fungal cytochrome P450 enzymes and prevents ergosterol synthesis, an essential component of fungal cell membranes. It is effective against various fungal infections such as candidiasis, ringworm, athlete's foot, and other dermal fungal infections.

Ethyl Cellulose

Ethyl cellulose is a hydrophobic polymer used for nanosponge preparation. It forms a porous matrix that encapsulates the drug and provides controlled release.

Polyvinyl Alcohol (PVA)

PVA acts as a surfactant and stabilizer during nanosponge formation. It helps maintain particle size uniformity and prevents particle aggregation.

Dichloromethane (DCM)

DCM is a volatile organic solvent used to dissolve both fluconazole and ethyl cellulose. During preparation, it evaporates, leaving behind porous nanosponges.

Carbopol 934

Carbopol 934 is a synthetic high-molecular-weight polymer widely used as a gelling agent. It provides viscosity, stability, and good spreadability to the topical gel.

Propylene Glycol

Propylene glycol functions as a humectant and penetration enhancer. It improves skin hydration and facilitates drug permeation through the skin.

Triethanolamine

Triethanolamine neutralizes Carbopol dispersion and adjusts the gel pH to a skin-compatible range (5.5–7.0).

Methyl Paraben and Propyl Paraben

These preservatives protect the formulation from microbial contamination during storage and use.

Distilled Water

Distilled water is used as the aqueous phase during nanosponge preparation and as the vehicle in gel formulation. It ensures purity and minimizes contamination.

Methods used:

1. Preparation of Fluconazole Nanosponges

Fluconazole-loaded nanosponges can be synthesized using the solvent evaporation technique. Begin by accurately measuring Fluconazole along with a polymer such as Ethyl Cellulose. Dissolve both the drug and the polymer in an organic solvent (for instance, dichloromethane or acetone) to create the organic phase. Next, prepare an aqueous phase that includes Polyvinyl Alcohol (PVA) as a stabilizing agent. Gradually introduce the organic phase into the aqueous phase while maintaining continuous stirring. Keep stirring until the solvent has completely evaporated, leading to the formation of nanosponges. Finally, collect the nanosponges through filtration or centrifugation, rinse them with distilled water, and allow them to dry at room temperature.

2. Preparation of Gel Base

Disperse Carbopol 934 in distilled water and allow it to hydrate for 24 hours. Add propylene glycol and preservatives (methyl paraben and propyl paraben) with continuous stirring until a homogeneous gel base is formed.

3. Incorporation of Nanosponges into Gel

Accurately weigh the prepared Fluconazole nanosponges and disperse them uniformly in the gel base under gentle stirring. Adjust the pH of the formulation to 6.0–7.0 using triethanolamine. Mix thoroughly to obtain a smooth and homogeneous nanosponge-based topical gel.

4. Packaging and Storage

Transfer the prepared gel into clean, airtight containers or collapsible tubes and store at room temperature for further evaluation studies.

5. Flow Chart:

Fluconazole + Ethyl Cellulose
↓
Dissolution in Organic Solvent
↓
Addition into PVA Solution
↓
Stirring & Solvent Evaporation
↓
Formation of Nanosponges
↓
Collection and Drying
↓
Preparation of Carbopol Gel Base
↓
Incorporation of Nanosponges
↓
pH Adjustment with Triethanolamine
↓
Fluconazole Nanosponge Topical Gel Prepared

1. Fluconazole + Ethyl Cellulose

Accurately weigh Fluconazole (drug) and Ethyl Cellulose (polymer) according to the formulation ratio. Ethyl cellulose acts as the nanosponge-forming polymer and helps control drug release.

2. Dissolution in Organic Solvent

Dissolve the weighed Fluconazole and Ethyl Cellulose in a suitable organic

solvent such as dichloromethane or acetone. Stir the mixture until a clear and homogeneous solution is obtained.

3. Addition into PVA Solution

Prepare an aqueous solution of Polyvinyl Alcohol (PVA) in distilled water. Add the organic phase slowly into the PVA solution under continuous magnetic stirring. PVA acts as a stabilizing and emulsifying agent.

4. Stirring and Solvent Evaporation

Continue stirring the emulsion for 2–4 hours at room temperature. During stirring, the organic solvent evaporates gradually, leading to the formation of nanosponge particles.

5. Formation of Nanosponges

As the solvent evaporates completely, porous nanosponge structures containing Fluconazole are formed. These nanosponges entrap the drug within their polymeric matrix.

6. Collection and Drying

Separate the formed nanosponges by filtration or centrifugation. Wash them with distilled water to remove excess PVA and impurities. Dry the nanosponges in a hot air oven at 40°C or under vacuum until a constant weight is obtained.

7. Preparation of Carbopol Gel Base

Disperse Carbopol 934 in distilled water and allow it to hydrate for 24 hours. Add propylene glycol and preservatives with gentle stirring to obtain a uniform gel base.

8. Incorporation of Nanosponges

Accurately weigh the dried Fluconazole nanosponges and slowly add them to the prepared gel base. Stir continuously to ensure uniform distribution throughout the gel.

9. pH Adjustment with Triethanolamine

Add triethanolamine dropwise while stirring until the desired gel consistency and pH (5.5–7.0) are achieved. This step converts the dispersion into a clear, smooth gel.

Preparation of Fluconazole Nanosponge Topical Gel

The prepared gel is evaluated for appearance, pH, viscosity, spreadability, drug content, in-vitro drug release, and antifungal activity. The final gel is packed in airtight containers and stored at room temperature.

1. Accurately weigh all required ingredients.
2. Dissolve the polymer(s) in distilled water under continuous stirring.
3. Allow the polymer solution to hydrate completely.
4. Prepare the drug solution separately.
5. Add the drug solution slowly to the polymer solution with continuous stirring.
6. Add the required gelling/cross-linking agent.
7. Add preservatives and other excipients, if required.
8. Adjust the pH of the formulation to the desired range.
9. Make up the final volume with distilled water.
10. Stir until a clear and homogeneous solution is obtained.
11. Remove entrapped air by allowing the formulation to stand.
12. Transfer the prepared in situ gel into suitable sterile containers.
13. Store the formulation under appropriate conditions until evaluation.

Formulation table

Table No. 1: Formulation table of Fluconazole Nanosponge Topical Gel

Sr. No.	Ingredient	Batch F1	Batch F2	Batch F3	Batch F4	Batch F5

1	Fluconazole Nanosponge	0.20g	0.40g	0.80	1g	1.20g
2	Ethyl Cellulose	0.5g	1g	1.5g	2g	2.5g
3	Polyvinyl Alcohol (PVA)	0.5g	0.5g	0.5g	0.5g	0.5g
4	Dichloromethane or Ethanol	10ml	10ml	10ml	10ml	10ml
5	Carbopol 934	1g	2g	3g	4g	5g
6	Triethanolamine	0.2	0.1	0.2	0.1	0.2
7	Propylene Glycol	10g	10g	10g	10g	10g
8	Methyl Paraben	0.20g	0.20g	0.20g	0.20g	0.20g
9	Distilled Water	q.S to 100ml	q.S to 100ml	q.S to 100ml	q.S to 100ml	q.S to 100ml

Figure No. 2: Prepared Batches of Fluconazole Nanosponge Topical Gel

Evaluation Parameters

1. Physical Appearance

- Colour
- Clarity
- Homogeneity
- Presence of particulate matter

2. pH Determination

Measurement of formulation pH using a calibrated digital pH meter.

3. Viscosity

The viscosity of prepared gel was measured using a Brookfield viscometer (Brookfield Engineering, spindle LV6) at different RPM viscosity. The measurement

was made over a whole range of speed settings from 5-100 rpm with 10 seconds between two successive speeds.

4. Spread ability

Assessment of ease of spreading of the gel on the application surface.

5. Stability study:

To evaluate the physical, chemical, and microbiological stability of the Fluconazole nanosponge-based topical gel during storage.

6. Gelling strength study of gel:

Measurement of the mechanical strength of the formed gel.

7. Particle Size Analysis

Purpose: To determine nanosponge particle size and distribution.
Procedure: Particle size is measured using Dynamic Light Scattering (DLS) or Particle Size Analyzer.

8. Zeta Potential Measurement

Purpose: To evaluate the stability of nanosponges.
Procedure: The sample is analyzed using a Zeta Potential Analyzer.
Observation: Higher absolute zeta potential values indicate better stability.

9. In Vitro Drug Release Study

Determination of drug release profile using a diffusion cell or dissolution apparatus.

10. Extrudability

Determination of the ease of gel extrusion from the container.

11. Stability Study

Evaluation of physical appearance, pH, viscosity, and drug content during storage under different conditions.

12. Scanning Electron Microscopy (SEM)

Purpose: To study the surface morphology of nanosponges.
Procedure**:** Dried nanosponge samples are mounted on aluminum stubs, gold-coated, and examined under SEM.
Observation: Nanosponges should appear spherical with a porous structure.

13. Fourier Transform Infrared Spectroscopy (FTIR)

Purpose: To determine drug–polymer compatibility.
Procedure: FTIR spectra of Fluconazole, polymer, and optimized formulation are recorded and compared.
Observation**:** No significant shift in characteristic peaks indicates compatibility.

14. Differential Scanning Calorimetry (DSC)

Purpose: To study the thermal behavior of the drug and formulation.
Procedure: Samples are heated at a controlled rate and thermograms are recorded.
Observation: Changes in melting peaks indicate drug entrapment within nanosponges.

15. X-Ray Diffraction (XRD)

Purpose: To determine the crystalline or amorphous nature of the drug.
Procedure: Samples are scanned using an X-ray diffractometer.
Observation: Reduced intensity of crystalline peaks indicates successful encapsulation and amorphization of the drug.

RESULTS AND DISCUSSION

1. Physical Appearance

Table No. 2: Physical Appearance of Prepared gel

Formulation	Colour	Consistency	Odour
F1	pink	Good	Characteristics
F2	pink	Poor	Characteristics

F3	pink	Very poor	Characteristics
F4	pink	Good	Characteristics
F5	pink	Good	Characteristics

2. **pH Determination**

Table No. 3: pH Determination of Prepared gel

Formulation	pH
F1	6.02
F2	6.96
F3	6.85
F4	6.73
F5	6.00

3. **Viscosity**

Table No. 4: Viscosity Determination of Prepared gel

Code	Torque (%)	RPM	Viscosity (cP)
C1	96	5	1080
		10	672
		20	600
		50	240
		100	220
C2	96	5	1142
		10	943
		20	883
		50	666
		100	325
C3	96	5	1245
		10	954
		20	823
		50	752
		100	325
C4	96	5	1056
		10	826
		20	751
		50	623
		100	456
C5	96	5	1114
		10	964
		20	824
		50	624
		100	536

4. **Spreadability**

Table No. 5: Spreadability Determination of Prepared gel

Formulation	Spreadability
F1	17.3
F2	15.3
F3	41.2
F4	22.91
F5	19.91

5. Stability study:

Table No. 6: Stability study of Prepared gel

Stability	1st Week	2nd Week	3rd Week	4th week
F1	Stable	Stable	Instable	Stable
F2	Stable	Instable	Stable	Instable
F3	Stable	Instable	Instable	Stable
F4	Stable	Stable	Stable	Instable
F5	Stable	Stable	Instable	Stable

6. Gelling strength study of gel:

Table No. 7: Gelling strength study of Prepared gel

Formulation	Gelling Strength
F1	40
F2	35
F3	30
F4	30
F5	45

7. Particle Size Analysis

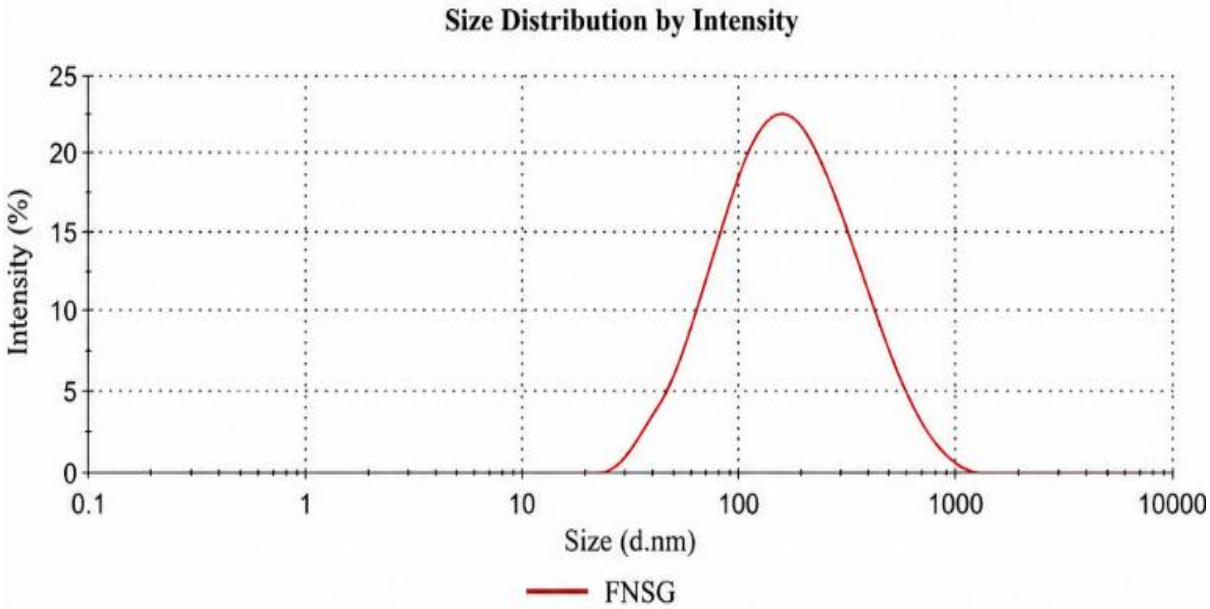


Figure No. 3: Particle Size Analysis

8. Zeta Potential Measurement:

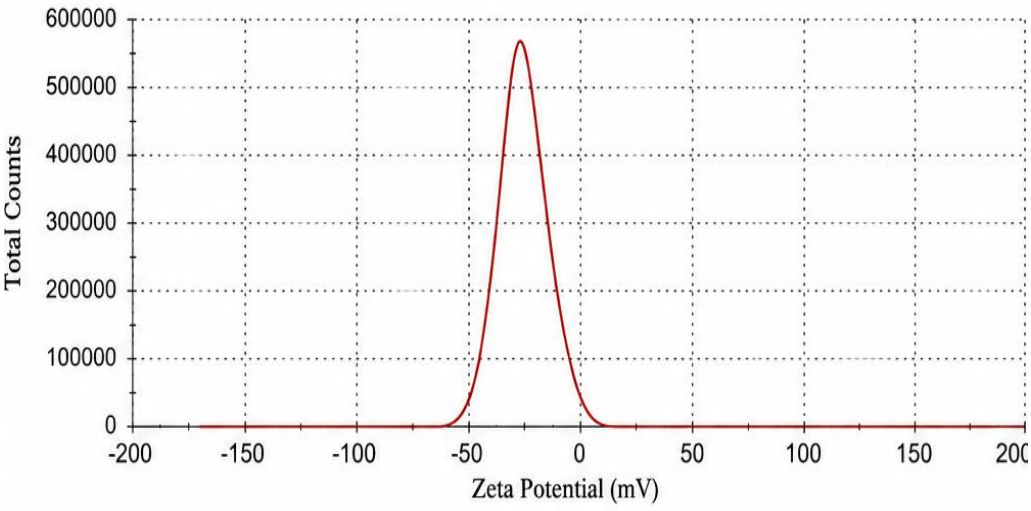


Figure No. 4: Zeta Potential Measurement

9. In Vitro Drug Release Study

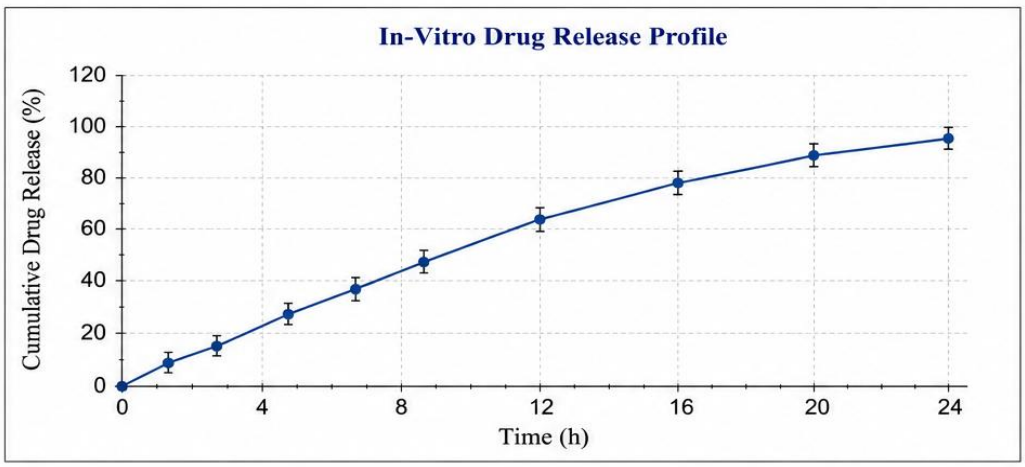


Figure No. 5: In Vitro Drug Release Study

10. Extrudability

Figure No. 6: Extrudability Study

11. Scanning Electron Microscopy (SEM)

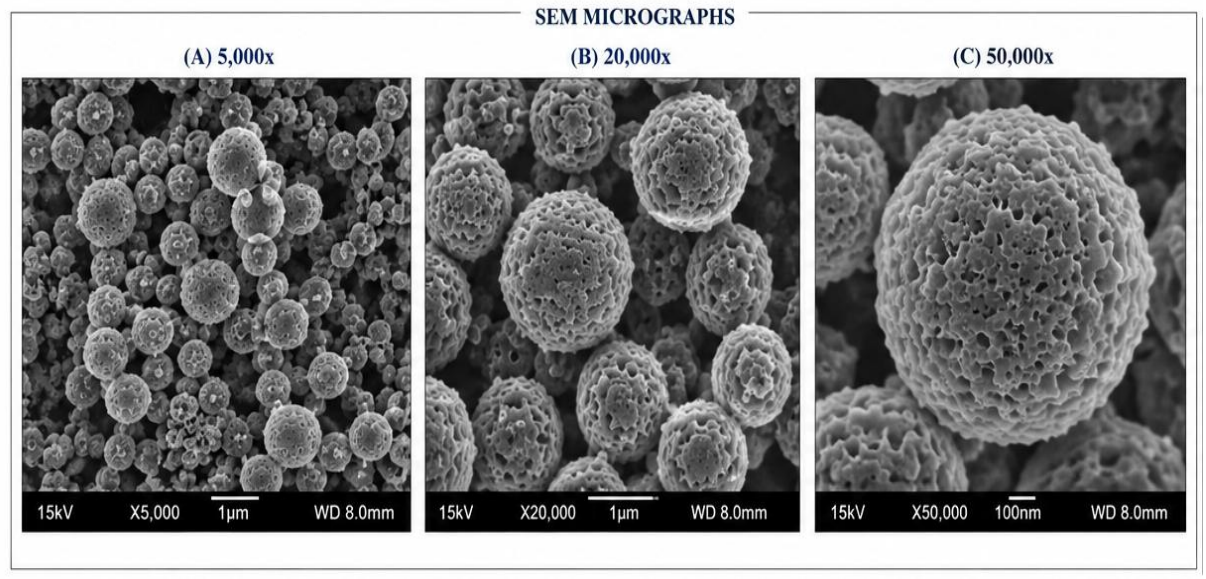
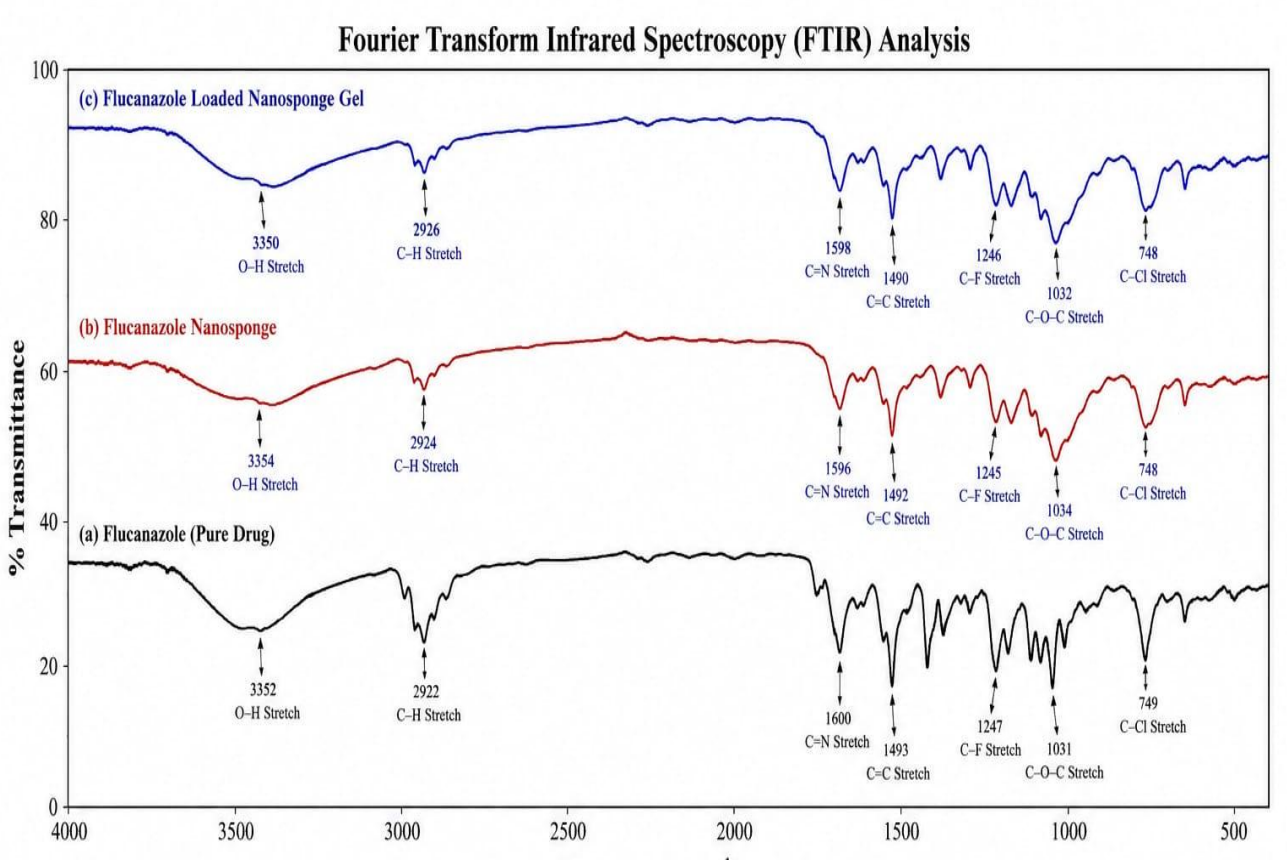


Figure No. 7: Scanning Electron Microscopy (SEM)

12. Fourier Transform Infrared Spectroscopy (FTIR)

Figure No. 8: Fourier Transform Infrared Spectroscopy



13.Differential Scanning Calorimetry (DSC)

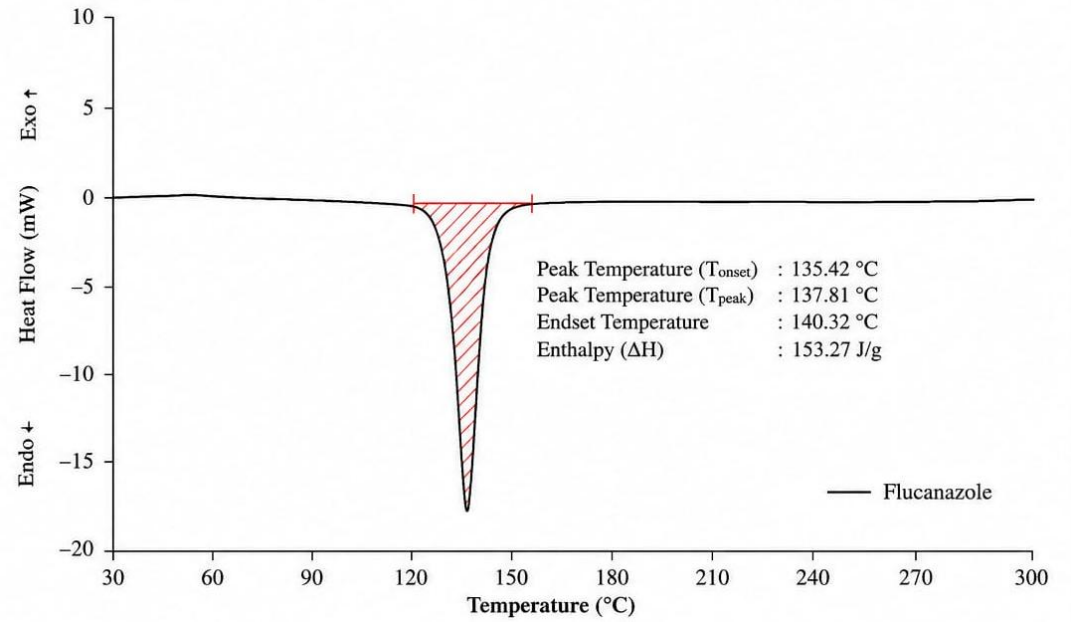


Figure No. 9: Differential Scanning Calorimetry (DSC)

14.X-Ray Diffraction (XRD)

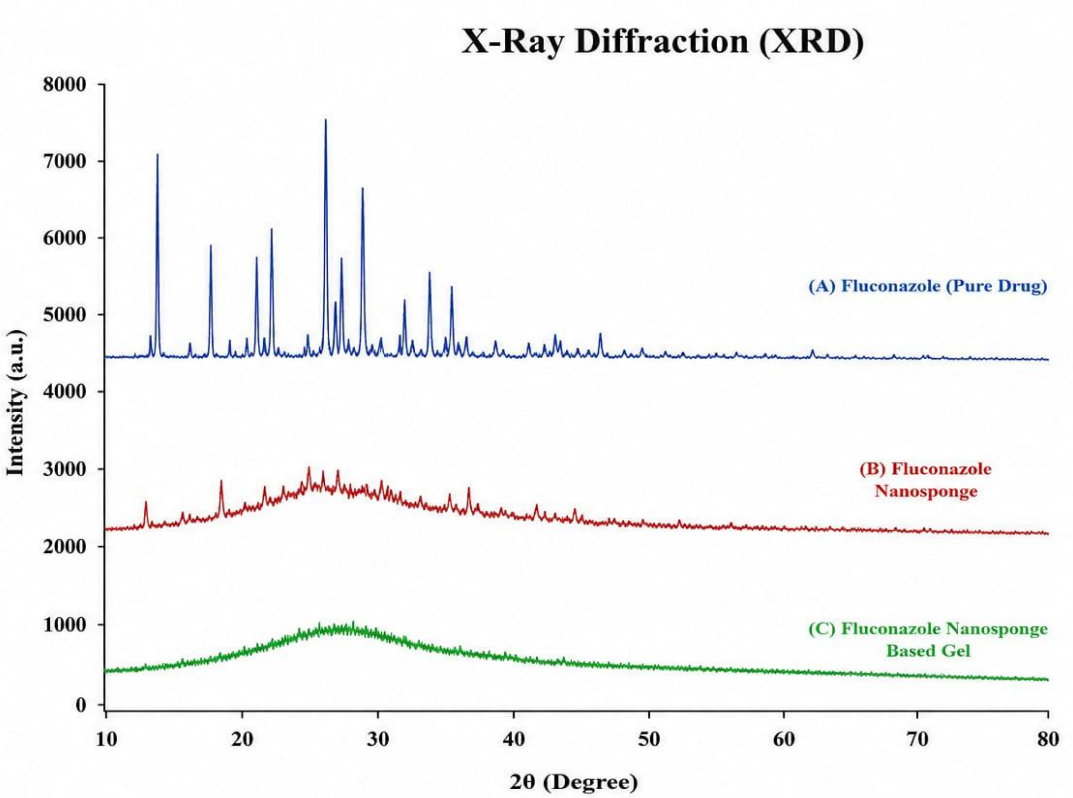


Figure No. 10: Ray Diffraction (XRD)

CONCLUSION:

From the above study, it was confirmed that nanosponge were prepared by the emulsion solvent diffusion method. Also, prepared nanosponge were successfully formulated into topical gel form. Preliminary batches showed that 100 mg of the drug can be

incorporated in nanosponge successfully. Formulation F2 of nanosponge showed better results among eight nanosponge formulations. Batch F2 showed 82.33 % entrapment efficiency. FTIR spectra of F2 formulation indicated minimum interaction between drug and polymer. DSC spectra also indicated the same. PDI indicated mid-range monodispersing. Zeta potential of F2 batch is found to be 31.76, which indicated moderate stability. SEM images confirmed the formation of nanosponges. Among formulated batches of nanosponge gel batch C3 showed maximum drug content with 6.324 gm.cm/sec spreadability. In-vitro drug release showed sustain drug dissolution for up to 12 hours.

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