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Development and Characterization of β-Cyclodextrin Assisted Neomycin Microsponge Gel for Sustained Topical Delivery

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Abstract

This study focuses on the formulation, development, and optimization of β-cyclodextrin-based neomycin-loaded microsponge gel for effective topical drug delivery. Preformulation studies confirmed neomycin's suitable physicochemical properties, including good solubility in polar solvents, acceptable pH, and stability. Microsponge formulations exhibited porous, spherical morphology with nanoscale particle size and high drug entrapment efficiency, with formulation MSF4 showing optimal performance. The prepared gel demonstrated desirable characteristics such as smooth texture, appropriate viscosity, skin-compatible pH, excellent spreadability, and absence of irritation. In vitro drug release followed zero-order kinetics, indicating sustained and controlled release. Antimicrobial studies revealed significant activity against Staphylococcus aureus and Escherichia coli. Stability studies confirmed minimal changes under various storage conditions. Overall, the optimized β-cyclodextrin-based microsponge gel presents a stable, effective, and promising system for localized treatment of bacterial skin infections with enhanced therapeutic efficacy and patient compliance.

Keywords; Neomycin, Microsponge drug delivery, β-cyclodextrin, Topical gel formulation, Controlled drug release.

INTRODUCTION

Topical drug delivery systems are designed either to produce a localized therapeutic effect or to deliver drugs into systemic circulation through the skin, which acts as a portal of entry. Various formulations such as creams, gels, lotions, and transdermal delivery systems (TDS) are commonly available. However, conventional topical preparations for local action have certain limitations, including rapid absorption leading to reduced duration of action and decreased therapeutic effectiveness. Achieving effective therapy often requires high concentrations of active ingredients, which may cause skin irritation, uncontrolled evaporation of the drug, and potential incompatibility with formulation components (Singla et al., 2012).

Topical drug delivery involves applying drug-containing formulations directly to the skin to treat cutaneous disorders. Among dermatological products, semisolid formulations are widely used, with gels gaining popularity due to their transparency, ease of application, and patient acceptability. Gels are formed by entrapping large amounts of aqueous or hydroalcoholic liquids within a network of colloidal particles, enabling faster drug release compared to creams and ointments. Despite these advantages, gels face limitations in delivering hydrophobic drugs (Charde et al., 2013). To overcome this issue, emulgels have been developed, combining the properties of emulsions and gels, thereby enhancing the delivery of hydrophobic drugs.

Transdermal drug delivery systems aim to deliver drugs into systemic circulation using penetration enhancers, while in some cases, retaining the drug within skin layers for localized action is preferred. Conventional formulations often exhibit drawbacks such as greasiness, stickiness, unpleasant odor, and potential allergic reactions. Therefore, advanced carrier systems and microparticle technologies are being explored to improve drug retention in the skin and enhance therapeutic efficacy.

The Human Skin

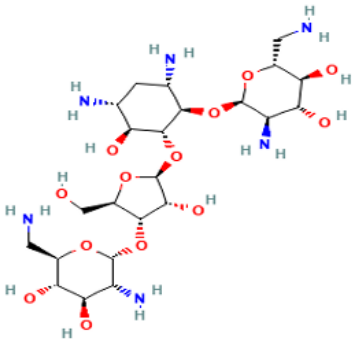
The skin is the largest organ of the human body, accounting for about 15% of total body weight. It serves multiple vital functions, including protection against physical, chemical, and biological threats, prevention of excessive water loss, and regulation of body temperature. Acting as a sensory interface, the skin can be considered a "brain on the outside," continuously interacting with the external environment. It is a complex biological system that integrates blood supply, nerve networks, immune responses, muscle activity, endocrine functions, and sensitivity to ultraviolet radiation. These components collectively maintain skin homeostasis and contribute to the overall balance of the body. Positioned between the external and internal environments, the skin plays a crucial protective and regulatory role. Despite its adaptability, skin disorders remain a significant health concern, ranking among the leading causes of nonfatal disease burden, with increasing prevalence linked to aging and lifestyle factors such as inactivity, poor diet, smoking, and alcohol consumption (Karimkhani et al., 2017).

Microsponges

Microsponges are microscopic, porous polymeric spheres designed to absorb skin secretions and reduce oiliness while delivering drugs in a controlled manner. These tiny, inert particles, typically 5–300 µm in size, do not penetrate the skin but accumulate on its surface and within small crevices. They can hold up to four times their weight in secretions and entrap a wide range of active ingredients such as emollients, fragrances, sunscreens, and anti-infective agents. Unlike conventional topical formulations that release drugs rapidly and may cause irritation due to high concentrations, microsponge systems provide a sustained and uniform release, improving efficacy and minimizing side effects. The interconnected porous structure allows significant drug retention, with high pore density and extensive internal channels. Drug release is triggered by factors such as rubbing or increased skin temperature. These microsponges can be incorporated into creams, lotions, and powders, making them effective carriers for prolonged topical drug delivery (Pradhan, 2011).

DRUG PROFILE

- **Generic name:** Neomycin
- **Molecular weight:** 614.644 g/mol
- **Chemical Formula:** C23H46N6O13
- **IUPAC name:** (2R,3S,4R,5R,6R)-5-amino-2-(aminomethyl)-6-[(1R,2R,3S,4R,6S)-4,6 diamino-2-[(2S,3R,4S,5R)-4-[(2R,3R,4R,5S,6R)-3-amino-6 (aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5 (hydroxymethyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxyoxane-3,4-diol
- **Structure:**



- **Synonyms:** Neomycin Sulfate, Neomycin, Sulfate (salt), Mycifradin, Neo-fradin. Neo-Rx, Xenobiotic.
- **Description:** Neomycin is an aminoglycoside antibiotic that targets both gram-negative aerobic and anaerobic bacteria and like rifaximin is not absorbed in the gastrointestinal tract. Neomycin targets the bacteria that produce ammonia; therefore, it is used in patients with hepatic encephalopathy. To date, prescription of neomycin is limited due to its known side effects, where nausea, vomiting, and diarrhea are common, and ototoxicity is serious but less common (Catalet al., 2018).

MATERIAL AND METHOD

The Materials and Methods section outlines the resources, instrumentation, and experimental protocols utilized during the research work. This comprehensive detailing ensures the reproducibility of the study and validates the procedural reliability. It encompasses the design, techniques, and standardizations followed in the formulation of beta-cyclodextrin-based microsponges loaded with neomycin, incorporated into a topical gel.

Selection of drug and chemical

Table 1: list of chemical

S. No.	Name of Chemicals	Company
1	Neomycin	Lexicare Pharma Pvt. Ltd
2	Methanol	Rankem
3	Ethanol	Merck India Ltd. Mumbai, India
4	Acetonitrile	Merck India Ltd. Mumbai, India
5	Ethyl cellulose	Sigma eldritch
6	PVA	Sigma eldritch
7	Carbopol 934	Sulab
8	Triethanolamine	Loba
9	Propylene glycol	Merck
10	Methyl paraben	Merck
11	Distilled water	Vizag Chemicals

Table 2: List of glassware's

S. No	List of Glassware's	Type/Company
1	Test tubes	Borocil
2	Petri dishes	Borocil
3	Glass rod	Borocil
4	Beaker	Borocil
5	Conical flask	Borocil
6	Measuring cylinder	Borocil
7	glass slide	Borocil
8	Volumetric flask	Borocil

Table 3: List of instruments used

S. No	Name of Instrument	Company
1	Electronic Weighing Balance	A & D Company HR 200
2	Mechanical Stirrer	Remi Motors, India.
3	Magnetic Stirrer	MC Dalal & amp; Co India
4	UV Visible Spectrophotometer	Shimadzu 1700
5	Stability Chamber	InlabEquipments Madras PVT (LTD)

Pre formulation studies of neomycin

Preformulation studies of neomycin are essential for developing a stable and effective topical formulation. These studies evaluate key physicochemical properties such as solubility, stability, and drug–excipient compatibility to enhance therapeutic efficacy, bioavailability, and shelf life (Srinivasan et al., 2025).

Neomycin's Organoleptic Properties

Organoleptic evaluation includes assessment of appearance, odor, and texture. Neomycin is examined for color uniformity, absence of degradation signs, acceptable odor, and smooth, non-gritty consistency to ensure quality and user acceptability (Del Rosso et al., 2013).

Neomycin's Solubility Study

Solubility is determined by dissolving neomycin in solvents like water, methanol, and ethanol. Observations such as clarity and absence of undissolved particles help identify suitable solvents for formulation (Kumar and Rao 2022).

Neomycin's pH Determination

A dilute neomycin solution is prepared, and its pH is measured using a calibrated pH meter. This helps evaluate stability and compatibility with excipients (Zuberi et al., 2023).

Neomycin's Melting Point Determination

The melting point is determined using a capillary method to assess purity and physical characteristics of the drug (Singh et al., 2022).

Spectroscopic Analysis and Calibration of Neomycin

Lambda (λ) max analysis: UV spectrophotometry (200–400 nm) is used to identify the wavelength of maximum absorbance (Gupta et al., 2021).

Calibration Curve: Standard solutions are prepared, and absorbance is measured at λmax to enable quantitative analysis (Pontes-Quero et al., 2021).

Preparation of Calibration Curve

Solutions of varying concentrations (2–12 µg/mL) are analyzed, and a linear calibration curve is plotted following Beer-Lambert's law (Kumar et al., 2021).

FT-IR Spectroscopy of Neomycin

FT-IR analysis (4000–400 cm^{-1}) using KBr pellets is conducted to identify functional groups and evaluate drug–excipient compatibility (Srisayam et al., 2014).

Preparation of Microsponges containing Neomycin

Neomycin Microsponges were prepared by quasi-emulsion solvent diffusion method. To prepare the internal phase, Neomycin was dissolved in 20 ml of dichloromethane: ethanol (1:1) mixture to dissolve both the drug and the polymer (Eudragit L 100) and to this add 0.5 ml dibutyl phthalate as a plasticizer The external phase containing 0.1 to 0.5 % of PVA in hot water. The external phase was placed in the vessel with propeller stirrer rotating at different rpm, to this add slowly internal phase. The system was thermally controlled at 25°C in a water bath. Agitations up to 30 min. permit the formation of microsponges and continue stirring for 8h to get desired rigid microsponges. After 8h stop stirring filter the rigid

micro sponges through the filter paper (Whatmann filter paper 0.45 µm),washed with distilled water and dried at room temperature Neomycin microsponges were prepared using various polymer and drug concentration i.e 100 to 500 mg and 0.5%. The formula of various microsponge is shown in table 4 (Hans et al., 2019).

Table 4: Composition of Microsponges Formulation

Formulation Code	Neomycin drug (mg)	Eudragit L 100 (mg)	Ethanol + Dichloro methane (20ml) Ratio	Polyvinyl alcohol (%)	Distilled Water (ml)	Dibutyl phthalate ml
MS1	500	100	1:1	0.1	100	0.5
MS2	500	200	1:1	0.2	100	0.5
MS3	500	300	1:1	0.3	100	0.5
MS4	500	400	1:1	0.4	100	0.5
MS5	500	500	1:1	0.5	100	0.5

Evaluation parameter of Neomycin loaded microsponges formulation

- **Physical Appearance:** The microsponges were visually examined for shape, color, and uniformity. Clarity of dispersion, surface texture, and absence of aggregation or phase separation were assessed to ensure formulation stability and quality (Penjuri et al., 2016).
- **Particle Size:** Particle size was determined using dynamic light scattering (DLS). The average size and polydispersity index (PDI) were measured to evaluate uniformity and suitability for topical or transdermal delivery (Abbas et al., 2018).
- **Zeta Potential:** Zeta potential was measured using a zeta analyzer to determine surface charge and colloidal stability. This parameter indicates the stability and dispersion behavior of the microsponges (Seema et al., 2015).
- **Scanning Electron Microscopy (SEM) Analysis:** SEM was used to study surface morphology, आकार, and संरचना of microsponges. Samples were metal-coated before imaging to obtain high-resolution images, providing insight into particle size distribution and surface characteristics (Pandya et al., 2019).
- **Drug Entrapment Efficiency:** Entrapment efficiency was determined by disrupting the microsponges using methanol, followed by filtration and UV spectrophotometric analysis. This evaluation measures the amount of drug successfully incorporated into the microsponges. The entrapment efficiency was calculated using the following formula:

Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc. × 100

This method provides an accurate estimation of how much neomycin was successfully encapsulated within the microsponges (Krishna et al., 2021).

Preparation of microsponges loaded Gel formulation

To prepare the microsponges gel, Carbopol 934 was first dispersed in 50 mL of warm distilled water (Phase A) and allowed to hydrate for 2 hours. The mixture was then stirred thoroughly using a magnetic stirrer at 600 rpm until a uniform dispersion was obtained. In a separate container, 50 mL of warm water (Phase B) was used to dissolve carboxymethyl cellulose and methyl paraben under continuous stirring to ensure complete solubilization. Phase A and Phase B were then slowly combined under constant agitation to form ahomogeneous gel base. To adjust the pH and initiate gelation, triethanolamine was added drop wise with continuous mixing. Once the desired pH was achieved, the previously prepared microsponges gel dispersion was incorporated into the gel base. Subsequently, propylene glycol was added as apermeation enhancer. The final mixture was stirred gently but thoroughly until a smooth, uniform, and lump free microsponges gel was obtained. The gel was then stored in airtight containers for further evaluation and application studies.

Table 5: Composition of microsponges gel formulation

S. No	Excipients	Quantity (gm)
1	Carbopol 934	1.00 gm
2	Carboxymethyl cellulose	1.00 gm
3	Propylene glycol	0.5 ml
4	Methyl paraben	0.2 ml
5	Micro sponges	100 mg
6	Triethanolamine	q.s
7	Distilled Water	100 ml

Characterization of micro sponges loaded gel formulation

- **Physical Properties:** The gel was visually evaluated for color, clarity, uniformity, and texture. Absence of grittiness, lumps, phase separation, or syneresis indicated good physical stability and suitability for topical use (Anwer et al., 2025).
- **Measurement of pH:** The pH of the gel was measured using a calibrated pH meter after dispersing it in distilled water. This test ensured

skin compatibility and minimized the risk of irritation.

- **Determination of Viscosity:** Viscosity was measured using a Brookfield viscometer to assess the gel's consistency and rheological behavior. Appropriate viscosity ensures good spreadability and ease of application.
- **Skin Irritation Test:** The formulation was applied to human volunteers and observed for 24–48 hours for signs of redness, itching, or irritation. Absence of adverse effects confirmed its dermatological safety.
- **Spreadability Study:** Spreadability was evaluated using the glass slide method. Better spreadability indicated ease of application and uniform distribution, enhancing user compliance and effectiveness. (Salem et al., 2018).

In Vitro Drug Release Study of Neomycin-Loaded Micro sponges gel

The in vitro release behavior of neomycin from the micro sponges gel formulation was assessed using the dialysis bag diffusion method. A defined quantity of the neomycin-loaded micro sponges suspension was introduced into a pre-soaked dialysis membrane, which was then suspended in 100 mL of phosphate buffer solution (pH 7.4) contained in a beaker. The setup was maintained at 37 ± 2 °C with constant stirring at 100 rpm using a magnetic stirrer to mimic physiological conditions. At predetermined time intervals, 2 mL aliquots were withdrawn from the release medium and immediately replaced with an equal volume of fresh buffer to maintain sink conditions. The collected samples were suitably diluted and analyzed using a UV-Visible spectrophotometer at a wavelength of 427 nm to quantify the amount of neomycin released over time. The resulting release data were then subjected to various kinetic modeling approaches (e.g., zero order, first-order, Higuchi, and Korsmeyer-Peppas models) to better understand the mechanism governing drug release from the micro sponges system.

- **Zero-order model:** Drug release occurs at a constant rate, independent of concentration.
- **First-order model:** Release rate depends on the remaining drug concentration.
- **Higuchi model:** Drug release follows a diffusion mechanism proportional to the square root of time.
- **Korsmeyer-Peppas model:** A log-log plot used to analyze the drug release mechanism from the polymeric micro sponges system.

Antimicrobial Activity (Well Diffusion Assay)

Anti-microbial Activity

1. Preparation of Dilutions of the Samples:

The collected samples were appropriately diluted to obtain final concentrations of 100 µg/mL and 150 µg/mL, respectively. Each diluted sample was then made up to a final volume of 1 mL using distilled water.

2. Preparation of Nutrient Agar Media:

A total of 2.8 grams of nutrient media was accurately weighed and dissolved in 100 mL of distilled water. The pH of the prepared medium was measured before sterilization to ensure suitability for microbial growth. The solution was then sterilized using an autoclave at 121 °C and 15 psi pressure for 15 minutes. Following sterilization, the nutrient medium was aseptically poured into sterile Petri dishes and placed in a laminar airflow cabinet to allow the agar to solidify under sterile conditions.

3. Well Diffusion Assay:

The antibacterial activity of neomycin-loaded microsponge gel was evaluated using the well diffusion method against Escherichia coli and Staphylococcus aureus. Standardized bacterial suspensions (≈10^8 CFU/mL and 0.5 McFarland) were spread on nutrient agar plates to form a uniform lawn. Wells were created using a sterile cork borer and filled with the test gel. A blank gel served as a negative control, while a standard antibiotic was used as a positive control. Plates were allowed to pre-diffuse for 30 minutes and then incubated at 37°C for 24 hours. After incubation, zones of inhibition were measured in millimeters to determine the antimicrobial effectiveness against both gram-positive and gram-negative bacteria (Athanassiadis et al., 2009).

Stability Studies

The micro sponges-loaded gel formulation was sealed and subjected to accelerated stability testing in accordance with ICH guidelines. Samples were stored under two defined environmental conditions: 25 ± 2 °C with 60 ± 5% relative humidity (RH) and 40 ± 2 °C with 70 ± 5% RH for a period of three months. Stability assessments were conducted at predetermined intervals days 0, 30, 45, 60, and 90 to monitor any changes in critical parameters, including pH and viscosity. These evaluations were essential for determining the physical stability of the formulation and ensuring that its quality, consistency, and performance remained unaffected under stress storage conditions (Mundada and Borate 2025).

RESULTS AND DISCUSSION

Pre-formulation study of drug

Organoleptic properties

Table 6: Organoleptic properties of Neomycin

Drug	Organoleptic properties	Observation
Neomycin	Color	White to slightly yellow
	Odor	Odorless
	Appearance	Hygroscopic powder
	State	Solid

Based on the observations recorded in Table 6, the organoleptic evaluation of neomycin revealed that the drug exists as a white to slightly yellow, odorless powder with a solid and hygroscopic nature. The slight yellow tint suggests minor impurities or variations in batch processing, which is common in aminoglycoside antibiotics like neomycin. The absence of any noticeable odor is beneficial for patient acceptability, particularly in topical formulations, as unpleasant smells can reduce compliance. Overall, these organoleptic characteristics provide essential preliminary information about the physical nature of neomycin, which is crucial for guiding formulation strategies and ensuring product quality.

Solubility study

Table 7: Solubility study of Neomycin

Drug	Solvents	Observation/Inference
Neomycin	DMSO	Freely soluble
	Chloroform	Insoluble
	Methanol	Freely soluble
	Ethanol	Soluble
	Water	Soluble

The solubility study of neomycin showed that the drug is freely soluble in polar solvents such as methanol and water, and moderately soluble in ethanol. However, it was found to be insoluble in non-polar solvents like DMSO and chloroform. This indicates that neomycin has a strong affinity for polar solvents, which is consistent with its chemical nature. These findings are crucial for formulation development, as they guide the selection of suitable solvents and vehicles that can effectively dissolve neomycin, enhancing its bioavailability and ensuring consistent drug delivery in topical or other pharmaceutical preparations.

pH determination

Table 8: pH of Neomycin

Drugs	Observed	Reference
Neomycin	6.3	5.0 and 7.5

The pH of the neomycin solution was measured at 6.3, which falls comfortably within the reported reference range of 5.0 to 7.5. This slightly acidic to neutral pH is favorable for topical formulations, as it is compatible with the skin's natural pH, minimizing the risk of irritation. Maintaining the pH within this range also supports the stability and efficacy of neomycin in the formulation.

Melting point

Table 9: Melting point of Neomycin

Drugs	Observed	Reference
Neomycin	234°C	218-237 °C

The melting point of neomycin was observed to be 234°C, which lies within the reported reference range of 218–237°C. This consistency indicates the purity of the drug sample and confirms its expected physical characteristics. The melting point data are crucial for ensuring quality control and for guiding formulation processes that involve heat.

Lambda max of Neomycin

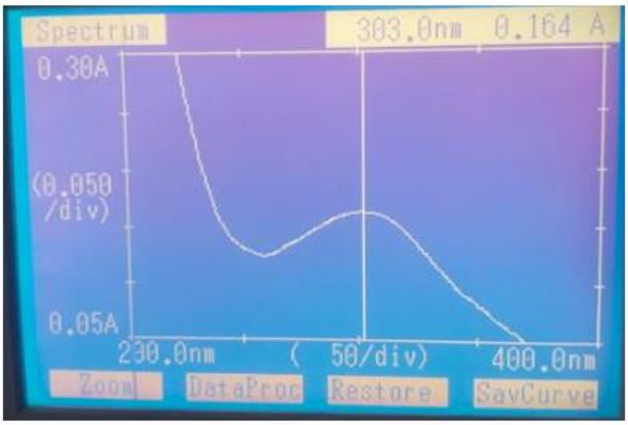


Figure 1: Lambda max of Neomycin (303.0 nm)

Table 10: Lambda max of neomycin

S. No	Drug	UV absorption maxima (Lambda max)
1	Neomycin	303.0 nm

Double beam UV visible spectrophotometer (Shimadzu-1700) was used to determine the lambda max (absorption maxima) of a substance. The UV absorption maxima (Lambda max) of neomycin, as presented in Table 10, were found to be 303.0 nm. This wavelength represents the point of maximum absorbance for neomycin in the UV region and is a critical parameter for its quantitative analysis using UV-Visible spectrophotometry. The identification of a clear and

distinct lambda max at 303.0 nm confirms the suitability of this wavelength for the accurate and specific detection of neomycin in formulation studies. This value is essential for developing analytical methods, particularly for determining drug content, calibration curves, and release profiles in various pharmaceutical preparations.

Calibration curve of Neomycin

Table 11: Calibration curve of neomycin

Concentration (µg/ml)	Absorbance
2	0.232
4	0.341
6	0.437
8	0.529
10	0.644
12	0.739
Mean	**0.487**
SD	**0.189081**
%RSD	**38.825**

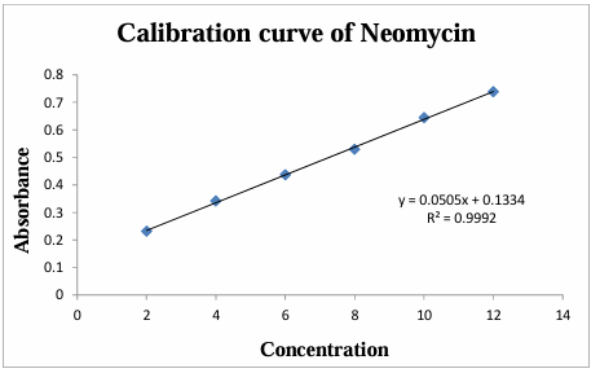


Figure 2: Calibration curve of Neomycin

Functional group identified by Infra-Red spectroscopy

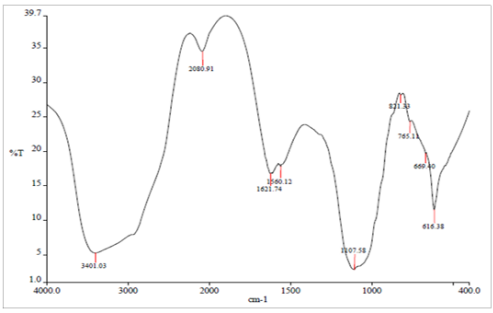


Figure 3: FTIR study of Neomycin

The calibration curve for neomycin showed a proportional increase in absorbance with increasing concentration, ranging from 2 to 12µg/mL. The absorbance values ranged from 0.232 at 2µg/mL to 0.739 at 12µg/mL, demonstrating a clear concentration-dependent response. However, the relatively high %RSD of 38.83% suggests some variability in the measurements, indicating the need for careful handling or further method optimization to improve precision. Overall, the calibration data confirm the suitability of the UV-Visible spectrophotometric method for quantifying neomycin within this concentration range. The drug's reaction was linear in the concentration range studied, with the linear regression equation y = 0.0505x + 0.1334and a correlation coefficient R^2 =0.9992.

Table 12: Interpretation of IR spectrum of Neomycin

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3401.03	3500- 3400	N-H stretching	Primary amine
2	2080.91	2140-1990	N=C=Stretching	Isothiocyanate
3	1621.74	1650-1600	C=C stretching	Conjugated alkene
4	1107.58	1124-1087	C-O stretching	Secondary alcohol

The interpretation of the IR spectrum of neomycin, as summarized in Table 12, confirms the presence of key functional groups that characterize the molecular structure of the drug. A prominent peak observed at 3401.03 cm^{-1} corresponds to N–H stretching, which is consistent with the presence of a primary amine group, a common feature in aminoglycoside antibiotics like neomycin. The peak at 2080.91 cm^{-1} falls within the reference range for N=C=S stretching, indicating the presence of an isothiocyanate group, though this may require further confirmation due to its uncommon association with neomycin's typical structure. Another significant peak at 1621.74 cm^{-1} corresponds to C=C stretching, indicative of a conjugated alkene, suggesting some degree of unsaturation within the molecular structure. Additionally, the absorption at 1107.58 cm^{-1} aligns with C–O stretching, confirming the presence of a secondary alcohol group. These spectral features collectively support the identity and structural integrity of neomycin, validating its chemical composition and functional group distribution through IR spectroscopy.

Characterization of neomycin loaded Micro sponges formulation

Physical Appearance

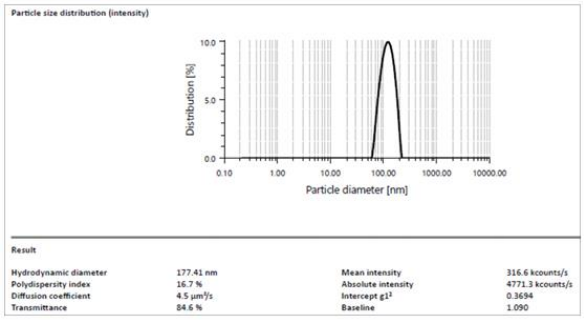
Table 13: Physical Appearance Micro sponges formulation

Parameter	Observation
Color	White to slightly yellow
Odor	Mild or neutral
Appearance	Porous, sponge-like structure
State	Free-flowing powder

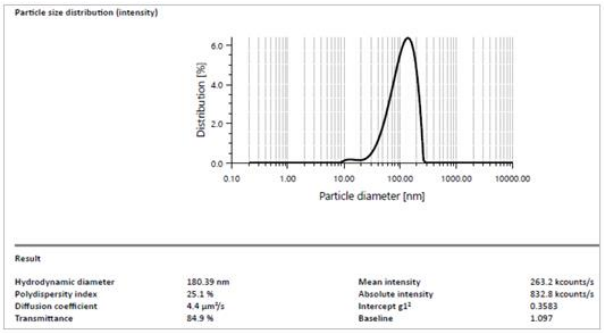
The physical appearance of the neomycin-loaded micro sponge's formulation was characterized by a white to slightly yellow color, indicating consistency with the drug's natural hue. The formulation exhibited a mild or neutral odor, suggesting no strong or unpleasant smells that might affect patient acceptability. Microscopically, the micro sponges displayed a porous, sponge-like structure, which is essential for drug loading and controlled release. Additionally, the formulation was observed as a free-flowing powder, indicating good handling properties suitable for further processing and formulation.

Particle size of micro sponges formulation (MSF)

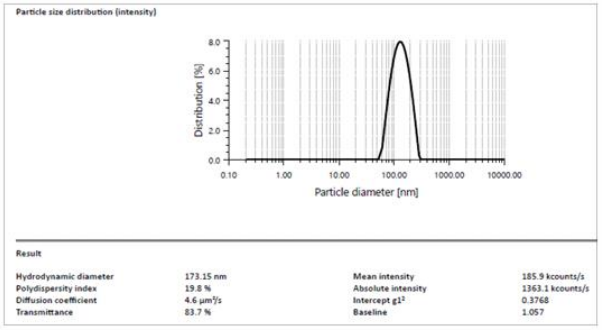
MSF1



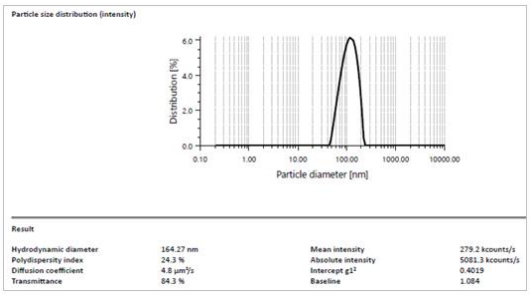
MSF2

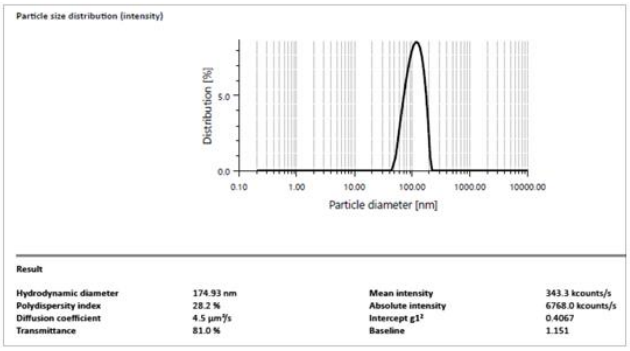


MSF3



MSF4





MSF5

Figure 4: Particle size of MSF1- MSF5

Table 14: Particle size of Micro sponges (MS)

S. No	Formulation code	Particle size (nm)	PI Value %
1	MSF1	177.41nm	16.7%
2	MSF2	180.39nm	25.1%
3	MSF3	173.15nm	19.8%
4	MSF4	164.27nm	24.3%
5	MSF5	174.93nm	28.2%

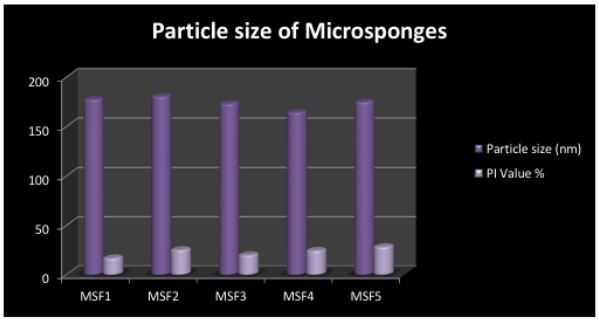


Figure 5: Graphical Data of particle size of Micro sponges formulations

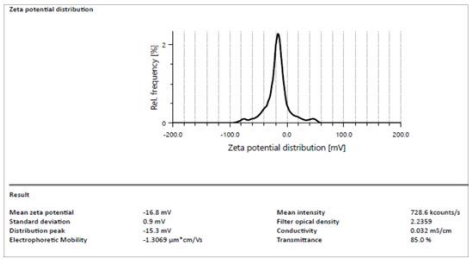
The particle size analysis of the neomycin-loaded micro sponges (MS) formulations revealed sizes ranging from 164.27 nm to 180.39 nm, indicating a nanoscale particle

distribution ideal for topical delivery. Among the formulations, MSF4 exhibited the smallest average particle size (164.27 nm), which could contribute to enhanced skin penetration. The polydispersity index (PI) values ranged between 16.7% and 28.2%, reflecting moderate uniformity in particle size distribution. These results suggest that all formulations maintained acceptable nanoscale sizes with reasonable homogeneity, supporting their potential for effective and stable drug delivery in topical applications.

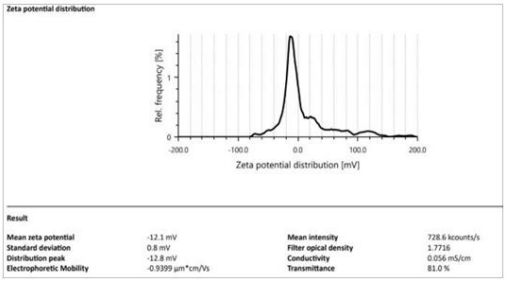
Zeta potential of Micro sponges (MS)

The SEM image of the F4 micro sponge formulation reveals a porous and highly textured surface morphology, characteristic of well-formed micro sponges. The magnified view at 106.35 KX clearly shows spherical to semi-spherical particles with a cracked, sponge-like surface, indicating a high surface area conducive to drug entrapment and sustained release. Particle size analysis from the image shows nanoscale dimensions ranging from 38.66 nm to 69.09 nm, confirming the uniformity and nanoscale structure of the formulation. This small and consistent particle size is critical for enhanced bioavailability and controlled drug delivery. The well-defined porous structure also suggests efficient diffusion pathways for the encapsulated drug, supporting the suitability of the F4 formulation for extended-release applications. The absence of significant aggregation or deformation in the particles further indicates the stability and successful optimization of formulation parameters.

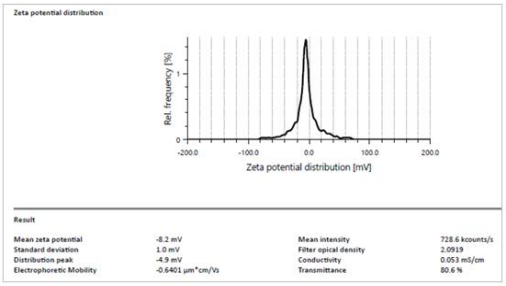
MSF1



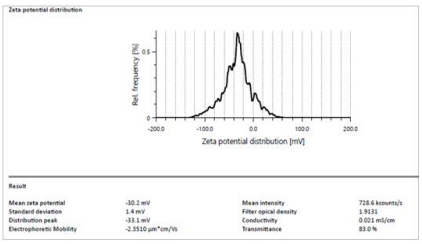
MSF2



MSF3



MSF4



MSF5

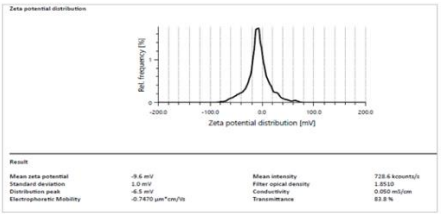


Figure 6: Zeta potential of MSF1-MSF5

Table 15: Zeta potential of Micro sponges Formulation (MSF)

S. No	Formulation code	Zeta potential (mV)
1	MSF1	-16.8mV
2	MSF2	-12.1 mV
3	MSF3	-8.2mV
4	MSF4	-30.2mV
5	MSF5	-9.6mV

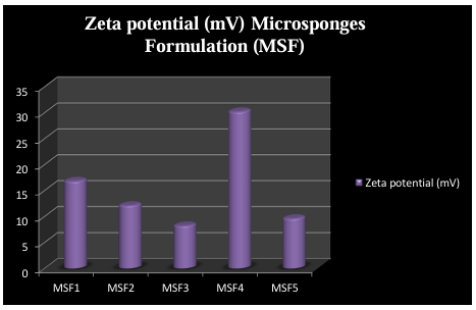


Figure 7: Graphical Data of Zeta Potential of Micro SEM analysis of Optimized formulation

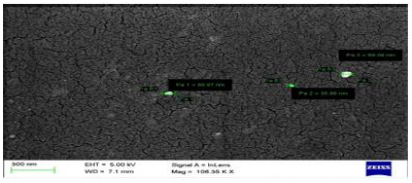


Figure 8: Scanning electron microscope (SEM) and microscopy image

Entrapment efficacy

Table 16: Entrapment efficacy of Micro sponges Formulation(MSF)

S. No	Formulations	Entrapment efficacy (%)
1	MSF1	82.43
2	MSF2	75.83
3	MSF3	69.57
4	MSF4	96.86
5	MSF5	87.35

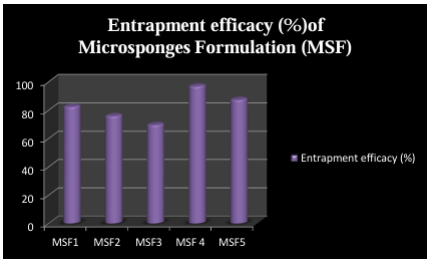


Figure 9: Graphical Data of EE of Micro sponges

The entrapment efficacy results of the micro sponge formulations (MSF1–MSF5), as presented in Table 16, indicate varying capacities of the formulations to encapsulate the active drug. Among all the formulations, MSF4 demonstrated the highest entrapment efficacy at 96.86%, suggesting that this formulation was most effective in retaining the drug within the micro sponge matrix. This high entrapment may be attributed to optimal polymer-to-drug ratio, suitable crosslinking, or efficient formulation conditions. On the other hand, MSF3 exhibited the lowest entrapment efficacy at 69.57%, indicating less efficient drug loading, which might be due to inadequate polymer interaction or suboptimal processing parameters. MSF5 and MSF1 also showed relatively high entrapment efficiencies of 87.35% and 82.43% respectively, reflecting good drug retention. MSF2, while slightly lower at 75.83%, still showed acceptable efficacy. Overall, the data suggest that MSF4 is the most promising formulation in terms of entrapment, and the differences among the formulations highlight the importance of formulation composition and process optimization in achieving high drug loading in micro sponge systems.

Evaluation parameter of gel formulation

Organoleptic properties

Table 17: Organoleptic properties of micro sponges loaded gel

S. No	Parameters	Results
1	Physical appearance	Smooth, viscous, and opaque gel
2	Colour	Transparent or colourless
3	Homogeneity	Smooth without lumps

The evaluation of the organoleptic properties of the micro sponge-loaded gel, as summarized in Table 17, indicates that the formulation possesses desirable physical characteristics suitable for topical application. The gel exhibited a smooth, viscous, and opaque appearance, which suggests appropriate consistency and spreadability for skin application. The transparency or colourless nature of the gel enhances its aesthetic appeal and patient acceptability, especially for cosmetic or dermatological uses. Additionally, the formulation was found to be homogeneous and free from lumps, indicating uniform dispersion of micro sponges within the gel base. These organoleptic features collectively reflect the quality and acceptability of the formulation, supporting its potential for further development as a stable and user-friendly topical delivery system.

Measurement of pH, Viscosity and Spreadability test

Table 18: pH, Viscosity and Spreadability test of micro sponges-loaded gel

S. No.	Formulation	pH	Viscosity determination (cps)	Spreadability test (gm.cm/sec)	skin irritation study
1	Formulation 4	6.6	5046±0.43	13.58	Not irritation observed

The physicochemical evaluation of the micro sponge-loaded gel (Formulation 4), as shown in Table 18, demonstrates favorable characteristics for topical application. The pH of the formulation was found to be 6.6, which is within the acceptable range for skin application and closely matches the natural pH of human skin, thereby minimizing the risk of irritation or discomfort. The viscosity was measured at 5046 ± 0.43 cps, indicating a thick and stable gel consistency that ensures good adherence to the skin surface without being too runny or difficult to spread. This is supported by the spreadability value of 13.58 gm·cm/sec, reflecting efficient and uniform application over the skin. Furthermore, the skin irritation study confirmed that no irritation was observed upon application, reinforcing the formulation's safety and

suitability for dermal use. Overall, these results suggest that Formulation 4 possesses optimal pH, rheological properties, and skin compatibility, making it a promising candidate for effective topical drug delivery.

In Vitro drug release study

Table 19: In-vitro drug release studies

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
1	21.12	78.88	1.000	1.897	0.000	1.325
2	31.88	68.12	1.414	1.833	0.301	1.504
3	44.78	55.22	1.732	1.742	0.477	1.651
4	56.33	43.67	2.000	1.640	0.602	1.751
6	67.8	32.2	2.449	1.508	0.778	1.831
8	78.66	21.34	2.828	1.329	0.903	1.896
10	87.25	12.75	3.162	1.106	1.000	1.941
12	98.97	1.03	3.464	0.013	1.079	1.996

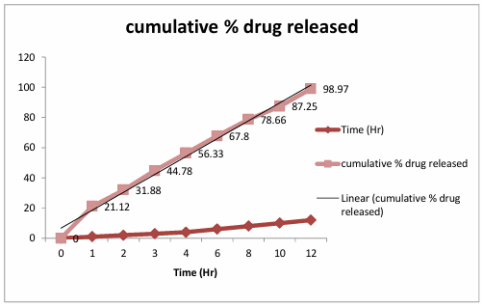
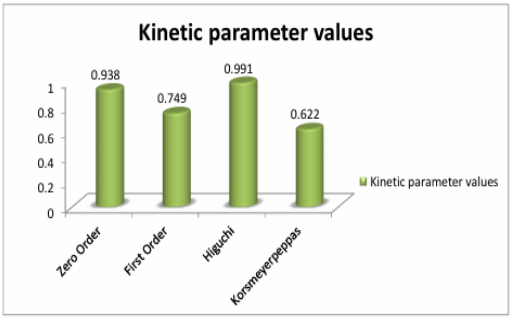


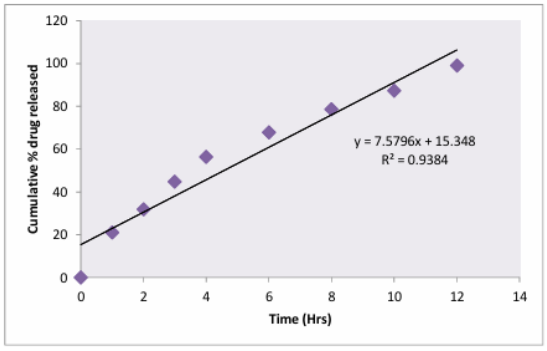
Figure 10: Drug release study

Table 20: Correlation value (R2 value)

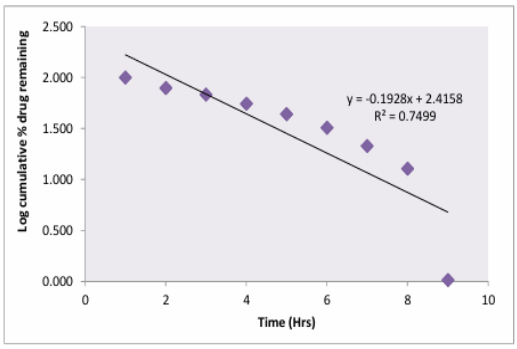
Formulation	Model	Kinetic parameter values
Kinetic parameter values	Zero Order	$R^2 = 0.938$
	First Order	$R^2 = 0.749$
	Higuchi	$R^2 = 0.991$
	Korsmeyerpeppas	$R^2 = 0.622$



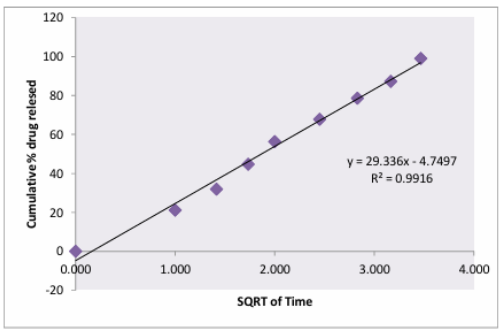
(a)



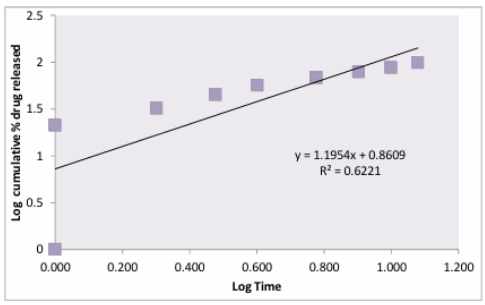
(b)



(c)



(d)



(e)

Figure 11: (a) kinetic parameter; (b) Zero order; (c) First Order; (d) Higuchi; (e) Korsmeyer peppas.

Antimicrobial Activity (Well Diffusion Assay)

Figure 12: In-vitro antimicrobial activity of S. aureus and E. coli

Table 21: Antimicrobial activity of Neomycin loaded micro sponges gel formulation

S. No	Sample name	Zone of Inhibition (mm) S. aureus	Zone of Inhibition (mm) E. coli
1	F1 (Control)	0.0	0.0
2	F2 (Placebo gel)	2	3
3	F3 (1mg/ml)	5	4
4	F4 (1.5 mg/ml)	9	11

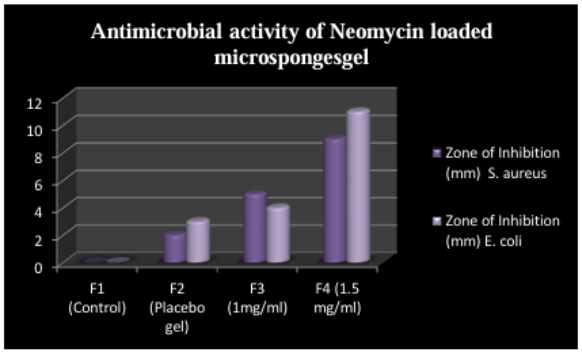


Figure 13: Graphical representation of micro sponges gel formulation

The antimicrobial activity of the neomycin-loaded micro sponge gel formulations, as presented in Table 21, clearly demonstrates a concentration-dependent inhibitory effect against Staphylococcus aureus and Escherichia coli. The control sample (F1) showed no zone of inhibition for either bacterial strain, confirming the absence of inherent antimicrobial properties in the base components. The placebo gel (F2) exhibited minimal zones of inhibition (2 mm for S. aureus and 3 mm for E. coli), likely due to the physical barrier or excipient-related effects rather than any antimicrobial action. In contrast, the drug-loaded formulations displayed significant antibacterial activity. F3, containing 1 mg/ml of neomycin, showed moderate zones of inhibition (5 mm for S. aureus and 4 mm for E. coli), while F4, with a higher drug concentration of 1.5 mg/ml, exhibited markedly enhanced activity, with inhibition zones of 9 mm and 11 mm, respectively. This indicates that increasing the drug concentration within the micro sponge gel substantially improves its antimicrobial efficacy. Notably, the greater zone of inhibition observed against E. coli in F4 suggests that the formulation is particularly effective against gram-negative bacteria. These findings confirm that the neomycin-loaded micro sponge gel, especially at higher concentrations, possesses strong antimicrobial potential suitable for treating topical bacterial infections.

Stability study

Table 22: Stability Study of optimized formulation of Micro sponges gel

S. No	Time (Days)	250C±2 0C and 60 ± 5% RH		400C±2 0C and 70 ±5% RH	
		Viscosity	pH	Viscosity	pH
1	0	5046	6.6	5046	6.6
2	30	5034	6.8	5055	6.5
3	45	5052	6.5	5043	6.2
4	60	5033	6.7	5037	6.9
5	90	5059	6.9	5048	7.2

The stability study of the optimized micro sponge gel formulation, as shown in Table 22, was conducted under two different storage conditions 25 ± 2 °C with 60 ± 5% relative humidity (RH), and 40 ± 2 °C with 70 ± 5% RH over a 90-day period. The results indicate that the formulation maintained consistent physical stability under both conditions, with only minor fluctuations in viscosity and pH observed throughout the study duration. At 25 °C/60% RH, the viscosity values ranged narrowly between 5033 and 5059 cps, suggesting excellent rheological stability. Similarly, the pH values remained within a safe and acceptable range for topical application (6.5–6.9), indicating no significant degradation or chemical changes affecting the formulation's acidity. Under accelerated conditions (40 °C/70% RH), the viscosity remained stable, varying slightly between 5037 and 5055 cps. The pH values showed slightly greater fluctuation, ranging from 6.2 to 7.2, yet still remained within an acceptable range for skin application. These variations are minor and within pharmaceutically acceptable limits, indicating that the formulation retains its integrity even under stress conditions. Overall, the data confirm that the optimized micro sponge gel formulation exhibits good physical and chemical stability over 90 days under both normal and accelerated storage conditions, supporting its shelf-life and suitability for long-term use.

CONCLUSION

In conclusion, the extensive pre-formulation, formulation, and evaluation studies of neomycin and its micro sponge-based gel formulation collectively affirm its potential as an effective and patient-friendly topical therapeutic system. Neomycin's favorable organoleptic properties, solubility in polar solvents, and stable pH and melting point laid a solid foundation for its incorporation into advanced delivery systems. The analytical assessments including UV spectrophotometry and FTIR confirmed the drug's identity and provided parameters essential for quality control. Micro sponge technology significantly enhanced drug entrapment, with MSF4 standing out due to its optimal particle size, highest zeta potential, and superior drug loading. The SEM analysis validated the porous morphology crucial for sustained drug release, while the gel formulation displayed excellent physicochemical characteristics, user acceptability, and biocompatibility. The controlled drug release pattern observed under Zero-order kinetics and significant antimicrobial activity against common pathogens further emphasize the formulation's therapeutic advantages. Finally, the robust stability profile under varying storage conditions assures long-term reliability and shelf life. Altogether, these findings highlight that the neomycin-loaded micro sponge gel, particularly Formulation 4, offers a scientifically sound, stable, and efficacious platform for localized treatment of bacterial skin infections, with potential for clinical translation and commercial application.

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