ARTICLE

Biomedical Engineering Communications 2025;4(3):19. https://doi.org/10.53388/BMEC2025019

Development and evaluation of nanosponge loaded hydrogel of Ceftriaxone for the treatment of anti-bacterial infections

Vaishali Goel[1], Ujjwal Nautiyal[2], Deepika Raina[1*]

[1]School of Pharmacy, Graphic Era Hill University, Dehradun 248002, India. [2]Himalayan School of Pharmaceutical Sciences, Swami Rama Himalayan University, Dehradun 248016 India.

[*]**Correspondence to:** Deepika Raina. School of Pharmacy, Graphic Era Hill University, 566/6, Bell Road, Dehradun 248002, India. E-mail: deepika709@gmail.com.

Author contributions

Vaishali Goel performed the experiment and wrote the manuscript. Vaishali Goel performed the data analyses. Vaishali Goel and Deepika Raina were involved in interpretation and data collection. Deepika Raina and Ujjwal Nautiyal contributed to the conception of the study.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Peer review information

Biomedical Engineering Communications thanks Xiao-Dong Zhu and other anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

FT-IR, fourier transform infrared; NS1, nanosponge formulation 1; NS2, nanosponge formulation 2; NS3, nanosponge formulation 3; SEM, scanning electron microscopy; TEM, transmission electron microscopy; PVA, polyvinyl alcohol; EC, ethyl cellulose; DCM, dichloromethane.

Citation

Goel V, Nautiyal U, Raina D. Development and evaluation of nanosponge loaded hydrogel of Ceftriaxone for the treatment of anti-bacterial infections. *Biomed Eng Commun*. 2025;4(3):19. doi: 10.53388/BMEC2025019.

Executive editor: .Meng-Meng Song.

Received: 19 February 2025; **Revised:** 12 March 2025; **Accepted:** 10 April 2025; **Available online:** 6 June 2025.

Abstract

Background: Nanosponges are an innovative type of material with microscopic particles that are characterized by cavities that measure just a few nanometers in width. These tiny sponges are capable of encapsulating various substances. The particles move through the body until they arrive at their designated location, where they bind to the surface and release their content. **Methods:** Using the Quasi-Emulsion Solution Diffusion technology, three different nanosponge formulations of Ceftriaxone can be created. These formulas are synthesized by crossing organic and inorganic substances. A surfactant, PVA, is added to the external phase to facilitate the formation of an emulsion by lowering the interfacial tension, thereby facilitating the formation of an emulsion. As a result of the incorporation of Carbopol 934 into a hydrogel matrix, the most effective formulation was nanosponge-loaded hydrogel. The prepared nanosponge hydrogel was subjected to different evaluations. **Results:** The skin irritation test revealed negligible irritation on the human skin. It was concluded that the hydrogel formulation caused no skin irritation and found compatible with skin. According to the antimicrobial investigation, both Gram-positive and Gram-negative bacteria are susceptible to the formulated product. Ex vivo and in vitro studies indicate that the drug is initially released rapidly, followed by a sustained and controlled release. The sustained effect is more pronounced with gel formulation. **Conclusion:** So, it can be concluded that prepared formulation provides a targeted, extended and sustained action for the treatment of skin infections.

Keywords: nanosponge; ethyl cellulose; carbopol 934; hydrogel; sustained action

Introduction

Nanosponges are minuscule particles, comparable in size to viruses, with diameters less than 1 μm, that show great potential for precise medication delivery purposes. Small sponges go around the body, attaching to specific locations where drugs are released methodically [1]. This precise delivery technique improves the effectiveness of the medication at the desired site while reducing overall exposure, perhaps resulting in better treatment results. Nanosponges has a small size and porous structure that allows them to effectively capture medicines with low solubility, hence improving their bioavailability [2]. They tackle diverse formulation obstacles by concentrating drug delivery on precise locations, protecting against drug and protein degradation, and ensuring a consistent and regulated release of the treatment [3]. Nanosponges are synthesized through appropriate cross-linking processes using both organic and inorganic materials, forming inclusion and non-inclusion complexes to encapsulate various molecules [4].

Our study aims to examine the efficacy of a nanosponge hydrogel containing Ceftriaxone for treating skin and soft tissue infections. Ceftriaxone, a third-generation cephalosporin antibiotic administered by injection, was encapsulated in nanosponges using the emulsion solvent diffusion process. It was then integrated into a hydrogel matrix containing Carbopol 934. In order to assess the efficiency of this hydrogel containing nanosponges in enhancing permeability, ex-vivo experiments were conducted utilising a Franz diffusion cell device. This formulation was assessed for its antibacterial efficacy against Staphylococcus aureus and Staphylococcus aureus, specifically targeting skin and soft tissue infections.

Materials and methods

Ceftriaxone drug was purchased from Akron India Pvt. Ltd., Paonta Sahib and Ethyl Cellulose, Polyvinyl Alcohol, Carbopol 934, Triethanolamine, Propylene Glycol was obtained from Grey Scientific, Ambala.

Method of preparation of Ceftriaxone loaded nanosponge

The Quasi-Emulsion Solvent Diffusion approach was employed to create nanosponges by utilising two separate phases that are unable to mix: an interior phase and an exterior phase. A surfactant is used to reduce the interfacial tension and facilitate the creation of the emulsion. The internal phase of the solution was initially made by the sonication of ethylcellulose in a mixture of 1:1 dichloromethane (DCM) and methanol in 20 milliliters. In order to prepare the external phase, the simultaneous measurement of Ceftriaxon and polyvinyl alcohol (PVA) was performed with 60 °C distilled water. After cooling at ambient temperature, the mixture is re-mixed. Using a magnetic stirrer, the burt was used to slowly introduce the inner phase to the outer phase, while the burt was used to gradually introduce the inner phase to the outer phase. The internal and external phases were thoroughly integrated during the night stirring process. Afterwards, the nanosponges were filtered and dried in a hot air furnace at 40 degrees C until they reached their final state [5–9].

Different formulations of nanosponge. To achieve the most effective nanosponge formulation, it was necessary to optimize the concentrations of the polymer, Ethyl Cellulose (EC), and the surfactant, Polyvinyl Alcohol. Therefore, three different formulations of nanosponge were prepared by standardising the concentration of EC and PVA.

Table 1 shows the different compositions of Nanosponge formulations with standardised concentration of EC and PVA.

Evaluations of nanosponge. Following are some parameters used for the evaluation of nanosponge:

Drug content. A mixture of 10 mL of phosphate buffer pH 7.2 was mixed with nanosponges to measure capture efficiency. After the sound, the mixture is filtered after the sound with a bathtub sonicator. In spectroscopy analysis, a portion of the 1 mL filter was diluted in phosphate buffer to 10 mL and analyzed at 254 nm using phosphate buffer. According to the provided equation, it is possible to calculate the encapsulation of drugs in nanospheres. The solution's absorbance was later measured using spectrophotometry at a wavelength of 254 nm. A blank nanosponge, manufactured using the same method as the sample, served as the reference for the measurement.

Drug entrapment efficiency. To evaluate the entrapment efficiency of the nanosponges, 10 mL of phosphate buffer with a pH of 7.2 was mixed with the nanosponges. The mixture was then subjected to sonication using a bath sonicator, followed by filtration. A 1 mL portion of the filtrate was diluted to 10 mL with phosphate buffer and analyzed spectrophotometrically at a wavelength of 254 nm. The amount of drug encapsulated within the nanosponges was calculated using the provided Equation:

$$\% \text{ EE} = (\text{Total Amount of Drug} - \text{Concentration of Drug})/\text{Total amount of Drug} \times 100 \quad (1)$$

Percentage yield. To determine the yield % of nanosponges, it is necessary to accurately document the beginning weight of the raw materials and then compare it to the end weight of the nanosponges.

$$\% \text{ Yield} = \text{Practical Weight of Nanosponge/Theoretical Weight} \times 100 \quad (2)$$

Physical and chemical properties. Fourier transform infrared (FT-IR) (Thermo Fisher Scientific Co., Ltd, Waltham, MA, USA). The FT-IR spectra of drug i.e. Ceftriaxone and excipient i.e. EC and formulated nanosponge was carried out by KBr technique using Thermo Scientific FT-IR instrument. Scanning electron microscopy (SEM), JSM 840A (JEOL Ltd., Tokyo, Japan). Through scanning electron microscopy, high-resolution images are generated and basic information is collected using electron optics. Microscope samples can be analyzed using this technique and can solve particles from 10–10 to 10–12 grams. The electron beams are used to expose the sample to the vacuum chamber. When the electron beam interacts with the sample, various physical reactions are generated and recorded to create detailed images and analyze the sample elements. The nanosponge formulations were studied using SEM (JSM 840 A) to determine surface characteristics. Before analysis, gold was sprinkled on the samples to add a layer of 25 nm thickness.

Preparation of nanosponges loaded hydrogel. The Carbopol 934, which forms a gel, was prepared after two hours in water. In order to achieve an equal distribution of the solution, a magnetic stirring machine was used to thoroughly mix the solution. After stirring immediately, the mixture was allowed to sit in its shape for 15 min without disturbance to release trapped air. The solution was extensively distributed and thereafter kept in a dark setting for 24 h to guarantee complete swelling of the Carbopol 934. Later, a 5% concentration of propylene glycol was added to the existing aqueous solution. To ensure a consistent distribution of the gel, an extra 50 millilitres of distilled water was added. Ultimately, the nanosponges were incorporated into the Carbopol gel combination. Finally, 2% v/v triethanolamine was gradually added to form the hydrogel [10, 11].

Evaluations of nanosponge loaded hydrogel. Following are some parameters for the evaluation of nanosponge loaded hydrogel:

pH determination. In a 25 millilitres container, 2.5 grams of gel were weighed and combined with 25 millilitres of distilled water. In order to determine the pH level of the blended product, a pH meter was pre-calibrated with buffer solutions set at pH 5.0 and 5.5 [12].

Viscosity measurement. The viscosity of the hydrogel loaded with nanosponges was measured using a Brookfield viscometer equipped with spindle No. 6, operating at various rpm settings, at a temperature of 37 ± 0.5 °C [12].

Skin irritation test. The method employd for skin irritation test was MTT test involves the usage of human skin cells. MTT assay test works on the principle of reduction of MTT reagent (yellow water-soluble tetrazolium dye) by mitochondrial dehydrogenases to form purple coloured formazan crystals. The sample was applied to 10,000 cells which are suspended in 100 μl of media and incubated with 10 μl of MTT reagent for approximately 3 h. The viable cells (metabolically active and alive) will reduce MTT reagent to formazan crystals which will produce purple colour. The resulted formazan crystals were

Table 1 Different formulations of nanosponge

Components	NS1	NS2	NS3
Drug (gm)	1	1	1
Ethyl Cellulose (gm)	2	2.5	3
Polyvinyl Alcohol (gm)	3	2.5	2
Distilled Water (mL)	150	150	150

NS1, nanosponge formulation 1; NS2, nanosponge formulation 2; NS3, nanosponge formulation 3.

solubilised by the addition of DMSO solvent and measured using a spectrophotometer at 570 nm wavelength. The purple-coloured intensity produced is directly proportional to the number of viable (metabolically active and live) cells [13]. As this research utilized secondary data sources, ethical approval was waived.

Drug content studies. Using a volume box containing 20 mL phosphate buffer at pH 5.5, 100 mg gel formula was poured into the box and agitated for 30 min. In particular for formulations containing nanosponges, the mixture was stirred for 30 min and then left without supervision for 24 h. After 24 h of incubation, a total of 100 mL of water was added to the mixture. Subsequently, the prepared solution was diluted with phosphate buffer at pH 5.5 to 50 mL. The absorption was measured using a 254 nm wavelength spectrophotometer [12].

Spreadability. In order to evaluate the extent to which the gel formulation can expand, the width of a 1 g gel sample was measured between two horizontal plates (20 × 5 cm) after a one-minute period of rest. A 100 g weight was applied to the upper plate for a duration of 5 min order to expel any trapped air and create a uniform, thin layer of gel between the plates. Once the weight was removed, any surplus gel at the edges was scraped away. Subsequently, a 20 g mass was meticulously affixed to the top plate. The duration for the top plate (which is capable of movement) to slide a distance of 6 cm and detach from the bottom plate (which remains stationary) due to the force of gravity was measured [14]. The gel's spreadability was subsequently determined using the following Equation:

$$\text{Spreadability} = m \times l/T \quad (3)$$

Where:
m = weight tied to the upper slides;
l = length of glass slide;
t = time taken.

Physical evaluation. The formulated gel underwent a thorough assessment to evaluate its visual aspects, including appearance, color, and scent, as well as its tactile properties when applied.

Drug entrapment efficiency. A quantity of hydrogel containing 100 mg of Ceftriaxone was immersed in 50 mL of distilled water and left to swell for 24 h. After swelling, the hydrogel was ground into a fine paste using an agate mortar and pestle. The resulting homogeneous mixture was then subjected to sonication for 2 min at a frequency of 60 MHz. The polymer mixture was precipitated by adding 50 mL of water. After centrifugation, the supernatant was separated and discarded after centrifugation at 10,000 rpm for 5 min. A UV spectrometer was used to measure the absorption rate at 254 nm to evaluate the concentration of Ceftriaxone in the remaining solution. After determining the efficiency of encapsulation, analytical techniques were applied [15].

$$\%\ EE = (\text{Total amount of drug} - \text{Concentration of drug})/\text{Total amount of drug} \times 100 \quad (4)$$

In-vitro release studies. The permeability of nanosponge hydrogels filled with Ceftriaxone was assessed by employing an artificial cellophane membrane. For this configuration, a quantity of 100 mg of the hydrogel was positioned in the donor compartment. A phosphate buffer solution was used to adjust the pH of the receptor compartment. The solution was continuously stirred with a magnetic bead to ensure thorough mixing. The experiment was conducted at a stable temperature of 37 ± 0.5 ˚C to replicate conditions akin to human skin. Each time a 1 mL sample was extracted from the receptor compartment at a designated time – specifically 0, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h – one mL of the sample was taken. After the replacement of these samples with fresh receptor solution, a constant volume of solution was maintained. To determine the concentration of the drug, spectral photometry was used at a wavelength of 254 nm to analyze the extracted samples. The quantity of the medication that was released was computed, and the proportion of the drug that was released as time progressed was graphed in order to evaluate the release pattern [16].

Ex-vivo permeation studies. The histology and biochemical characteristics of pig skin have been widely studied and found to closely resemble those of human skin [17]. In order to conduct penetration investigations, pig ear skin was acquired from a nearby abattoir. The skin underwent a meticulous cleansing procedure to eliminate hair and subcutaneous fat, followed by a rinse with Ringer's solution. After the skin was dried, it was enclosed in aluminium foil and placed in a polyethylene bag at a temperature of −2 ˚C for storage [18]. The drug release trials were done in vitro using a setup similar to that used in prior research. Permeation studies were conducted using Franz diffusion cells, each having a surface area of 3.14 cm^2. The receptor chamber was filled with a phosphate buffer solution at a pH of 7.2. The experiment was conducted at a temperature of 37 ± 0.5 ˚C, which was controlled by an external water circulator to replicate physiological circumstances. In order to mitigate the impact of boundary layer effects, the receptor media was consistently agitated using a compact magnetic stirrer. In this experiment, 100 mg hydrogel was applied to the skin surface of pigs and placed between the donors and the receptors. Three mL of the receptor solution was removed from the receptor compartment at a specified time interval (1, 2, 3, 4, 5, 6, 7, 8 h) and replaced with fresh receptor solution. The spectrophotometric analysis of these samples was carried out at 254 nm. All formulations were evaluated according to the release profile after 24 h, and the flow after 24 h was recorded [19].

TEM (transmission electron microscopy, FEI Tecnai G2 F20, Eindhoven, Netherland). The structure of the ceftriaxone-loaded nanosponge hydrogel was examined using optical microscopy. Additionally, TEM was employed for further analysis, utilizing the FEI Tecnai G2F20, based in the Netherlands. To prepare the sample, a drop of the diluted formulation was placed on a copper grid coated with a carbon film and subjected to negative staining using a 2% phosphotungstic acid solution. After staining, the sample was air-dried prior to observation under the TEM. The analysis was conducted at an operating voltage of 120 kV.

Determination of particle size and polydispersity index by zeta sizer, Beckmen Coulter Delsa™ Nano Version 3.73/2.30 (Brea, CA, USA). The characterization process for the Ceftriaxone-loaded nanosponge hydrogel includes assessing the particle size and Polydispersity Index using a Zetasizer device (Beckman Coulter Delsa™ Nano Version 3.73/2.30). This analysis was performed at a controlled temperature of 25 ˚C and a scattering angle of 90°, following the proper dilution of the sample with double-distilled water [20].

FT-IR spectral analysis. The FT-IR spectra of Ceftriaxone loaded Nanosponge Hydrogel was carried out by KBr technique using Thermo

Scientific FT-IR instrument.

Anti-microbial study. For this experiment, two types of bacteria were used: *Staphylococcus aureus* (ATCC, Manassas, VA, USA), which is a type of bacteria that stains purple when exposed to a certain dye, and *Staphylococcus aureus*, which is a type of bacteria that stains pink when exposed to the same dye. Clobetason-GM was used as the benchmark. A powdered medium of nutrient agar was reconstituted with distilled water in accordance with a specific formulation for cell growth: 15.0% of agar, 5.0% of animal tissue digest, 5.0% of sodium chloride, 1.5% of beef and 1.5% of yeast, with distilled water adjusted to 1000 milliliters. In this experiment, we fine-tuned the pH of the solution to 7.4 ± 0.2 at 25 °C. A 121 °C autoclave was used under 15 psi pressure to ensure the stability of the medium.

Method employed:

- Cup and Plate method: Sterile nutrient agar, warmed to a temperature between 40 and 50 °C, was meticulously poured into sterile Petri plates, forming a layer that was roughly 3 to 4 mm in thickness. The dishes were positioned on a flat surface to provide uniform dispersion. Once the agar was poured, it was allowed to harden. After the agar had solidified, 0.1 mL of a solution containing the test organism, produced using water meant for injection, was added to each plate. Subsequently, wells were created in the middle of every plate and subsequently filled with the gel. The plates were placed in an incubator set at a temperature of 37 °C for a duration of 24 h. After the incubation time, the diameter of the areas where microbial growth was inhibited surrounding each well was measured to assess the efficiency of the antimicrobial agent [21, 22].
- Preparation of media: Exactly 28 grammes of dried nutritional agar powder were measured and then dissolved in 1,000 millilitres of distilled water, which was held in a conical flask. Subsequently, the concoction was subjected to heating in a water bath to guarantee the thorough dissolution of the medium.
- Sterilization of media: The nutrient agar medium was put in a conical flask and sealed with a non-absorbent cotton stopper. Both the flask mouth and the cotton stopper were tightly sealed with aluminium foil. Afterwards, the medium was sterilised by autoclaving it at a pressure of 15 pounds per square inch for a period of 20 min.

Stability studies. Over a three-month period, different conditions were used to evaluate the stability of the nanosponge-encapsulated hydrogel formulations. These included storage at ambient temperature (30 ± 2 °C), in refrigerated conditions (4 ± 2 °C), and under accelerated stress conditions (40 ± 2 °C with 75% relative humidity) within a humidity chamber. Evaluations of the formulations were conducted at 30, 60, and 90 days to assess visual appearance and pH levels [23].

Result and discussion

Evaluations of nanosponge

Drug content. The drug content of three formulations of nanosponge is shown in Table 2.

The drug content was assessed across all three formulations. Variations in drug content were attributed to differences in the concentrations of the polymer and surfactant used. The formulation NS1 exhibited the highest drug content, which is likely due to the higher concentration of surfactant, enhancing the stability of the formulation.

Drug entrapment efficiency. The efficiency of drug entrapment was assessed for each of the three formulations, with the results detailed in Table 3.

The efficiency of drug entrapment was evaluated for each nanosponge formulation, uncovering significant variations mainly due to differences in polymer concentration and cross-linking levels. Among the formulations, NS1 exhibited the greatest entrapment efficiency. This superior performance is linked to its elevated polyvinyl alcohol content, combined with a comparatively lower amount of hydroxyethyl cellulose.

Percentage yield. The percentage yield of all three formulations is listed in Table 4.

According the obtained values of percentage yield of all three formulations, the second formulation i.e. NS2 has highest percentage yield followed by first formulation i.e. NS1 and lowest percentage yield is of third formulation i.e. NS3.

Physical and chemical properties: FT-IR spectrum analysis. The FT-IR spectroscopy was performed on the most effective nanosponge formulation, specifically the NS1 formulation, and compared with the FT-IR spectra of the drug and the excipients. The FT-IR spectrum of Ceftriaxone (Figure 1) reveals distinctive peaks at 1,219.45 cm^{-1}, which indicate the presence of the C-N functional group (associated with amines and amides), and at 772.53 cm^{-1}, which signifies the presence of the C-H functional group (typical of aromatic rings) as given in Table 5.

The FT-IR spectrum of EC (Figure 2) shows the characteristics peak at 2,932.57 and 2,851.76 confirmed the presence of C-H functional group (Alkanes), characteristics peaks at 1,736.21 and 1,707.60 confirmed the presence of C=O functional group, characteristics peaks at 1,522.60 confirmed the presence of C=C functional group (Aromatic rings), characteristic peaks at 1,219.39 and 1,166.78 confirmed the presence of C-O functional group (Alcohol, esters, carboxylic acid) and characteristic peak at 772.44 confirmed the presence of C-H functional group (Alkenes) as given in Table 6.

The FT-IR spectrum of Ceftriaxone loaded nanosponge (Figure 3) shows the characteristic peak at 3,440.06 confirmed the presence of N-H functional group (Amines and Amides), characteristic peaks at 1,629.54 confirmed the presence of C=C functional group (Alkenes), characteristic peak at 776.21, 772.66 and 768.80 confirmed the presence of C-H functional group (Aromatic and Alkenes) as given in Table 7.

Table 2 Drug content of nanosponge formulations

Formulations	% Drug content
NS1	70.26 ± 0.235
NS2	64.46 ± 0.094
NS3	63.56 ± 0.59

NS1, nanosponge formulation 1; NS2, nanosponge formulation 2; NS3, nanosponge formulation 3.

Table 3 Entrapment efficiency of nanosponge formulations

Formulations	% Entrapment efficiency
NS1	79.03 ± 0.09
NS2	77.3 ± 0.11
NS3	73.6 ± 0.11

NS1, nanosponge formulation 1; NS2, nanosponge formulation 2; NS3, nanosponge formulation 3.

Table 4 Percentage yield of nanosponge formulations

Formulations	Percentage yield (%)
NS1	84.3
NS2	85.1
NS3	77.5

NS1, nanosponge formulation 1; NS2, nanosponge formulation 2; NS3, nanosponge formulation 3.

Table 5 Interpretation of FT-IR of ceftriaxone

Reference peaks	Observed peaks	Functional group
1,180–1,360	1,219.45	C-N (Amides and Amines)
690–900	772.53	C-H (Aromatic Rings)

Table 6 Interpretation of FT-IR of ethyl cellulose

Reference peaks	Observed peaks	Functional group
2,850–2,970	2,932.57	C-H (Alkanes)
2,850–2,970	2,851.76	C-H (Alkanes)
1,690–1,760	1,736.21	C=O (Aldehydes, Ketones, Carboxylic acids, Esters)
1,690–1,760	1,707.60	C=O (Aldehydes, Ketones, Carboxylic acids, Esters)
1,500–1,600	1,522.60	C=C (Aromatic rings)
1,050–1300	1,219.39	C-O (Alcohols, Esters, Carboxylic Acids)
1,050–1,300	1,166.78	C-O (Alcohols, Esters, Carboxylic Acids)
675–995	772.44	C-H (Alkenes)

Table 7 Interpretation of FT-IR of Ceftriaxone loaded nanosponge

Reference peaks	Observed peaks	Functional group
3,300–3,500	3,440.06	N-H (Amines, Amides)
1,610–1,680	1,629.54	C=C (Alkenes)
1,180–1,360	1,219.48	C-N (Amines, Amides)
690–900	776.21	C-H (Aromatic)
690–900	772.66	C-H (Aromatic)
690–900	768.80	C-H (Alkenes)

The FT-IR Spectrum of Ceftriaxone loaded nanosponge shows the characteristic peaks at 772.66, 776.21 and 768.80 corresponding to C-H group which are present in FT-IR spectrum of Ceftriaxone and Ethyl Cellulose defining the presence of Aromatic, Alkanes and Alkenes as shown in Table 7. The FT-IR spectrum of Ceftriaxone loaded nanosponge shows peak at 1,219.48 corresponding to C-N group which is present in FT-IR spectrum of Ceftriaxone at 1,219.45 defining the presence of Amides and Amines. Also, FT-IR spectrum of Ceftriaxone loaded nanosponge shows the peak at 1,629.54 corresponding to C=C which is present in FT-IR spectrum of Ethyl cellulose at 1,522.60 defining the presence of Aromatic Alkenes. It means that the peak of drug and excipient are maintained in the nanosponge formulation, which means that drug was intact in the formulation and did not react with either of the polymer.

The SEM was carried out of the formulation having highest drug entrapment efficiency i.e. NS1 formulation of nanosponge. The nanosponge was evaluated and images are shown as below (Figure 4).

SEM images showed the nanosponges are porous and spherical in shape.

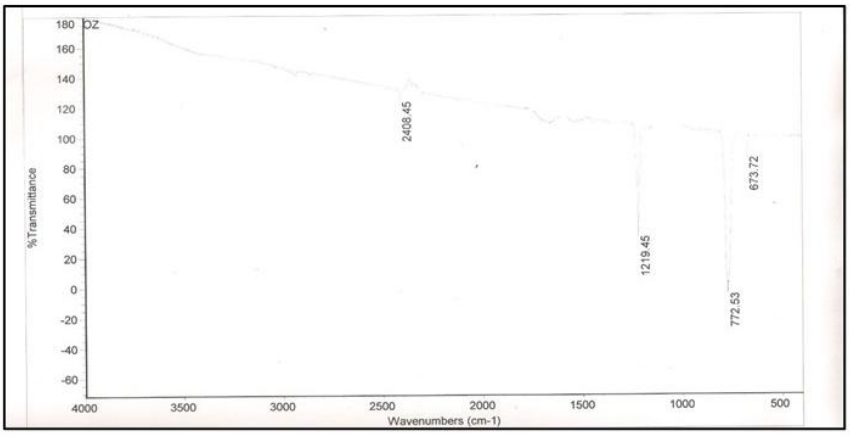

Figure 1 FT-IR spectrum of Ceftriaxone. FT-IR, fourier transform infrared.

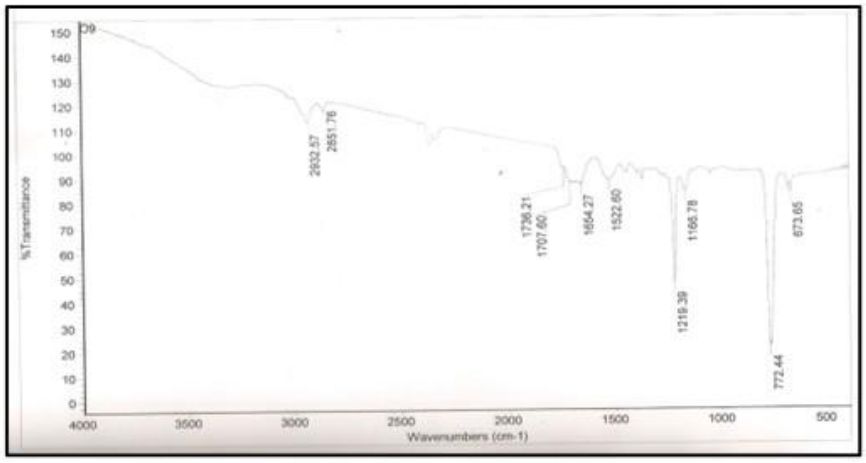

Figure 2 FT-IR spectrum of ethyl cellulose. FT-IR, fourier transform infrared.

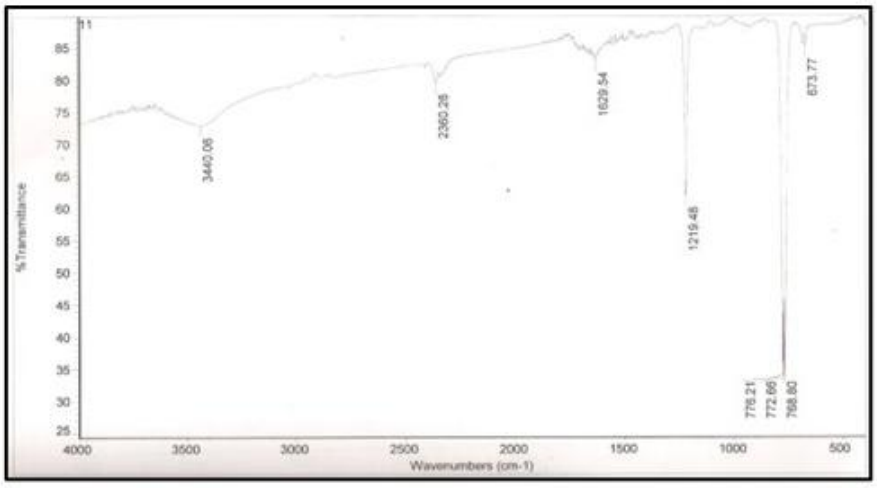

Figure 3 FT-IR spectrum of Ceftriaxone loaded nanosponge. FT-IR, fourier transform infrared.

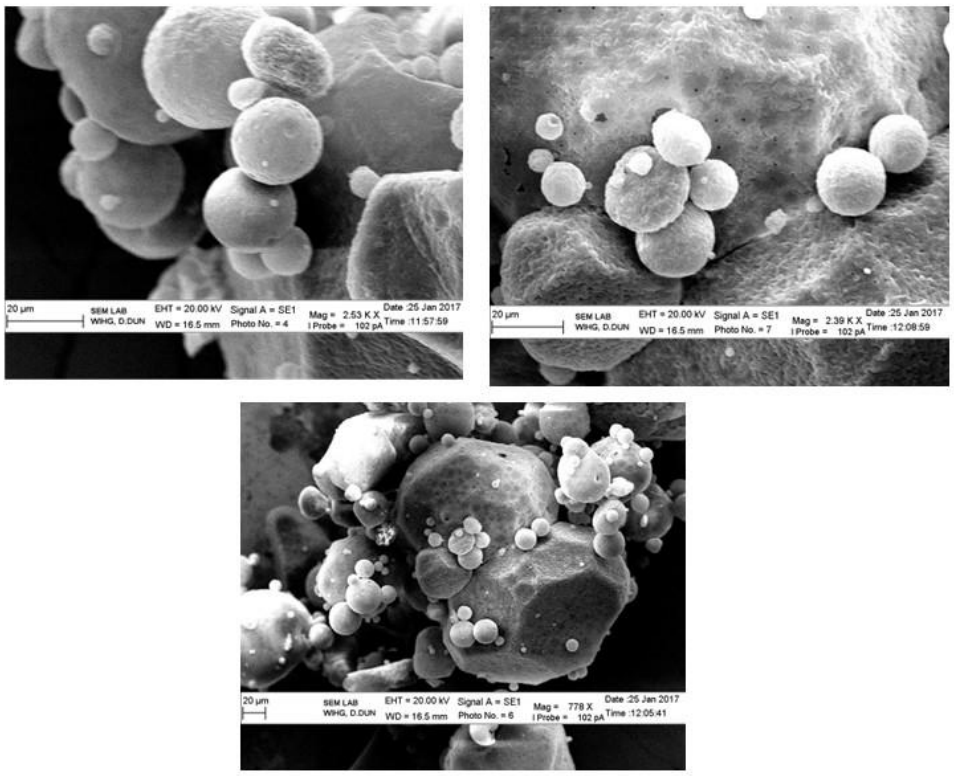

Figure 4 SEM images of Ceftriaxone loaded nanosponge. SEM, scanning electron microscopy.

Preparation of nanosponges loaded hydrogel

The preparation of hydrogels was done using nanosponges that provided the greatest entrapment efficiency. Below are the parameters used to assess the resulting gel after these nanosponges were incorporated into a Carbopol gel matrix.

Evaluations of nanosponge loaded hydrogel

pH determination. The pH of nanosponge loaded hydrogel was determined in triplicate and mean of all three values were taken. The pH of the hydrogel is mentioned in below Table 8.

Table 8 pH value of nanosponge loaded hydrogel

Mean value of pH	5.8 ± 0.008

Viscosity measurement. The viscosity of nanosponge loaded hydrogel was determined at different rpm and mean of all values were taken. The viscosity values are mentioned in below Table 9.

Table 9 Viscosity values of nanosponge loaded hydrogel

Average of viscosity (cp)	8,373.5

Skin irritation test. The method employed was MTT test for NS1 formulation as it shows maximum drug entrapment efficiency and the results are shown in below Table 10.

Drug content studies. The drug content values of nanosponge loaded hydrogel was taken in triplicate and averages of all three values were taken. The average percentage drug content is shown below in Table 11.

Table 11 Percentage drug content of nanosponge loaded hydrogel

Average % drug content	69.96 ± 0.094

Spreadability. The spreadability of nanosponge loaded hydrogel was taken in triplicate and averages of all three values were taken. The spreadability of hydrogel is given in below Table 12.

Table 12 Spreadability of nanosponge loaded hydrogel

Average value of spreadability (g × cm/cm)	80 ± 1.22

Physical evaluation. The nanosponge loaded hydrogel was appeared translucent, smooth feel on application and homogenous and results are listed in below Table 13.

Table 13 Physical evaluation of hydrogel

Appearance	Feel on application	Odour
Whitish and translucent	smooth	none

Drug entrapment efficiency. The drug entrapment efficiency was assessed in triplicate to assure precision, and the percentage of drug entrapment efficiency was determined based on these results. The mean percentage of drug entrapment efficiency was then calculated. The comprehensive findings are displayed in Table 14 below.

Table 14 Drug entrapment efficiency of nanosponge loaded hydrogel

Average % DDE of hydrogel	77.4 ± 0.1

DDE, Degree of Drug Entrapment.

In-vitro release studies. The in-vitro release profile of the nanosponge hydrogel was evaluated at intervals of 1 h, extending up to 6 h, and finally at 24 h. The findings are detailed in Table 15, with the corresponding release percentages illustrated in Figure 5.

From the above graph, it can be concluded that the drug released from Nanosponges gel was characterized by initial burst release in first few hours and later on providing a sustained release profile. The graph also illustrates the concept of a dual-phase release system in which the nanosponge porosity allows for drug loading and retention while the hydrogel swelling regulates the release rate. This technique is appropriate for situations when initial therapeutic levels are

required fast, afterwards adding a maintenance dose over time.

Ex-vivo permeation studies. The ex-vivo release of nanosponge hydrogel was carried out after 1 h of interval up to 6 hours and lastly by 24 h. The results are shown in below Table 16 and the graph representing the percentage release is shown in below Figure 6.

From the above graph, it can be concluded that the drug released from nanosponges gel was characterized by initial burst release in first few hours and later on providing a sustained release profile. But the amount of release after 24 h was less than that of the in-vitro drug release profile.

Transmission electron microscopy. The morphology was shown by optical microscopy and by Transmission Electron Microscopy. The prepared slide then analysed under digital microscope under 40X magnification as shown in Figure 7. The TEM image of Nanosponge loaded Hydrogel is shown in Figure 8.

The TEM image of nanosponge loaded hydrogel justified that the particles in the hydrogel were in nanometric size range and were nearly spherical in shape.

Determination of particle size and polydispersity index by zeta sizer. At a temperature of 25 °C, the mean particle size and the polydispersity index of the hydrogel formulation containing nanosponges were evaluated using an analytical measurement system. The average particle size of the hydrogel was determined to be 289.2 nm, while the polydispersity index (P. I.) was measured at 0.279, as illustrated in Figure 9.

Table 10 MTT assay

Compound ID	Concentration (µm)	Absorbance at 570 nm			% Cell death			Mean % cell death	IC50 (µm)	% Cell Viability			Mean % cell viability	SEM
NS1	25	0.116	0.211	0.226	54.3	16.9	11.0	27.4	No cytotoxicity	45.7	83.1	89.0	72.6	13.6
	50	0.183	0.21	0.208	28.0	17.3	18.1	21.1		72.0	82.7	81.9	78.9	3.4
	100	0.178	0.151	0.192	29.9	40.6	24.4	31.6		70.1	59.4	75.6	68.4	4.7
	200	0.218	0.236	0.237	14.2	7.1	6.7	9.3		85.8	92.9	93.3	90.7	2.4
Standard	0.005	0.218	0.222	0.201	14.2	12.6	20.9	15.9	1.817034502	85.8	87.4	79.1	84.1	2.5
	0.5	0.12	0.155	0.207	52.8	39.0	18.5	36.7		47.2	61.0	81.5	63.3	10.0
	5	0.105	0.121	0.127	58.7	52.4	50.0	53.7		41.3	47.6	50.0	46.3	2.6
	50	0.047	0.083	0.056	81.5	67.3	78.0	75.6		18.5	32.7	22.0	24.4	4.3

Table 15 In-vitro release profile of nanosponge loaded hydrogel

Time (h)	% drug release
1	3.21 ± 0.18
2	7.35 ± 0.008
3	12.38 ± 0.008
4	24.34 ± 0.01
5	40.38 ± 0.03
6	62.56 ± 0.03
24	88.85 ± 0.01

Table 16 Ex-vivo release of nanosponge loaded hydrogel

Time (h)	% drug release
1	2.88 ± 0.004
2	6.82 ± 0.003
3	11.78 ± 0.003
4	21.01 ± 0.02
5	33.99 ± 0.03
6	54.29 ± 0.09
24	77.15 ± 0.03

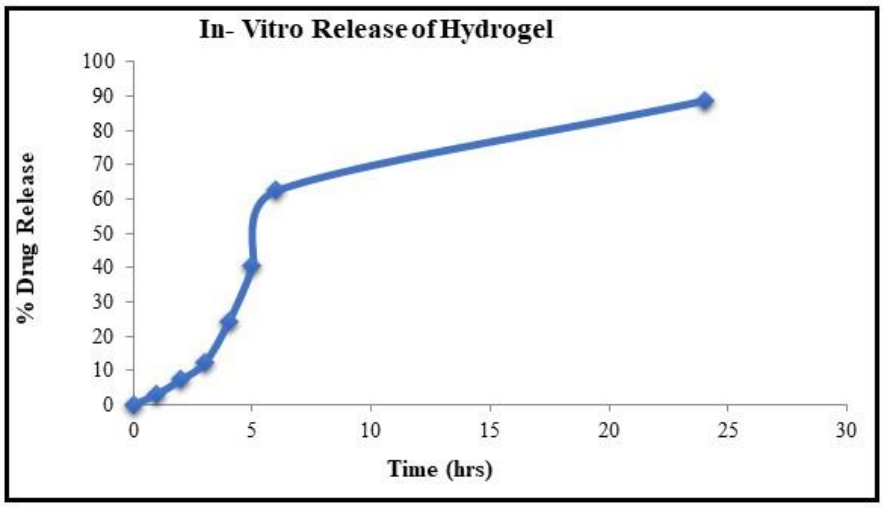

Figure 5 In-vitro release profile of nanosponge loaded hydrogel

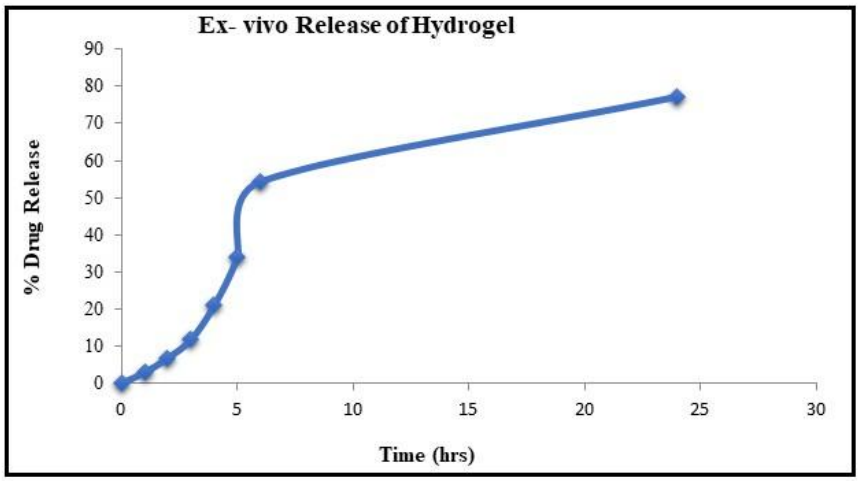

Figure 6 Ex-vivo release profile of nanosponge loaded hydrogel

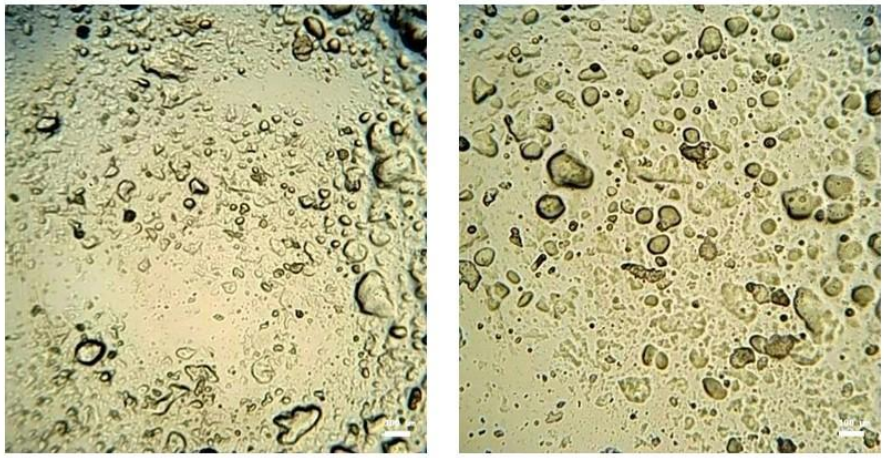

Figure 7 Optical microscopy of nanosponge loaded hydrogel

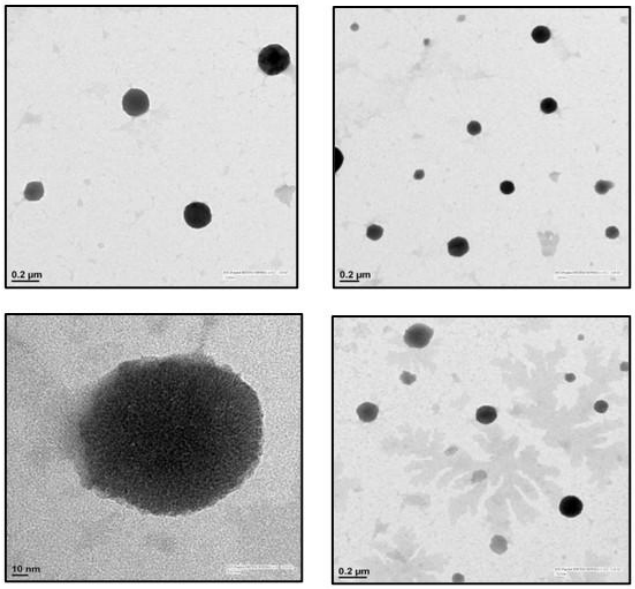

Figure 8 TEM images of nanosponge loaded hydrogel. TEM, Transmission Electron Microscopy.

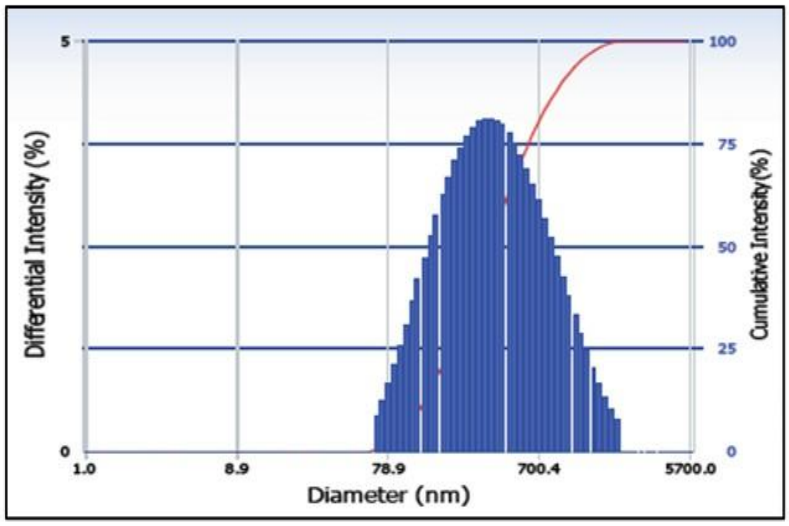

Figure 9 Particle size determination by zeta sizer

FT-IR spectral analysis. The FT-IR spectrum of nanosponge loaded Hydrogel was carried out and was compared with FT-IR spectrum of Carbopol 934 and Ceftriaxone loaded Nanosponge. The FT-IR spectrum of Carbopol 934 (Figure 10) shows the characteristic peaks at 2,917.10, 1,371.05 and 2,849.39 and confirmed the presence of C-H functional group (Alkanes), characteristic peak at 1,738.03 confirmed the presence of C=O (Aldehyde, Ketone and Carboxylic acids), characteristics peak at 1,237.78 confirmed the presence of C-N functional group (Amides) as given in Table 17. The FT-IR spectrum of Ceftriaxone loaded nanosponge (Figure 11) shows the characteristic peak at 3,440.06 confirmed the presence of N-H functional group (Amines and Amides), characteristic peaks at 1,629.54 confirmed the presence of C=C functional group (Alkenes), characteristic peak at 776.21, 772.66 and 768.80 confirmed the presence of C-H functional group (Aromatic and Alkenes) as given in Table 18. The FT-IR Spectrum of nanosponge loaded Hydrogel (Figure 12) shows the characteristics peak at 3,279.04 confirmed the presence of H-bonded alcohols (phenols), characteristic peak at 2,924.77 and 1,364.36 confirmed the presence of C-H functional group (Alkanes), characteristic peak at 1,612.07 confirmed the presence of N-H functional group (Amines, Amides), characteristic peak at 1,239.92 confirmed the presence of C-N functional group (Amines, Amides), characteristic peak at 759.59 confirmed the presence of C-H functional group (Alkenes) as given in Table 19.

The FT-IR Spectrum of Nanosponge loaded Hydrogel shows the characteristic peak at 2,924.77, 1,364.36 corresponding to C-H group which are present in FT-IR Spectrum of Carbopol 934 defining the presence of Alkanes. The FT-IR spectrum of the hydrogel loaded with nanosponges reveals a distinct peak at 759.59 cm^{-1}, which is indicative of the C-H group, a feature also observed in the FT-IR spectra of both Carbopol and Ceftriaxone-loaded nanosponges, signifying the presence of alkenes. Additionally, the spectrum for the nanosponge-loaded hydrogel displays a prominent peak at 1,239.98 cm^{-1}, corresponding to the C-N group. This peak is similarly detected in the FT-IR spectra of Carbopol and Ceftriaxone-loaded nanosponges, highlighting the presence of amines and amides. It means that the peak of drug and excipient are maintained in the nanosponge formulation, which means that drug was intact in the formulation and did not react with either of the polymer.

Anti-microbial study. The anti-microbial study was carried out for nanosponge loaded hydrogel and Clobetacon which is taken as standard (in triplicate). The results involve evaluation and measurement of zone of inhibition of hydrogel and standard against gram positive and gram-negative bacteria. The zone of inhibition

against Staphylococcus aureus (gram positive) is shown in Figure 13 and zone of inhibition against Staphylococcus aureus (gram negative) is shown in Figure 14. The diameter of zone of inhibition is shown in Table 20.

According to the measurement of zone of inhibition of both hydrogel and standard, it can be concluded that prepared gel has approximately same zone of inhibition as that of standard. Consequently, the formulated product demonstrates effective antimicrobial properties against both Gram-positive and Gram-negative bacterial strains.

Stability Studies. Stability assessments were carried out to evaluate the formulation's resilience against fluctuations in temperature. Over a period of three months, the formulation's stability was evaluated by monitoring any alterations in its appearance and pH levels. Initially, the formulation remained stable for up to two months, maintaining consistent appearance and pH. However, after three months, noticeable changes in both appearance and pH were observed in samples stored under accelerated and room temperature conditions. The specific changes in appearance for the formulation after three months under refrigerated, room, and accelerated conditions are illustrated in Figure 15 and detailed in Table 21. The variations in pH levels after three months under these conditions are documented in Table 22.

Based on the observations noted, it can be concluded that the formulation exhibited the highest stability when stored under refrigerated conditions, in contrast to room temperature or accelerated conditions. Therefore, refrigeration is deemed the most suitable condition for the storage of this formulation.

Table 17 Interpretation of FT-IR spectrum of carbopol 934

Reference peaks	Observed peaks	Functional group
2,850–2,970	2,917.10	C-H (Alkanes)
2,850–2,970	2,849.39	C-H (Alkanes)
1,690–1,760	1,738.03	C=O (Aldehyde, Ketone, Carboxylic Acids)
1,340–1,470	1,371.05	C-H (Alkanes)
1,150–1,366	1,237.78	C-N (Amines)
675–995	719.61	C-H (Alkenes)

FT-IR, fourier transform infrared.

Table 18 Interpretation of FT-IR of Ceftriaxone loaded nanosponge

Reference peaks	Observed peaks	Functional group
3,300–3,500	3,440.06	N-H (Amines, Amides)
1,610–1,680	1,629.54	C=C (Alkenes)
1,180–1,360	1,219.48	C-N (Amines, Amides)
690–900	776.21	C-H (Aromatic)
690–900	772.66	C-H (Aromatic)
690–900	768.80	C-H (Alkenes)

FT-IR, fourier transform infrared.

Table 19 Interpretation of FT-IR spectrum of nanosponge loaded hydrogel

Reference peaks	Observed peaks	Functional group
3,200–3,600	3,279.04	H-bonded Alcohols (Phenols)
2,850–2,970	2,924.77	C-H (Alkanes)
1,610–1,680	1,612.07	N-H (Amines, Amides)
1,340–1,470	1,364.36	C-H (Alkanes)
1,180–1,360	1,239.98	C-N (Amines, Amides)
675–995	759.59	C-H (Alkenes)

FT-IR, fourier transform infrared.

Table 20 Measurement of zone of inhibition (cm)

Formulation applied	Staphylococcus aureus	Mean	Staphylococcus aureus	Mean
Nanosponge loaded hydrogel	2.5, 2.4, 2.4	2.4	2.5, 2.6, 2.5	2.5
Clobetacon	2.4, 2.5, 2.4	2.4	2.4, 2.5, 2.5	2.4

Table 21 Effect of temperature on stability (physical appearance)

Temperature	Physical appearance		
	After 1 month	After 2 months	After 3 months
Room temperature	No Change	No Change	Blackish Pigment Appeared
Refrigerated temperature	No Change	No Change	No Change
Accelerated temperature	No Change	No Change	Blackish Pigment Appeared

Table 22 Effect of temperature on stability (pH)

Temperature	pH of formulation		
	After 1 month	After 2 months	After 3 months
Room temperature	5.8	5.82	5.84
Refrigerated temperature	5.8	5.8	5.8
Accelerated temperature	5.8	5.85	5.99

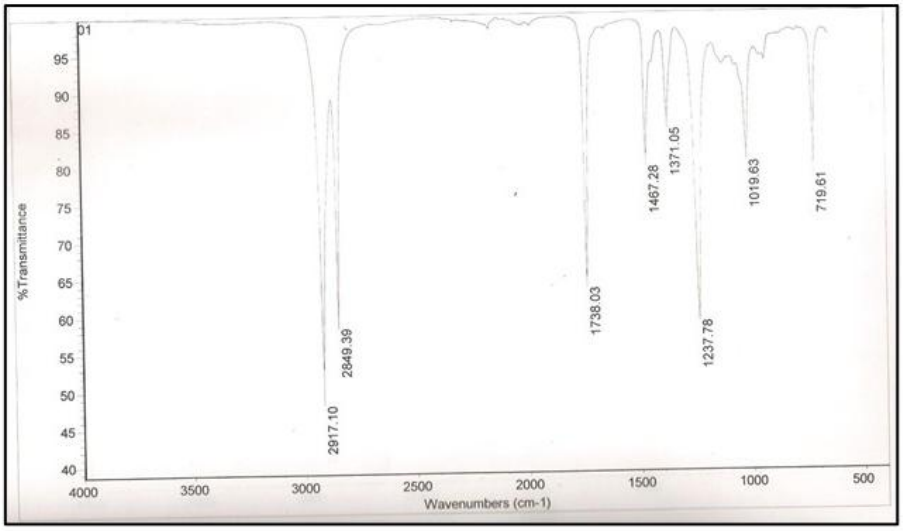

Figure 10 FT-IR spectrum of carbopol 934. FT-IR, fourier transform infrared.

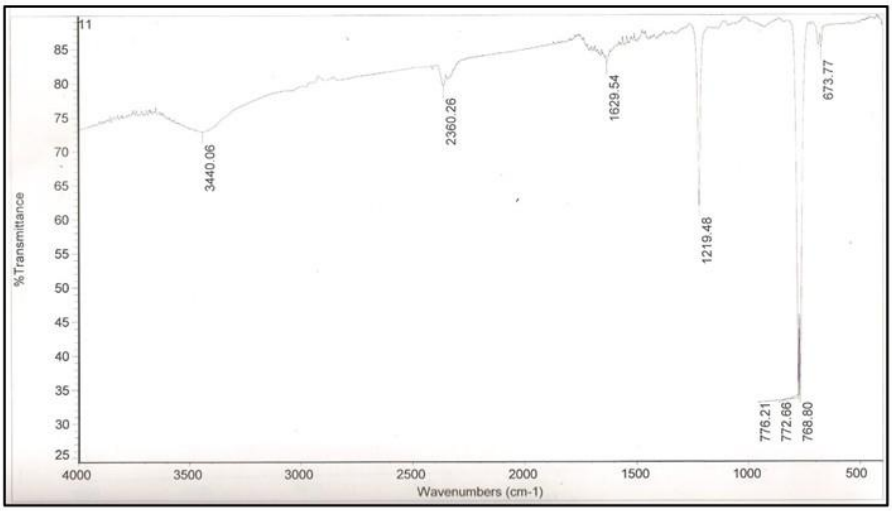

Figure 11 FT-IR spectrum of Ceftriaxone loaded nanosponge. FT-IR, fourier transform infrared.

Biomedical Engineering Communications 2025;4(3):19. https://doi.org/10.53388/BMEC2025019

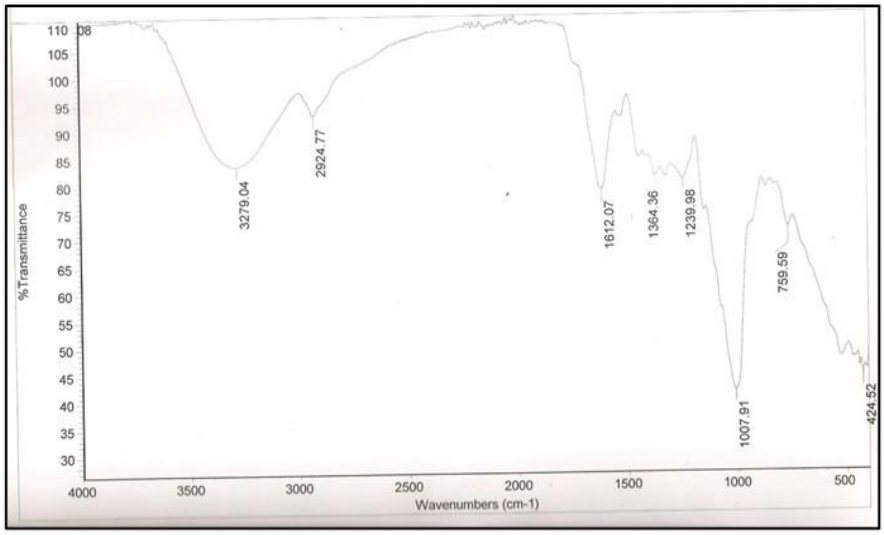

Figure 12 FT-IR spectrum of nanosponge loaded hydrogel. FT-IR, fourier transform infrared.

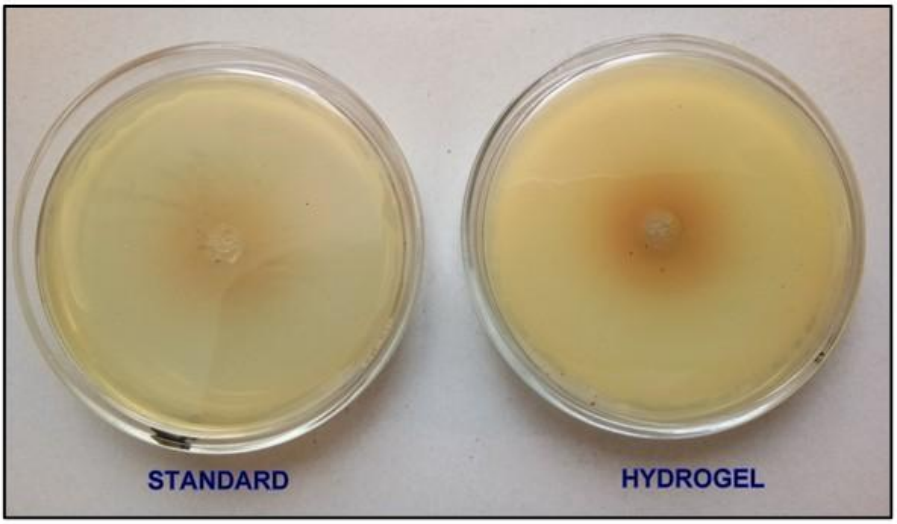

Figure 13 Zone of inhibition against Staphylococcus aureus

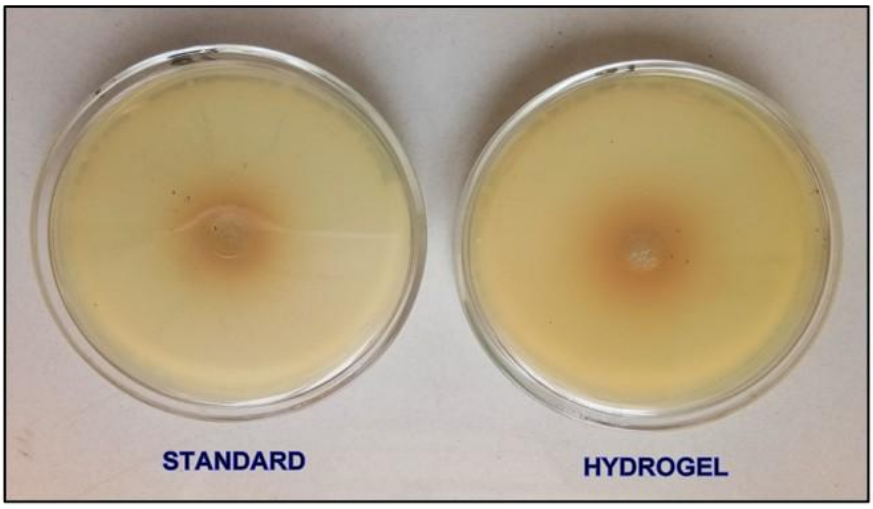

Figure 14 Zone of inhibition against Staphylococcus aureus

Figure 15 Changes in physical appearance after 3 months. F, Freeze conditions; R, Room Conditions; H, High Temperature conditions.

Conclusion

In this research, Ceftriaxone was embedded within nanosponges, and among the different formulations tested, the NS1 batch emerged as the most efficient. This particular formulation exhibited the highest drug load at 70.26% and achieved the greatest entrapment efficiency, reaching 79.03%. SEM analysis of the NS1 batch revealed that the nanosponges are both spherical and porous. Consequently, the NS1 formulation was chosen for integration into Carbopol to develop a nanosponge-infused hydrogel. The hydrogel was subjected to a skin irritation assessment, which indicated minimal irritation and confirmed its compatibility with the skin. Furthermore, antimicrobial evaluations demonstrated that the formulation was highly effective against both Gram-positive and Gram-negative bacteria. Drug release studies, conducted in both in vitro and ex vivo environments, showed an initial burst release followed by a controlled release phase. The gel formulation exhibited a more sustained release, enhancing its effectiveness for delivering targeted and prolonged treatment for skin infections.

References

1. Shivani S, Poladi K. Nanosponges-novel emerging drug delivery system: A review. *Int J Psychol Relig.* 2015;6(2):529–540. Available at: https://ijpsr.com/bft-article/nanosponges-novel-emerging-drug-delivery-system-a-review/
2. Liang L, Liu DP, Liang CC. Optimizing the delivery systems of chimeric RNA.DNA oligonucleotides. *Eur J Biochem.* 2002;269(23):5753–5758. Available at: http://doi.org/10.1046/j.1432-1033.2002.03299.x
3. Embil K, Nacht S. The Microsponge® Delivery System (MDS): a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for the release of actives. *J Microencapsulation.* 1996;13(5):575–588. Available at: http://doi.org/10.3109/02652049609026042
4. S S, S A, Krishnamoorthy K, Rajappan M. Nanosponges: A Novel Class of Drug Delivery System – Review. *J Pharm Pharm Sci.* 2012;15(1):103–111. Available at: http://doi.org/10.18433/J3K308
5. Beckenbach L, Baron JM, Merk HF, Löffler H, Amann PM. Retinoid treatment of skin diseases. *Eur J Dermatol.* 2015;25(5):384–391. Available at: http://doi.org/10.1684/ejd.2015.2544
6. Amrutiya N, Bajaj A, Madan M. Development of Microsponges for Topical Delivery of Mupirocin. *AAPS PharmSciTech.* 2009;10(2):402–409. Available at: http://doi.org/10.1208/s12249-009-9220-7
7. Jelvehgari M, Siahi-Shadbad MR, Azarmi S, Martin GP, Nokhodchi A. The microsponge delivery system of benzoyl peroxide: Preparation, characterization and release studies. *Int J Pharm.* 2006;308(1–2):124–132. Available at: http://doi.org/10.1016/j.ijpharm.2005.11.001
8. Bothiraja C, Gholap AD, Shaikh KS, Pawar AP. Investigation of Ethyl Cellulose Microsponge Gel for Topical Delivery of Eberconazole Nitrate for Fungal Therapy. *Ther Deliv.* 2014;5(7):781–794. Available at: http://doi.org/10.4155/tde.14.43
9. Jain V, Singh R. Design and characterization of colon-specific drug delivery system containing paracetamol microsponges. *Arch Pharm Res.* 2011;34(5):733–740. Available at: http://doi.org/10.1007/s12272-011-0506-4
10. Moghimipour E, Salami A, Monjezi M. Formulation and Evaluation of Liposomes for Transdermal Delivery of Celecoxib. *Jundishapur J Nat Pharm Prod.* 2015;10(1):e17653. Available at: http://doi.org/10.17795/jjnpp-17653
11. Shi S, Aggarwal N. Preparation of hydrogels of griseofulvin for dermal application. *Int J Pharm.* 2006;326(1–2):20–24. Available at: http://doi.org/10.1016/j.ijpharm.2006.07.001
12. Dineshmohan S, Gupta VRM. Formulation and in-vitro evaluation of fluconazole loaded microsponge gel for topical sustained delivery. *IOSR-JPBS.* 2015;10(6):15-20. Available at: https://www.iosrjournals.org/iosr-jpbs/papers/Vol10-issue6/Version-3/C010631520.pdf
13. Sagheer R, Gupta A, Luqman S, et, al. Antiproliferative and antioxidant potential of Tridax procumbens extracts against various human cancer cell lines: An insight for medicines from weeds. *J King Saud Univ Sci.* Available at: https://doi.org/10.1016/j.jksus.2024.103474
14. Kesavanarayanan K, Nappinnai M, Ilavarasan R. Topical dosage form of valdecoxib: Preparation and pharmacological evaluation. *Acta Pharm.* 2007;57(2):199–209. Available at: http://doi.org/10.2478/v10007-007-0016-6
15. Agnihotri SA, Jawalkar SS, Aminabhavi TM. Controlled release of cephalexin through gellan gum beads: Effect of formulation parameters on entrapment efficiency, size, and drug release. *Eur*

J Pharm Biopharm. 2006;63(3):249–261. Available at: http://doi.org/10.1016/j.ejpb.2005.12.008

16. Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. *Eur J Pharm Biopharm.* 2000;50(1):27–46. Available at: http://doi.org/10.1016/S0939-6411(00)00090-4
17. Ansari KA, Vavia PR, Trotta F, Cavalli R. Cyclodextrin-Based Nanosponges for Delivery of Resveratrol: In Vitro Characterisation, Stability, Cytotoxicity and Permeation Study. *AAPS PharmSciTech.* 2011;12(1):279–286. Available at: http://doi.org/10.1208/s12249-011-9584-3
18. El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: From drug delivery to model membranes. *Eur J Pharm Sci.* 2008;34(4–5):203–222. Available at: http://doi.org/10.1016/j.ejps.2008.05.002
19. Dragicevic Curic N, Scheglmann D, Albrecht V, Fahr A. Temoporfin-loaded invasomes: Development, characterization and in vitro skin penetration studies. *J Control Release.* 2008;127(1):59–69. Available at: http://doi.org/10.1016/j.jconrel.2007.12.013
20. Kaur K, Nautiyal U, Singh D. Nanostructured lipid carrier for bioavailability enhancement. *IJARST*. 2015;2(1):1–9. Available at: https://www.ijrast.com/index.php/ijrast/article/view/42
21. Gupta VK, Fatima A, Faridi U, et al. Antimicrobial potential of Glycyrrhiza glabra roots. *J Ethnopharmacol.* 2008;116(2):377–380. Available at: http://doi.org/10.1016/j.jep.2007.11.037
22. Sobeh M, Braun MS, Krstin S, Youssef FS, Ashour ML, Wink M. Chemical Profiling of the Essential Oils of Syzygium aqueum, Syzygium samarangense and Eugenia uniflora and Their Discrimination Using Chemometric Analysis. *Chem Biodivers.* 2016;13(11):1537–1550. Available at: http://doi.org/10.1002/cbdv.201600089
23. Roy N, Saha N, Saha P. Stability Study of Novel Medicated Hydrogel Wound Dressings. *Int J Polym Mater Polym Biomater.* 2013;62(3):150–156. Available at: http://doi.org/10.1080/00914037.2011.641697