

DEVELOPMENT AND CHARACTERIZATION OF NANOSPONGES LOADED
HYDROGEL FORMULATION CONTAINING NYSTATINKhush Nandan Kumar^{*1}, Naveen Gupta², Hemant Agrawal³¹Student- Madhyanchal Professional University (MPU), Bhopal (M.P).²Dean - Madhyanchal Professional University (MPU), Bhopal (M.P).³Associate Professor - Madhyanchal Professional University (MPU), Bhopal (M.P).***Corresponding Author: Khush Nandan Kumar**

Student- Madhyanchal Professional University (MPU), Bhopal (M.P).

DOI: <https://doi.org/10.5281/zenodo.23118325>**How to cite this Article:** Khush Nandan Kumar^{*1}, Naveen Gupta², Hemant Agrawal³. (2026). Development And Characterization of Nanosponges Loaded Hydrogel Formulation Containing Nystatin. European Journal of Pharmaceutical and Medical Research, 13(10), 361–370.

This work is licensed under Creative Commons Attribution 4.0 International license.



Article Received on 05/09/2026

Article Revised on 25/09/2026

Article Published on 01/10/2026

ABSTRACT

The present study was aimed at the development and evaluation of a Nystatin-loaded nanosponge gel formulation for enhanced topical antifungal therapy. Nystatin is a polyene antifungal drug widely used in the treatment of fungal infections; however, its conventional formulations are associated with limitations such as poor permeability, low retention time, and limited stability. To overcome these drawbacks, nanosponge technology was employed to achieve controlled drug release, improved stability, and enhanced topical delivery. Pre-formulation studies of Nystatin were carried out to determine its physicochemical properties, including organoleptic characteristics, solubility, melting point, pH, UV spectroscopic analysis, and FTIR studies. The λ -max of Nystatin was found to be 290.0 nm, and the calibration curve exhibited excellent linearity within the concentration range of 10–60 μ g/ml. FTIR studies confirmed the presence of characteristic functional groups. Nystatin-loaded nanosponges were prepared by the hot melt method using β -cyclodextrin as the polymer and diphenyl carbonate as the cross-linking agent. The prepared formulations were evaluated for particle size, zeta potential, surface morphology, and entrapment efficiency. Among all formulations, F2 showed the most promising results with a particle size of 120.41 nm, zeta potential of -35.4 mV, and entrapment efficiency of 98.57%. The optimized nanosponge formulation was incorporated into a carbopol-based gel and further evaluated for pH, viscosity, spreadability, skin irritation, and in-vitro drug release. The gel formulation exhibited satisfactory physicochemical properties with good homogeneity, suitable viscosity, excellent spreadability, and no signs of skin irritation. In-vitro drug release studies demonstrated sustained and controlled release behavior, with formulation F2 showing the highest cumulative drug release of 97.43% over 15 hours. Stability studies conducted under accelerated conditions confirmed the stability of the formulation with minimal changes in pH and viscosity. The findings of the study concluded that the developed Nystatin-loaded nanosponge gel formulation is a promising topical drug delivery system capable of enhancing antifungal efficacy, improving drug stability, prolonging drug release, and increasing patient compliance.

KEYWORDS: Nystatin; Nanosponges; Topical drug delivery; β -cyclodextrin; Controlled drug release; Entrapment efficiency; Carbopol gel; In-vitro drug release; Stability studies.**1. INTRODUCTION**

Fungal infections of the skin and mucosal tissues are among the most common infectious disorders affecting millions of individuals worldwide. The increasing prevalence of fungal infections, particularly those caused by *Candida* species, has created a significant demand for effective and safe topical antifungal therapies. Nystatin is a widely used polyene antifungal drug known for its

broad-spectrum antifungal activity and low systemic toxicity. However, conventional formulations of Nystatin are associated with limitations such as poor aqueous solubility, inadequate skin penetration, instability, and shorter residence time at the site of application, which may reduce therapeutic efficacy and patient **compliance** (Fantini et al., 2020; Ansari et al., 2022).

Pre-formulation studies play a crucial role in the development of stable and effective pharmaceutical formulations by providing important information regarding the physicochemical and compatibility characteristics of the drug substance. Parameters such as organoleptic properties, melting point, solubility, pH, UV spectroscopy, and Fourier Transform Infrared (FTIR) spectroscopy are essential for understanding the behavior of the drug (Vilegave *et al.*, 2013). FTIR spectroscopy, in particular, is widely utilized for identifying functional groups and evaluating drug–excipient interactions in pharmaceutical systems (Lakshminarayanan and Balakrishnan, 2020).

Nanotechnology-based drug delivery systems have emerged as promising approaches to improve the therapeutic effectiveness of poorly soluble drugs. Among these systems, nanosponges have attracted considerable attention because of their porous structure, high drug-loading capacity, controlled drug release characteristics, and improved stability. β -cyclodextrin-based nanosponges are especially advantageous due to their biocompatibility, non-toxicity, and ability to encapsulate lipophilic drugs efficiently (Kumar *et al.*, 2021). Nanosponges also enhance topical drug delivery by improving drug retention, sustained release behavior, and penetration through biological membranes (Đorđević *et al.*, 2022).

In the present investigation, Nystatin-loaded nanosponges were prepared using β -cyclodextrin and diphenyl carbonate through the hot melt method and further incorporated into a topical gel formulation. The prepared nanosponges were evaluated for particle size, zeta potential, surface morphology, and entrapment efficiency to determine their suitability as an advanced drug delivery system. Scanning Electron Microscopy (SEM) and other characterization techniques have been widely employed for evaluating the morphology and performance of nanosponge formulations (Anwer *et al.*, 2019).

Furthermore, the nanosponge-loaded gel formulation was assessed for important physicochemical parameters such as pH, viscosity, spreadability, homogeneity, and skin irritation potential to ensure patient acceptability and topical compatibility. Evaluation of topical gel formulations is essential to confirm appropriate consistency, stability, and ease of application (Salem *et al.*, 2018). The in-vitro drug release studies demonstrated sustained and controlled release of Nystatin from the nanosponge gel system, suggesting improved drug availability and prolonged antifungal activity. Therefore, the developed Nystatin-loaded nanosponge gel formulation may serve as a promising alternative to conventional topical antifungal preparations with enhanced therapeutic efficacy, stability, and patient compliance.

2. MATERIAL AND METHODS

2.1 pre-formulation studies of Nystatin

Pre-formulation studies of Nystatin were conducted to establish its fundamental physical and chemical properties prior to the development of the final formulation. These studies included the evaluation of solubility in various solvents, determination of melting point, pH measurement, stability assessment, and FTIR analysis.

2.1.1 Organoleptic Properties of Nystatin

The organoleptic properties of Nystatin were examined in order to evaluate its visual characteristics, including color, odor and appearance. (Fantini *et al.* 2020).

2.1.2 Solubility study of Nystatin

The solubility study of Nystatin was conducted by dissolving 1 mg of the drug in 1 ml of various polar and non-polar solvents, followed by visual inspection. Each mixture was thoroughly shaken and then allowed to stand undisturbed at room temperature. After the settling period, the solutions were visually examined for clarity, turbidity, or the presence of un-dissolved particles. (Ansari *et al.*, 2022).

2.1.3 Melting Point of Nystatin

The melting point of Nystatin was determined using a standard melting point apparatus.

2.1.4 pH determination of Nystatin

The pH of Nystatin was determined using a calibrated digital pH meter. A suspension was prepared by dispersing an appropriate quantity of the drug in distilled water. Prior to measurement, the pH meter was calibrated using standard buffer solutions (pH 4.0, 7.0, and 9.2) to ensure accuracy. (Awan *et al.* 2022).

2.1.5 Determination of Maximum Wavelength (λ_{max})

2.1.5.1 Preparation of Nystatin standard stock solution in methanol

The standard stock solution of Nystatin was prepared by accurately weighing 10 mg of the drug and dissolving it in 5 ml of methanol in a 10 ml volumetric flask. The solution was then made up to the mark with methanol to obtain a stock concentration of 1000 μ g/ml. From this stock, 1 ml was pipetted out and further diluted to 10 ml with methanol to prepare a working standard solution of 100 μ g/ml. This solution was used for further spectrophotometric analysis and calibration curve preparation (Singh *et al.* 2008).

2.1.5.2 Lambda max of Nystatin

To determine the λ_{max} of Nystatin, a 20 μ g/ml solution was prepared by transferring 2 ml of a 100 μ g/ml stock solution into a 10 ml volumetric flask and diluting it to volume with methanol. The resulting solution was scanned in the UV-visible spectrophotometer over a wavelength range of 200 to 400 nm, using methanol as a blank. (Kumbhar and Salunkhe, 2013).

2.1.5.3 Linearity and Calibration Curve of Nystatin

Standard solutions of Nystatin were prepared at concentrations of 10, 20, 30, 40, 50 and 60 µg/mL by diluting the 100 µg/mL working stock solution with methanol. Each dilution was accurately transferred into 5 mL volumetric flasks and made up to volume with methanol. The absorbance of these solutions was measured at 290.0 nm using a UV-visible spectrophotometer, with methanol serving as the blank. (Singh *et al.* 2020).

2.1.6 Functional group identified by FTIR

For the analysis of Nystatin, the sample was prepared using the potassium bromide (KBr) pellet method. Approximately 1 mg of Nystatin was finely mixed with 100 mg of spectroscopic-grade KBr. The mixture was then thoroughly ground, dried, and compressed under high pressure to form a clear, transparent disc. This disc was scanned using an FTIR spectrometer across a spectral range of 400 to 4000 cm⁻¹. The obtained FTIR spectrum offered valuable insight into the functional groups and possible molecular interactions within

Nystatin, aiding in its structural confirmation and purity assessment (Lakshminarayanan and Balakrishnan 2020).

2.2 Preparation of nanosponge's formulation

Beta-cyclodextrin nanosponges were prepared by 'hot melt method'. Various ratios comprising Diphenyl carbonate (DPC cross-linker) and Beta- cyclodextrin (β-CD polymer) (Ratios of DPC and β-CD are given in table no.4) were chosen for the nanosponges preparation. Anhydrous polymer (β-CD) and crosslinker (DPC) (finely homogenised) were gradually heated (90 to 100 °C) with magnetic stirring for 3 hours. The substrate mixture (β-CD and DMC) was allowed to react for 3h so as to ensure completion of cross linking reaction amongst them; resulting in formation of nanosponges. The obtained reaction mixture was subsequently cooled at ambient temperature. The solid thus obtained was washed repeatedly using double distilled water (to remove unreacted β-CD). Finally, the placebo nanosponges obtained were dried (at 40 °C) and stored in a desiccator, till further use (Kumar *et al.*, 2021).

Table 1: Composition of nano sponge's formulation.

Ingredients	Formulation Code				
	F1	F2	F3	F4	F5
Nystatin (%)	1.0	1.0	1.0	1.0	1.0
Diphenyl carbonate (DPC cross-linker) (Ratio)	100	100	100	100	100
Beta- cyclodextrin (β-CD polymer) (Ratio)	50	100	150	200	250
Temperature	80 ⁰ C	85 ⁰ C	90 ⁰ C	95 ⁰ C	100 ⁰ C
Distilled water	q.s	q.s	q.s	q.s	q.s

• Loading of Nystatin in nano sponges

Placebo NS (1 gm) was dispersed in double-distilled water (50 mL) with help of magnetic stirrer. Approximately 1.0 % of drug was added to above dispersion. The obtained dispersion was then, sonicated (for 10 minutes), and subsequently kept for 1-6 hours (under stirring). The resulting suspensions were centrifuged (1000 rpm; 10 minutes) in order to separate the un-entrapped drug, as a residue below the colloidal supernatant. Next, the supernatant was lyophilized at -81 °C and 0.0010 mbar (pressure). The dried drug loaded NS powder was stored for future use (Kumar *et al.*, 2018).

2.3 Evaluation parameter of Nystatin loaded nanosponges formulation

2.3.1 Size Distribution and Zeta potential

Zeta potential and particle was measured by Malvern zeta sizer instrument. Sample was sonicated before analysis for avoid particle aggregation (Singh and Vingkar 2008). (Đorđević *et al.* 2022).

2.3.2 Scanning Electron Microscopic (SEM)

To analyze the surface morphology of Nystatin-loaded nanosponges, Scanning Electron Microscopy (SEM) was utilized. Prior to imaging, the sample was coated with a thin metallic layer (approximately 2–20 nm) using a sputter coater under vacuum conditions. Metals such as

gold, platinum, or palladium were used to enhance the sample's conductivity and improve image resolution. The coated sample was then exposed to a focused electron beam, causing the emission of secondary electrons, including Auger electrons, as a result of interactions between the beam and the atoms on the sample's surface. Electrons scattered at an angle of 90° were selectively detected and processed according to principles based on Rutherford and Kramer's Law. This enabled the generation of detailed surface topography images, revealing the morphological characteristics of the Nystatin-loaded nanosponges (Anwer *et al.* 2019).

2.3.3 Entrapment efficiency

To accurately determine the entrapment efficiency of the Nystatin-loaded nanosponges, 10 milliliters of the nanosponge formulation were mixed with 5 milliliters of methanol in a volumetric flask. The mixture was vortexed for 1 minute to ensure complete drug extraction. The volume was then adjusted to 10 milliliters with methanol. The resulting solution was filtered and appropriately diluted. The concentration of Nystatin in the filtrate was measured using UV-Visible spectrophotometry at a wavelength of 250.0 nm to quantify the amount of drug entrapped within the nanosponges (Swetha *et al.* 2011).

Loading efficiency = Actual drug content in Nanosponge / Theoretical drug content $\times$ 100

2.4 Preparation of nanosponges loaded Gel formulation

Initially carbopol-940 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. In separate container carboxymethyl cellulose and methyl paraben was added

into 50 ml warm water (B) and stirred continuously to make stiff gel. Both the mixtures A and B were mixed with the continuous stirring. Then triethanolamine (Drop wise) was added to neutralize the pH and Nanosponges formulation was incorporated into the dispersion to obtained gel. At this stage, permeation enhancer (Propylene glycol) was added. The final dispersion was agitated until smooth gel was formed without lumps (Silpa *et al.*, 2021).

Table 2: Composition of herbal gel formulation.

S. No	Name of Ingredient	Formulation I
1	Carbopol 940	0.5 gm
2	Carboxymethyl cellulose	0.5 gm
3	Propylene glycol	0.25 ml
4	Methyl paraben	0.1 ml
5	Nanosponges	100 mg
6	Triethanolamine	q.s
7	Water	50 ml

2.5 Characterization of drug loaded nanosponges gel formulation

2.5.1 Physical properties drug loaded nanosponges gel formulation

The physical characteristics of the prepared drug loaded nanosponges gel formulation were assessed through careful visual observation. (Anwer *et al.*, 2025).

2.5.2 Measurement of pH drug loaded nanosponges gel formulation

The pH of the drug loaded nanosponges gel formulation was measured using a calibrated digital pH meter to confirm its compatibility with the skin and overall formulation stability.

2.5.3 Determination of Viscosity drug loaded nanosponges gel formulation

The viscosity of the drug loaded nanosponges gel formulation was assessed using a Brookfield viscometer to determine its rheological behavior and ensure suitable consistency for topical application.

2.5.4 Skin irritation test of drug loaded nanosponges gel formulation

The skin irritation study for the drug loaded nanosponges gel formulation was conducted on human volunteers to assess its dermal safety and compatibility.

2.5.5 Spreadability study of drug loaded nanosponges gel formulation

A measured amount of the gel was placed between two glass slides, and a specific weight was applied to the top slide for a defined duration to facilitate spreading. After the allotted time, the extent of gel spread measured by the diameter or area was recorded. Greater spreadability indicated that the gel could be applied effortlessly, promoting uniform coverage. This property is crucial for enhancing user comfort, ensuring proper dosing, and improving the overall performance of the topical formulation (Salem *et al.*, 2018).

2.6 In-vitro drug release of drug loaded nanosponges gel formulation

The in vitro drug release of Nystatin-loaded nanosponge gel formulations was evaluated using the dialysis bag diffusion method. A measured amount of the Nystatin-loaded nanosponges gel was placed inside a pre-soaked dialysis membrane, which was then immersed in a beaker containing 100 mL of phosphate buffer solution at pH 7.4. The beaker was positioned on a magnetic stirrer, and the temperature was maintained at $37 \pm 2^\circ\text{C}$ throughout the study. The stirring speed was kept constant at 100 rpm to ensure uniform mixing. At predetermined time intervals, 2 mL of the release medium was withdrawn and replaced with an equal volume of fresh pH 7.4 buffers to maintain sink conditions.

The collected samples were suitably diluted and analyzed using a UV-Visible spectrophotometer at a wavelength of 290.0 nm to determine the concentration of Nystatin released over time.

2.7 Stability studies of drug loaded nanosponges gel formulation

The gel was sealed in airtight containers and stored in a stability chamber at two ICH-recommended accelerated conditions: $25 \pm 2^\circ\text{C}$ with $60 \pm 5\%$ relative humidity (RH) and $40 \pm 2^\circ\text{C}$ with $70 \pm 5\%$ RH, for duration of three months. Samples were withdrawn at predetermined intervals—0, 30, 45, 60, and 90 days—and evaluated for critical parameters such as physical appearance, pH, and viscosity, which are essential indicators of the gel's stability and usability.

3. RESULTS AND DISCUSSIONS

3.1 pre-formulation study of Nystatin

3.1.1 Organoleptic evaluation

Table 3: Organoleptic evaluation of Nystatin.

S. No	Physical parameter	Observation
1	Color	White
2	Odor	Odorless
3	State	Solid powder
4	Appearance	Smooth and consistent texture

3.1.2 Melting Point

Table 4: Melting Point of Nystatin.

Drugs	Observed	Reference
Nystatin	164 °C	160°C to 165°C

3.1.3 Determination of pH

Table 5: pH determination of Nystatin.

S. No.	Drug	Observed	Reference
1	Nystatin	6.7	6.0 and 8.0

3.1.4 Solubility study of the drug

Table 6: Solubility study of Nystatin.

Drug	Solvents	Observation/Inference
Nystatin	Methanol	Freely Soluble
	Acetone	Soluble
	Water	Soluble
	Ethanol	Slightly soluble
	DMSO	Freely soluble

3.2 Determination of λ max by UV spectroscopy

The λ max of Nystatin was determined using UV-visible spectroscopy. A solution of Nystatin was scanned in the range of 200–400 nm using methanol as the solvent. The maximum absorbance was observed at 290.0 nm, indicating the characteristic peak of Nystatin.

3.2.1 Standard calibration curve

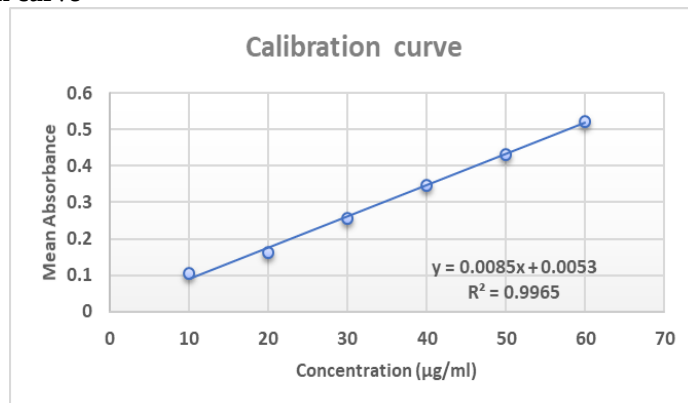


Figure 1: Calibration curve of Nystatin.

3.2.2 Functional group identified by Infra-Red spectroscopy

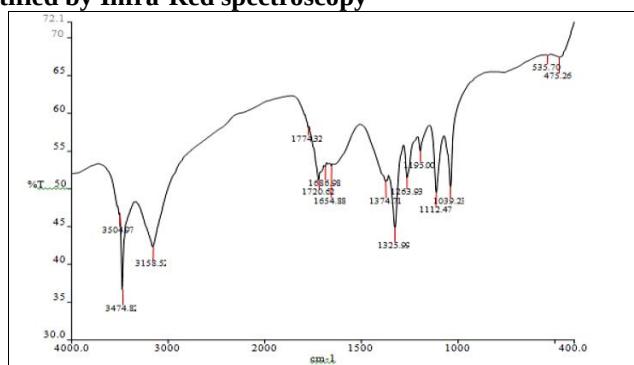


Figure 2: FTIR study of Nystatin.

Table 7: Interpretation of IR spectrum of Nystatin.

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3474.41	3500- 3400	N-H stretching	Primary amine
2	1774.32	1770-1780	C=O stretching	vinyl / phenyl ester
3	1374.21	1385-1380	C-H bending	Alkenes
4	1263.93	1275-1200	C-O stretching	alkyl aryl ether
5	1039.25	1050-1040	CO-O-CO stretching	anhydride

7.3 Characterization of Nystatin loaded Nanosponges formulation

7.3.1 Physical Appearance of Nanosponges

Table 8: Physical Appearance of Nanosponges.

Formulation	Parameters	Observation
Nanosponges	Colour	White
	Odour	Characteristic
	Appearance	Solid

7.3.2 Particle Size

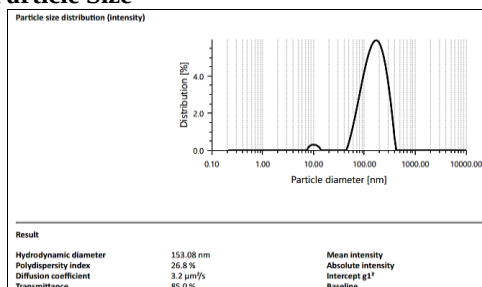


Figure 3: Particle size (F1)

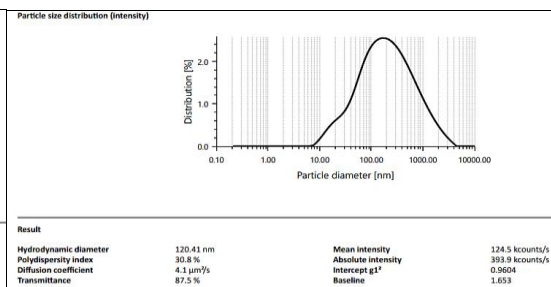


Figure 4: Particle size (F2)

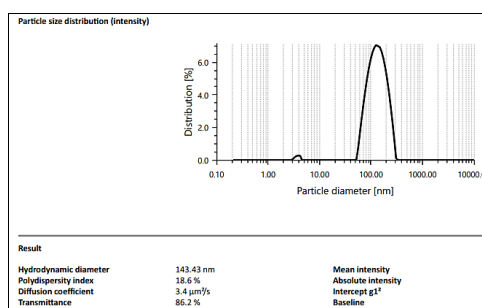


Figure 5: Particle size (F3)

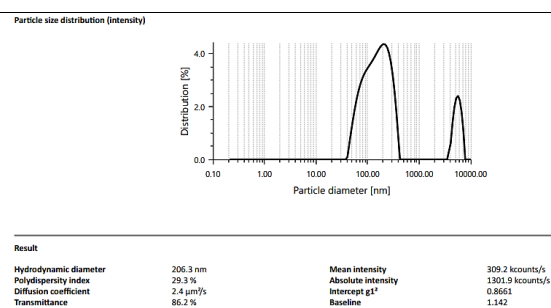


Figure 6: Particle size (F4).

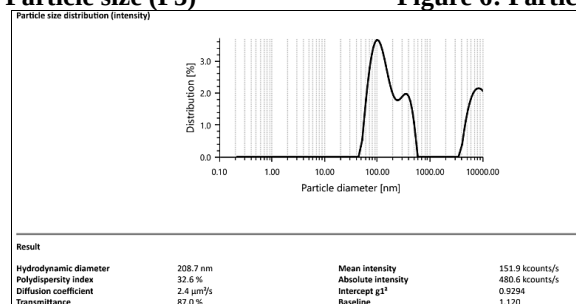


Figure 7: Particle size (F5).

7.3.3 Zeta potential

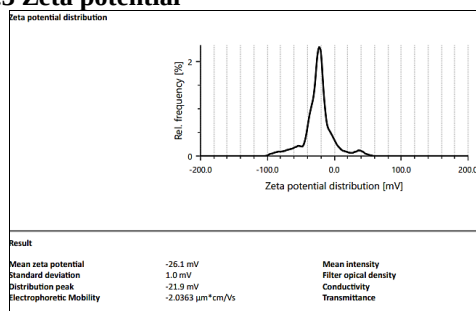


Figure 8: Zeta potential (F1).

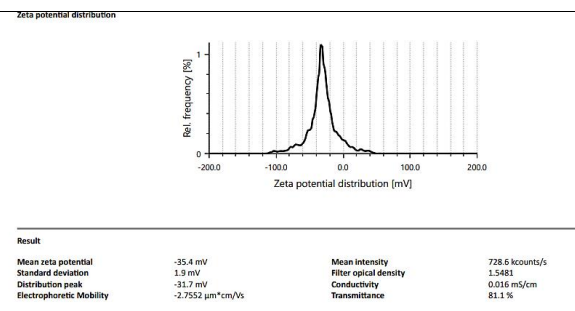


Figure 9: Zeta potential (F2).

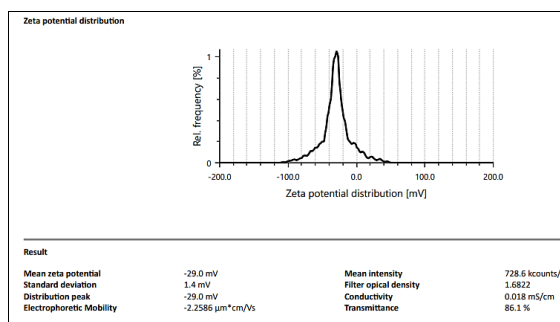


Figure 10: Zeta potential (F3).

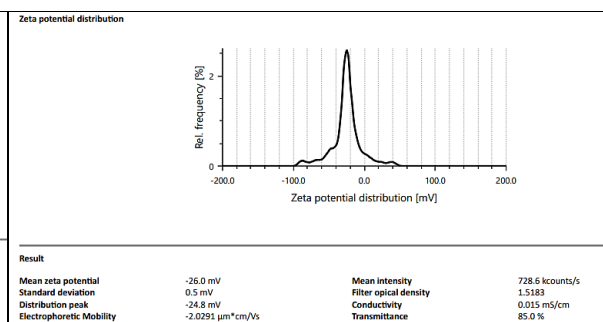


Figure 11: Zeta potential (F4).

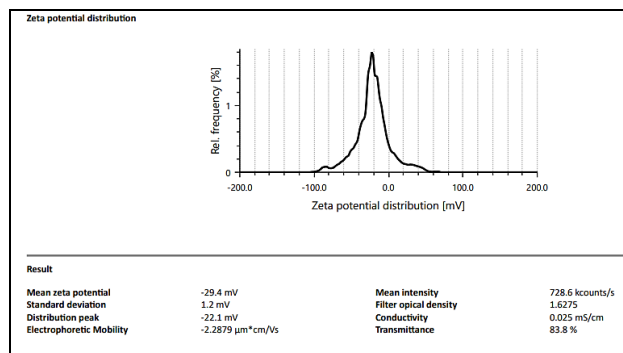


Figure 12: Zeta potential (F5).

Table 9: Particle size and Zeta potential of nanosponges.

S. No	Formulation Code	Particle size (nm)	Zeta potential mV
1.	F1	153.08 nm	-26.1
2.	F2	120.41 nm	-35.4
3.	F3	143.43 nm	-29.0
4.	F4	206.3 nm	-26.0
5.	F5	208.7 nm	-29.4

7.3.4 Scanning electron microscope (SEM)

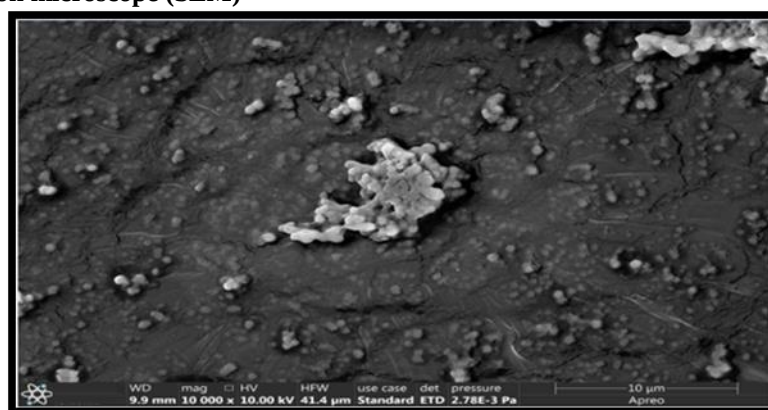


Figure 13: Scanning electron microscope (SEM).

7.3.5 Entrapment efficacy

Table 10: Entrapment efficacy.

S. No	Formulations (F1-F5)	Entrapment efficacy (%)
1.	Nanosponges (F1)	83.32
2.	Nanosponges (F2)	98.57
3.	Nanosponges (F3)	82.21
4.	Nanosponges (F4)	79.41
5.	Nanosponges (F5)	76.69

7.4 Evaluation parameter of gel formulation

7.4.1 Organoleptic properties

Table 11: Organoleptic properties of Nystatin-loaded nanosponges gel.

S. No	Parameters	Results
1.	Physical appearance	Clear, transparent, and smooth gel
2.	Colour	Off-white to white
3.	Homogeneity	Homogeneous without lumps

7.4.2 Measurement of pH, Viscosity and Spreadability test

Table 12: pH, Viscosity and Spreadability test of Nystatin-loaded nanosponges gel.

S. No	Formulation	pH	Viscosity determination (cps)	Spreadability test (gm.cm/sec)	skin irritation study
1.	Gel formulation	6.3	4247±0.52	13.83	Not irritation observed

7.5 In-vitro drug release studies of Nystatin-loaded nanosponge gel formulation

Table 13: In-vitro drug release studies.

Time (hrs)	F 1	F 2	F 3	F 4	F 5
0	0	0	0	0	0
2	14.06	28.82	15.53	20.69	24.36
4	22.07	37.56	29.86	31.75	33.19
6	29.73	44.8	30.76	33.34	39.13
8	44.47	60.19	47.57	39.59	57.59
10	59.79	68.72	60.8	63.31	66.83
12	71.23	79.41	73.29	70.25	75.17
14	80.07	84.11	81.25	81.21	82.41
15	82.13	97.43	88.94	91.08	92.25

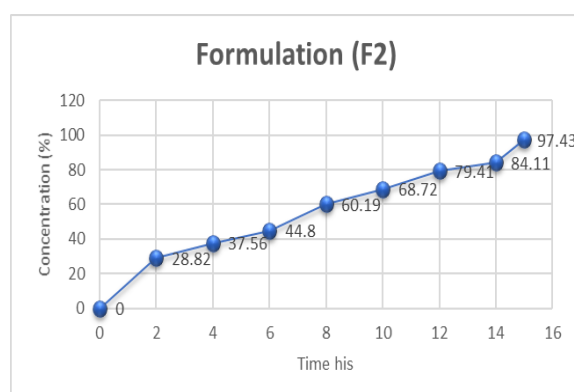
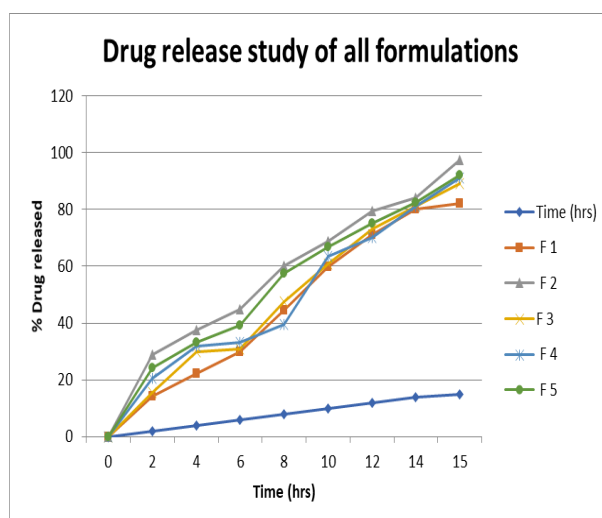


Figure 14: Drug release of final optimized formulation (F2).

7.7 Stability study

Table 14: Stability Study of Nystatin-loaded nanosponges gel formulation.

S. No	Time (Days)	25 ⁰ C±2 ⁰ C and 60 ± 5% RH		40 ⁰ C±2 ⁰ C and 70 ±5% RH	
		pH	Viscosity	pH	Viscosity
1.	0	6.3	4247	6.3	4227
2.	30	6.0	4221	6.9	4230
3.	45	6.4	4220	6.6	4225
3.	60	6.2	4219	6.1	4221
4.	90	6.5	4224	6.6	4231

DISCUSSION

The present study successfully developed and evaluated a Nystatin-loaded nanosponge gel formulation for topical antifungal delivery. Pre-formulation studies confirmed the purity, compatibility, and suitability of Nystatin for formulation development. The drug showed good solubility in methanol and DMSO, while UV and FTIR studies confirmed its characteristic properties and absence of drug–excipient interactions.

The prepared nanosponge formulations exhibited nanoscale particle size, good stability, and high entrapment efficiency. Among all formulations, F2 showed the best results with minimum particle size (120.41 nm), highest zeta potential (–35.4 mV), and maximum entrapment efficiency (98.57%). SEM analysis revealed spherical and porous nanosponges, supporting efficient drug loading and controlled release.

The nanosponge-loaded gel showed satisfactory physicochemical properties, including suitable pH, viscosity, spreadability, and absence of skin irritation. In-vitro drug release studies demonstrated sustained release behavior, with formulation F2 showing the highest drug release (97.43%) over 15 hours. Stability studies indicated that the optimized formulation remained stable under accelerated storage conditions.

4. CONCLUSION

The study concluded that Nystatin-loaded nanosponge gel can be successfully formulated for effective topical antifungal delivery. The optimized formulation (F2) demonstrated excellent particle size, entrapment efficiency, stability, and sustained drug release properties. The developed gel was stable, non-irritant, and suitable for topical application. Therefore, the nanosponge-based gel formulation may serve as a promising alternative to conventional Nystatin formulations by improving drug release, stability, and therapeutic efficacy.

5. REFERENCES

- Kumar, S., Sharma, P., Gupta, V., & Singh, R. (2021). Preparation and evaluation of β -cyclodextrin based nanosponges for drug delivery applications. *Journal of Drug Delivery Science and Technology*, 61: 102245.
- Vilegave, K., Vidyasagar, G., & Chandankere, R. K. (2013). Preformulation studies and compatibility analysis of pharmaceutical dosage forms. *International Journal of Pharmaceutical Sciences Review and Research*, 18(2): 112–118.
- Fantini, M., et al. (2020). Physicochemical characterization and pharmaceutical applications of Nystatin. *Journal of Pharmaceutical Sciences and Research*, 12(4): 556–562.
- Ansari, M., Ahmad, S., & Ali, J. (2022). Solubility enhancement approaches for poorly soluble antifungal drugs: A review. *Asian Journal of Pharmaceutics*, 16(1): 45–53.
- Lakshminarayanan, R., & Balakrishnan, P. (2020). FTIR spectroscopy in pharmaceutical analysis and drug characterization. *International Journal of Pharmaceutical Investigation*, 10(3): 201–208.
- Salem, H., Kharshoum, R., & Halawa, A. (2018). Evaluation parameters of topical gel formulations and spreadability studies. *International Journal of Pharmaceutical and Clinical Research*, 10(5): 145–152.
- Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., et al. (2022). Nanosponges as emerging drug delivery systems for topical applications. *Pharmaceutics*, 14(3): 567.
- Anwer, M. K., Jamil, S., Ibnouf, E. O., & Shakeel, F. (2019). Characterization and evaluation of nanosponge formulations for controlled drug delivery. *Saudi Pharmaceutical Journal*, 27(3): 389–397.
- Anwer, M. K., Jamil, S., Ibnouf, E. O., Shakeel, F., & Imam, S. S. (2019). Development and evaluation of nanosponge-based drug delivery systems for controlled topical application. *Drug Development and Industrial Pharmacy*, 45(8): 1285–1294.
- Anwer, M. K., et al. (2025). Evaluation and characterization of nanosponge-based topical gel formulations. *International Journal of Pharmaceutical Sciences and Research*, 16(2): 210–218.
- Ansari, M., Ahmad, I., & Aqil, M. (2022). Solubility enhancement approaches for poorly soluble antifungal drugs. *Journal of Drug Delivery Science and Technology*, 68: 102987.
- Awan, Z. A., Alharbi, K. S., & Shakeel, F. (2022). Physicochemical evaluation and pH determination of pharmaceutical formulations. *Saudi Pharmaceutical Journal*, 30(4): 412–419.
- Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., Kostić, I., Komes, D., Bugarski, B., & Nedović, V. (2022).

- Nanosponges as emerging drug delivery systems for topical applications. *Pharmaceutics*, 14(5): 1054.
14. Fantini, M., Faccendini, A., & Rossi, S. (2020). Physicochemical characterization and pharmaceutical applications of Nystatin. *Journal of Pharmaceutical Sciences*, 109(9): 2756–2764.
 15. Kumar, S., Sharma, P., & Gupta, R. (2018). Drug loading and characterization of cyclodextrin nanosponges for controlled drug delivery. *Asian Journal of Pharmaceutics*, 12(3): 145–152.
 16. Kumar, S., Verma, A., & Singh, P. (2021). Preparation and evaluation of β -cyclodextrin based nanosponges for drug delivery applications. *International Journal of Applied Pharmaceutics*, 13(4): 85–92.
 17. Kumbhar, D. D., & Salunkhe, K. S. (2013). Development and validation of UV spectrophotometric method for estimation of Nystatin. *International Journal of Pharmaceutical Chemistry and Analysis*, 2(1): 15–19.
 18. Lakshminarayanan, R., & Balakrishnan, P. (2020). FTIR spectroscopy in pharmaceutical analysis and drug characterization. *International Journal of Pharmaceutical Research*, 12(3): 1180–1187.
 19. Salem, H., Kharshoum, R. M., & Abou-Taleb, H. A. (2018). Evaluation parameters of topical gel formulations and spreadability studies. *Journal of Cosmetic Dermatology*, 17(5): 950–958.
 20. Silpa, K., Priyanka, G., & Reddy, M. S. (2021). Formulation and evaluation of nanosponge loaded topical gel for controlled drug delivery. *World Journal of Pharmacy and Pharmaceutical Sciences*, 10(7): 1120–1132.
 21. Singh, A., & Vingkar, S. K. (2008). Particle size and zeta potential analysis of nanosponge formulations. *Indian Journal of Pharmaceutical Education and Research*, 42(3): 234–240.
 22. Singh, P., Kumar, R., & Sharma, V. (2020). Development of calibration curve and analytical method validation of Nystatin using UV spectroscopy. *International Journal of Pharmaceutical Sciences Review and Research*, 61(1): 45–50.
 23. Singh, R., Patel, M., & Shah, D. (2008). Preparation of standard stock solutions and spectrophotometric estimation of antifungal drugs. *Asian Journal of Chemistry*, 20(6): 4321–4326.
 24. Swetha, M., Gowthami, M., & Rao, Y. M. (2011). Determination of entrapment efficiency in nanosponge drug delivery systems. *Research Journal of Pharmacy and Technology*, 4(11): 1758–1762.
 25. Vilegave, K., Vidyasagar, G., & Chandrasekhar, K. B. (2013). Preformulation studies and compatibility analysis in pharmaceutical dosage forms. *International Journal of Pharmaceutical Sciences Review and Research*, 18(2): 45–52.