Formulation and Evaluation of Neomycin Sulphate-Loaded Nanosponge Hydrogel

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Abstract

Infected diabetic foot ulcers (DFUs) are a major complication of diabetes mellitus, often associated with delayed wound healing and an increased risk of severe infection. Conventional topical formulations may release the drug rapidly, limiting prolonged drug availability at the wound site. The present study aimed to formulate and evaluate neomycin sulphate-loaded nanosponge hydrogel for the topical management of infected diabetic foot ulcers. Neomycin sulphate-loaded nanosponges were prepared using the solvent emulsion diffusion method, employing ethyl cellulose as the polymer and polyvinyl alcohol as the stabilizer. Nine formulations were developed and evaluated for drug content, entrapment efficiency, particle size, zeta potential, and in-vitro drug release. The optimized formulation was incorporated into a hydrogel base containing Carbopol 940 and carboxymethyl cellulose and assessed for physicochemical characteristics, drug release kinetics, and antimicrobial activity. Formulation F5 demonstrated an entrapment efficiency of 87.60%, a drug content of 97.92%, a particle size of 332 nm, and a zeta potential of −32.38 mV. In-vitro release studies showed 65.11% drug release from F5 over seven hours, compared with 94.47% from the conventional neomycin gel. The optimized formulation also demonstrated in-vitro antibacterial activity against *Staphylococcus aureus*. These findings suggest that neomycin sulphate-loaded nanosponge hydrogel is a promising platform for sustained topical antibiotic delivery. Further safety, stability, and in-vivo studies are required to establish its suitability for infected diabetic foot ulcer management.

Keywords: Neomycin sulphate; nanosponges; hydrogel; diabetic foot ulcer; sustained drug release; antimicrobial activity.

INTRODUCTION

Diabetic foot ulcer (DFU) is a serious complication of diabetes mellitus, commonly associated with peripheral neuropathy, impaired circulation, reduced immune function, and delayed wound healing. These factors contribute to the development of chronic wounds that are susceptible to microbial colonization and infection. Infected DFUs can progress to deeper tissue involvement, osteomyelitis, and, in severe cases, amputation. Therefore, effective infection control and appropriate wound management are essential for reducing complications and promoting healing.

The management of infected DFUs generally involves wound assessment, debridement, pressure offloading, appropriate dressings, glycaemic control, and antimicrobial therapy when indicated. Topical antibiotics may be useful in selected cases; however, their performance can be limited by wound exudate, frequent dressing changes, and inadequate drug retention at the wound surface. In addition, the complex wound environment and formation of bacterial biofilms may contribute to persistent infection and reduced antimicrobial susceptibility. These challenges highlight the need to investigate localized drug delivery systems that can maintain drug availability at the wound site for an extended period. Topical formulations should complement, rather than replace, established DFU management and systemic antibiotic treatment when clinically indicated. **(Armstrong DG et al.,2012)**

Neomycin sulphate is an aminoglycoside antibiotic with activity against various susceptible Gram-positive and Gram-negative bacteria. Its topical use makes it a candidate for investigation in localized antimicrobial delivery systems. However, conventional topical formulations may not provide prolonged drug retention or sustained release under wound conditions. Moreover, the use of topical antibiotics must be guided by appropriate clinical assessment and susceptibility considerations to minimize unnecessary exposure and the risk of antimicrobial resistance. Developing a suitable carrier system may help improve the delivery characteristics of neomycin sulphate and provide a basis for evaluating its potential in infected wound management. **Vijaya Patil et al., (2014)**

Nanosponges are porous, three-dimensional polymeric drug carriers that can entrap therapeutic agents within their internal structure and facilitate their gradual release. Their properties depend on the polymer composition, stabilizer concentration, and preparation conditions. Incorporating a drug into nanosponges may improve its retention within a formulation and modify its release profile compared with a conventional preparation. Ethyl cellulose can serve as a polymeric carrier, while polyvinyl alcohol can act as a stabilizer during nanosponge preparation. **Peters et al. (2017)**

Hydrogels are three-dimensional polymeric networks capable of retaining water and maintaining a moist environment. They are widely investigated for topical and wound-care applications because they can provide a suitable vehicle for drug administration and conform to the application site. Combining drug-loaded nanosponges with a hydrogel matrix offers a potential strategy to integrate the drug-retaining properties of the nanosponge carrier with the application and hydration benefits of the gel. Carbopol 940 and carboxymethyl cellulose can be used to develop a hydrogel base with appropriate physicochemical characteristics. **Wu H et al. (2020)**

Based on this rationale, the present study aimed to formulate and evaluate neomycin sulphate-loaded nanosponges and incorporate the optimized formulation into a hydrogel for potential topical application in infected diabetic foot ulcers. Nanosponges were prepared using the solvent emulsion diffusion method with ethyl cellulose and polyvinyl alcohol. The formulations were evaluated for relevant physicochemical and drug-loading characteristics, followed by incorporation into a hydrogel base. The optimized nanosponge hydrogel was assessed for its physical properties, in-vitro drug release, release kinetics, and in-vitro antibacterial activity, with comparison against a conventional neomycin sulphate

gel. The study was intended to determine whether the nanosponge–hydrogel system could provide a modified drug-release profile and demonstrate antibacterial activity under the experimental conditions.

AIM AND OBJECTIVES

Aim

To formulate and evaluate neomycin sulphate-loaded nanosponge hydrogel for the topical management of infected diabetic foot ulcers.

Objectives

- To formulate neomycin sulphate-loaded nanosponges using the solvent emulsion diffusion method.
- To characterize the prepared nanosponges for particle size, polydispersity index, zeta potential, entrapment efficiency, and drug content.
- To incorporate the optimized nanosponge formulation into a hydrogel base and evaluate its physicochemical properties.
- To investigate the in-vitro drug release profile and release kinetics of the optimized formulation.
- To evaluate the in-vitro antibacterial activity of the formulated hydrogel against *Staphylococcus aureus*.

MATERIALS REQUIRED

S. No.	Material	Purpose
1	Neomycin sulphate	Active pharmaceutical ingredient
2	Ethyl cellulose	Nanosponge-forming polymer
3	Dichloromethane	Organic solvent
4	Polyvinyl alcohol (PVA)	Stabilizing agent
5	Carbopol 940	Hydrogel-forming agent
6	Carboxymethyl cellulose (CMC)	Hydrogel-forming agent
7	Glycerine	Humectant
8	Triethanolamine	Neutralizing agent
9	Ethanol	Solvent used during the study

METHODOLOGY

Preformulation Studies

Description of Drug

Neomycin sulphate was examined for its physical appearance, colour and odour.

Determination of Melting Point

The melting point of neomycin sulphate was determined using the open capillary method with a digital melting point apparatus.

Solubility Studies

The solubility of neomycin sulphate was evaluated in distilled water and various solvents by observing its dissolution.

Determination of Partition Coefficient

The partition coefficient of neomycin sulphate was evaluated to assess its distribution between an aqueous phase and an organic phase.

Preparation of Phosphate Buffer (pH 7.4)

Phosphate buffer of pH 7.4 was prepared using potassium dihydrogen phosphate and sodium hydroxide, with distilled water used to make up the final volume.

Determination of Maximum Absorption (λmax)

A neomycin sulphate solution (10 µg/mL) was scanned from 200–400 nm using a UV–Visible spectrophotometer. The maximum absorption wavelength was found to be 205 nm.

Preparation of Standard Calibration Curve

Standard solutions of neomycin sulphate (10–50 µg/mL) were prepared in phosphate buffer (pH 7.4). Absorbance was measured at 205 nm against the buffer blank, and a calibration curve was plotted.

Drug–Excipient Compatibility Studies (FTIR)

FTIR spectra of neomycin sulphate and the selected excipients were recorded over 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using the KBr pellet method.

Formulation of Neomycin Sulphate-Loaded Nanosponges

Neomycin sulphate-loaded nanosponges were prepared using the solvent emulsion diffusion method, employing ethyl cellulose as the polymer, dichloromethane as the organic solvent, polyvinyl alcohol (PVA) as the stabilizer, and distilled water as the aqueous phase.

Neomycin sulphate and ethyl cellulose were dissolved in dichloromethane to form the organic phase. The aqueous phase was prepared by dissolving PVA in distilled water with heating. The organic phase was slowly added to the aqueous phase under magnetic stirring and stirred for 30 minutes. The resulting nanosponges were collected by filtration and dried in a hot-air oven at 45°C.

Characterisation of Neomycin Sulphate-Loaded Nanosponges

Determination of Drug Content

An accurately weighed quantity of neomycin sulphate-loaded nanosponges was dispersed in methanol and sonicated to ensure complete dissolution. The dispersion was filtered through Whatman filter paper, and the filtrate was diluted with phosphate buffer (pH 7.4). The absorbance was measured at 205 nm using a UV–Visible spectrophotometer, and drug content was calculated using the standard calibration curve.

Determination of Entrapment Efficiency (EE%)

Entrapment efficiency was determined by separating the unentrapped drug from the neomycin sulphate-loaded nanosponges by centrifugation. The amount of free drug in the supernatant was estimated spectrophotometrically at 205 nm. Entrapment efficiency was calculated using the following equation:

Determination of Particle Size

The particle size of the optimized nanosponge formulations was determined using a particle size analyser based on the light-scattering technique. The mean particle size was recorded to assess the nanosponge dimensions and size distribution.

Determination of Polydispersity Index (PDI)

The polydispersity index was measured using a particle size analyser to evaluate the uniformity of particle size distribution. A lower PDI indicates a more uniform particle population.

Determination of Zeta Potential

The zeta potential of the selected nanosponge formulations was measured using a zeta potential analyser. The measured surface charge was used to assess the electrostatic stability of the nanosponge dispersion.

Scanning Electron Microscopy (SEM)

The surface morphology of the optimized nanosponge formulation was examined using a scanning electron microscope (ZEISS) to evaluate particle shape, surface characteristics and morphology.

Formulation of Neomycin Sulphate-Loaded Nanosponge Hydrogel

The hydrogel base was prepared by dispersing Carbopol 940 in distilled water under stirring. Carboxymethyl cellulose (CMC) was separately dispersed in distilled water and allowed to swell for 24 hours. Both dispersions were mixed to form the gel base, and the neomycin sulphate-loaded nanosponges were incorporated with continuous stirring. Triethanolamine was used to adjust the pH, and the prepared hydrogel was sonicated for 15 minutes to remove air bubbles.

Evaluation of Neomycin Sulphate-Loaded Nanosponge Hydrogel

Physical Appearance

The prepared hydrogel was visually examined for colour, clarity, consistency and the presence of foreign particles or aggregates.

Determination of pH

The pH of the prepared hydrogel was measured using a calibrated pH meter to assess its suitability for topical application.

Determination of Viscosity

The viscosity of the hydrogel was measured using a Brookfield viscometer to evaluate its consistency and flow properties.

Determination of Spreadability

The spreadability of the hydrogel was evaluated using the glass-slide method to assess its ease of application over the skin.

Determination of Swelling Index

The swelling behaviour of the hydrogel was evaluated by recording its weight before and after exposure to the specified medium. The swelling index was calculated from the change in weight.

In-vitro Drug Release Study

The in-vitro drug release study was performed using a Franz diffusion apparatus with phosphate buffer (pH 7.4) as the receptor medium. The assembly was maintained at 37°C, and samples were withdrawn at predetermined time intervals up to 420 minutes. Sink conditions were maintained by replacing the withdrawn volume with fresh buffer. The samples were analysed at 205 nm using a UV–Visible spectrophotometer.

In-vitro Drug Release Kinetics

The drug release data were fitted to suitable kinetic models to determine the release pattern and mechanism of neomycin sulphate from the optimized nanosponge hydrogel.

Drug–Excipient Compatibility Study (FTIR)

FTIR analysis was performed to assess the compatibility of neomycin sulphate with the selected excipients by comparing their characteristic absorption peaks and identifying any major peak shifts or disappearance.

In-vitro Antimicrobial Activity

The antimicrobial activity of the optimized nanosponge hydrogel and conventional neomycin sulphate gel was evaluated against Staphylococcus aureus using the agar well diffusion method. Mueller–Hinton agar plates were incubated at 37°C for 24 hours, and antibacterial activity was assessed by measuring the zones of inhibition.

RESULTS AND DISCUSSION

Preformulation Studies

Description of Drug

Neomycin sulphate was observed as a yellowish-white, odourless powder and complied with the manufacturer's Certificate of Analysis.

Determination of Melting Point

The melting point of neomycin sulphate was found to be 189–192°C, confirming its characteristic thermal property.

Solubility Studies

Neomycin sulphate was found to be very soluble in water and soluble in ethanol, chloroform, and phosphate buffer (pH 7.4).

UV–Visible Spectrophotometric Studies

Determination of Maximum Absorption (λmax)

The maximum absorption wavelength of neomycin sulphate was determined to be 205 nm in phosphate buffer (pH 7.4).

Calibration Curve of Neomycin Sulphate

Neomycin sulphate obeyed Beer–Lambert's law over the concentration range of 10–50 µg/mL, with a reported correlation coefficient of 0.9988. The calibration data are presented below.

CONCENTRATION (µg/ml)	ABSORBANCE at 205 (nm)±SD
0	0.000
10	0.061±0.0016
20	0.085±0.0021
30	0.124±0.0016
40	0.162±0.0021
50	0.198±0.0355
Regression value (r^2)	0.9988

Drug Content and Entrapment Efficiency

Formulation	Drug content (%) ± SD	Entrapment efficiency (%) ± SD
F1	90.25 ± 0.42	62.93 ± 0.13
F2	91.18 ± 0.38	67.50 ± 0.20
F3	92.34 ± 0.47	71.03 ± 0.20
F4	93.12 ± 0.51	80.91 ± 0.15
F5	96.05 ± 0.44	87.60 ± 0.35
F6	97.04 ± 0.49	84.16 ± 0.08
F7	96.14 ± 0.36	83.77 ± 0.07
F8	93.72 ± 0.41	85.07 ± 0.23
F9	97.05 ± 0.39	82.06 ± 0.20

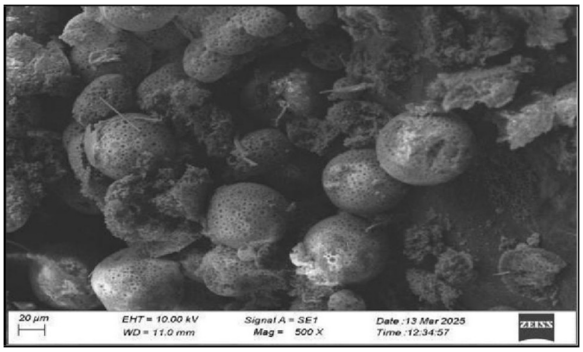
Drug content and entrapment efficiency were evaluated to determine the amount of neomycin sulphate present in the prepared nanosponges and the percentage of drug successfully entrapped within the polymeric matrix.

The drug content of the formulations ranged from 90.25% to 97.05%, indicating satisfactory drug incorporation. Entrapment efficiency ranged from 62.93% to 87.60%, with formulation F5 showing the highest efficiency. F5, prepared using a drug-to-polymer ratio of 1:4 and 1% PVA, demonstrated a

favourable balance between drug incorporation and entrapment. Therefore, F5 was selected as the optimized nanosponge formulation for further characterization and hydrogel development.

Morphological Characterization

The surface morphology of the optimized F5 neomycin sulphate-loaded nanosponges was examined using scanning electron microscopy (SEM).



SEM analysis revealed the morphological features of the prepared nanosponges, including their shape, surface characteristics, and porosity. The observed morphology supported the formation of nanosponge particles and their suitability as a carrier system for neomycin sulphate. The porous polymeric structure may facilitate drug entrapment and influence the release profile of the formulation.

Particle Size, Polydispersity Index (PDI) and Zeta Potential

Particle size, PDI, and zeta potential were evaluated to characterize the nanosponge formulations and assess their particle-size distribution and physical stability.

Formulation	Particle size (nm)	PDI	Zeta potential (mV)
F4	216	0.22	−28.79
F5	332	0.185	−32.38
F6	250	0.25	−23.45

The particle sizes of the selected formulations ranged from 216 to 332 nm. Formulation F5 showed a PDI of 0.185, indicating a relatively narrow particle-size distribution. Its zeta potential of −32.38 mV suggests favourable electrostatic repulsion between particles, which may help reduce aggregation. Considering its highest entrapment efficiency and satisfactory drug content, F5 was selected as the optimized formulation for incorporation into the hydrogel base.

Formulation of Neomycin Sulphate-Loaded Nanosponge Hydrogel

The optimized F5 nanosponge formulation was incorporated into a hydrogel base containing Carbopol 940 and carboxymethyl cellulose (CMC). Carbopol 940 was dispersed in distilled water under stirring, while CMC was separately dispersed and allowed to swell for 24 hours. Both dispersions were mixed to form a uniform gel base. The neomycin sulphate-loaded nanosponges were then incorporated with continuous stirring to obtain a 1% drug-loaded nanosponge hydrogel. The pH was adjusted using triethanolamine, and the formulation was sonicated for 15 minutes to remove entrapped air bubbles.

Evaluation of Neomycin Sulphate-Loaded Nanosponge Hydrogel

The prepared hydrogel was evaluated for physical appearance, homogeneity, pH, viscosity, spreadability, swelling index, drug content, in-vitro drug release, and antimicrobial activity.

Physical Appearance, Homogeneity and pH

The prepared hydrogel was clear, smooth, and free from foreign particles. It exhibited uniform homogeneity without visible aggregates. The pH values ranged from 6.4 to 6.7, indicating suitability for further topical evaluation.

Viscosity, Spreadability and Swelling Index

Viscosity was measured using a Brookfield viscometer, spreadability was assessed by the parallel-plate method, and swelling index was determined using phosphate buffer (pH 7.4).

Evaluation parameter	Observed range
Viscosity	4463–5653 cps
Spreadability	5.16–6.35 cm
Swelling index	310–440%

The results indicate that the prepared hydrogels possessed suitable consistency, spreading characteristics, and swelling capacity, supporting their potential application as topical formulations.

Drug Content

The drug content of the optimized F5 nanosponge hydrogel was compared with that of the plain neomycin gel.

Formulation	Drug content (%)
Plain neomycin gel	92.07
F5 nanosponge hydrogel	96.05

The higher drug content observed in F5 indicates satisfactory drug incorporation and distribution within the nanosponge hydrogel matrix.

***In-vitro* Drug Release**

In-vitro drug release was evaluated using a Franz diffusion cell with phosphate buffer (pH 7.4) over seven hours. The optimized F5 nanosponge hydrogel showed 65.11% cumulative drug release, compared with 94.47% for the plain gel at the same time point. This difference indicates slower drug release from the nanosponge hydrogel, supporting its potential for controlled and sustained topical delivery.

Time (min)	Plain gel (% drug release) ± SD	F5 nanosponge hydrogel (% drug release) ± SD
0	0.00	0.00
30	13.38 ± 0.24	12.12 ± 0.09

60	25.79 ± 0.31	18.30 ± 0.10
120	37.11 ± 0.22	28.13 ± 0.14
180	49.47 ± 0.18	37.32 ± 0.24
240	61.32 ± 0.42	46.11 ± 0.35
300	73.95 ± 0.09	54.22 ± 0.21
360	85.26 ± 0.29	60.05 ± 0.29
420 (7 h)	94.47 ± 0.34	65.11 ± 0.14

The plain neomycin gel exhibited 94.47% drug release after 7 hours, whereas the optimized F5 nanosponge hydrogel released 65.11% during the same period. The slower release from F5 may be attributed to the entrapment of neomycin sulphate within the ethyl cellulose nanosponge matrix, which acts as a barrier to drug diffusion. These findings demonstrate the potential of F5 to provide controlled and sustained topical drug release.

Antimicrobial Activity

Antimicrobial activity was evaluated against Staphylococcus aureus using the agar diffusion method.

Formulation	**Zone of inhibition at 100 µg (mm)**	**Zone of inhibition at 50 µg (mm)**
Standard	16.6	12.5
Plain gel	15.3	12.1
F5 nanosponge hydrogel	16.1	12.8

The F5 formulation demonstrated antibacterial activity against S. aureus, with inhibition zones comparable to the standard under the tested conditions. These findings support further investigation of the formulation for infected diabetic foot ulcer management.

Conclusion

The present study successfully formulated and evaluated neomycin sulphate-loaded nanosponge hydrogel as a potential topical drug delivery system for the management of infected diabetic foot ulcers. The optimized F5 formulation demonstrated satisfactory physicochemical characteristics, effective drug entrapment, and promising in-vitro antibacterial activity against *Staphylococcus aureus*. Compared with the conventional neomycin sulphate gel, the nanosponge hydrogel exhibited a slower and more sustained drug-release profile, highlighting the potential of the ethyl cellulose-based nanosponge matrix to regulate drug release and prolong drug availability at the application site. Incorporation into a hydrogel base further offers a suitable semisolid formulation for topical application.

Overall, these findings support the potential of neomycin sulphate-loaded nanosponge hydrogel as an alternative approach to conventional topical antibiotic delivery for infected diabetic foot ulcers. However, its clinical usefulness cannot be established from in-vitro findings alone. Further investigations involving formulation stability, skin and wound compatibility, cytotoxicity, antimicrobial

efficacy against relevant wound pathogens, and in-vivo wound-healing studies are required to establish its safety, therapeutic performance, and suitability for future clinical application.

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