RESEARCH PAPER

Gallic Acid–Loaded Nanosponge Hydrogel for Targeted Topical Delivery in Seborrheic Dermatitis: Formulation, Characterization and Evaluation

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ABSTRACT

Background
Seborrheic dermatitis (SD) is a chronic inflammatory skin disorder requiring prolonged topical therapy. Conventional formulations often suffer from poor retention, inadequate penetration, and dose-related irritation. Gallic acid, a natural polyphenolic compound with antioxidant and anti-inflammatory properties, presents therapeutic potential; however, its clinical utility is limited by instability and rapid release.

Objective
To develop and evaluate a gallic acid-loaded nanosponge-based hydrogel for controlled topical delivery aimed at improving therapeutic efficacy and patient compliance in seborrheic dermatitis.

Methods
Gallic acid-loaded nanosponges were prepared using the quasi-emulsion solvent diffusion method and incorporated into a hydrogel base. The formulation was evaluated for physicochemical properties including viscosity, pH, spreadability, extrudability, and drug content. In vitro drug release studies were performed over 12 hours. Stability studies were conducted under different temperature and humidity conditions (25°C/60% RH, 30°C/65% RH, and 40°C/75% RH).

Results
The optimized formulation (B4) exhibited a viscosity of 13,453 ± 209 cP, indicating suitable consistency for topical application. The pH range (5.0–5.5) was compatible with skin physiology, minimizing irritation risk. The gel showed good spreadability and extrudability (80–85%), ensuring ease of application. Drug content was found to be 97.38%, indicating uniform drug distribution. The in vitro release profile demonstrated a controlled and sustained drug release over 12 hours, with an initial slow release phase (6.77–22.02% within 0.5–2 h), followed by a moderate release phase (22.02–56.99% within 2–6 h), and a sustained release reaching 82.94% at 12 hours. This biphasic release pattern suggests diffusion-controlled drug release from the nanosponge-hydrogel system. Stability studies revealed no significant changes in physical parameters, including clarity, color, odor, pH, viscosity, spreadability, and extrudability under all tested conditions, confirming formulation stability.

Conclusion
The developed gallic acid-loaded nanosponge hydrogel demonstrated desirable physicochemical properties, high drug content, controlled release behavior, and excellent stability, making it a promising topical delivery system for seborrheic dermatitis. The sustained release profile and stability of the formulation may enhance therapeutic efficacy, reduce dosing frequency, and improve patient compliance.

Keywords: Gallic acid, Nanosponges, Hydrogel, Seborrheic dermatitis, Controlled release, Stability studies.
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1) INTRODUCTION

Seborrheic dermatitis (SD) is a common chronic inflammatory skin disorder that most commonly affects young adults, and less often children [1,2]. In adolescents and adults, SD clinical presentation may range from mild scalp scaling to diffuse white or yellowish patches in regions rich in sebaceous glands, such as the scalp, face, and trunk [3]. In infants, SD mainly occurs on the scalp as yellowish, scaly patches, with varying degrees of inflammation, configuring the so-called “cradle cap. Although it can be associated with human immunodeficiency virus infection and neurologic diseases (e.g., cerebrovascular event, Parkinson's disease), seborrheic dermatitis typically occurs in healthy individuals. Its prevalence ranges from 1% to 3% in the general population and from 34% to 83% in immunocompromised individuals [4-7]. It has a bimodal distribution, with peaks at two to 12

months of age and in adolescence and early adulthood. It is more common in men and is typically more severe in cold and dry climates and during periods of increased stress[8,9].

Current treatment of the seborrheic dermatitis mainly involves Topical Antifungals, Topical Corticosteroids, and Topical Calcineurin Inhibitors or solid (e.g. tablets, suppositories) systems. However, these present drawbacks can impair user acceptability and adherence to treatment, including the inability to provide a permanent cure, risk of adverse effects from long-term medication use, and high rates of relapse [10-12]. Thus, Phytoconstituents especially emerge as the potential candidate for the management of Seborrheic Dermatitis. Gallic acid, a naturally occurring polyphenol, exhibits potent antioxidant, anti-inflammatory, antimicrobial, and keratolytic properties, making it a promising candidate for the management of seborrheic dermatitis[13,14]. These characteristics make gallic acid a promising candidate for the management of inflammatory skin disorders like seborrheic dermatitis.

Gallic acid has a number of advantages over the currently existing drugs, such as reduced chances of adverse effects, natural source, and multi-targeting of oxidative stress and microbial growth. In comparison to corticosteroids, it does not result in skin thinning or hormonal imbalance, and, in comparison to synthetic antifungal agents, it can possibly decrease the possibility of resistance development. Despite its therapeutic potential, the clinical application of gallic acid is often hindered by its rapid degradation and poor skin permeability which reduces its therapeutic efficacy when applied in conventional formulations [15-18]. To overcome these biopharmaceutical challenges, **nanosponges** have emerged as a powerful delivery platform, offering a high load-bearing capacity and a controlled release profile that can enhance the localised efficacy of Gallic acid at the site of seborrheic lesions [19]. Nanosponges are nanosized porous carriers, which can entrap both lipophilic and hydrophilic drugs, enhancing their stability, controlled release and skin penetration. They offer topical delivery, reduce systemic absorption and improve treatment outcomes [20,21].

1.1) Rationale of the Study

Since Seborrheic dermatitis is a chronic inflammatory skin condition that requires safe and effective long-term therapy, Conventional topical formulations of antioxidants and anti-inflammatory agents, such as gallic acid, are limited by poor solubility, low stability, rapid degradation, and inadequate skin penetration. These drawbacks reduce therapeutic efficacy and necessitate frequent application, which may lead to poor patient compliance. Also, its clinical utility in topical therapy is limited by its hydrophilicity, instability under physiological and environmental conditions, and poor skin retention. To overcome these limitations, we have developed nanosponges—porous, polymeric, nanosized carriers that offer a novel approach to encapsulate gallic acid, enhancing its stability, controlled release, and skin permeability while minimising systemic exposure and irritation. Incorporating gallic acid-loaded nanosponges into topical therapeutic systems (such as gels) can provide sustained, localised drug delivery, ensuring prolonged therapeutic action at the target site.

2) MATERIALS AND METHODOLOGY

2.1) Materials

Gallic acid was obtained from Orison Pharma. Ethyl Cellulose, Beta-Cyclodextrin, Carbopol 934, Polyvinyl Alcohol, Propyl Paraben, Triethanolamine, and Methyl Paraben were purchased from a local vendor (Vk Chemicals, Ambala)

2.2) Methodology

a) Preparation of Gallic acid-Loaded Nanosponges

Nanosponges were prepared using the Quasi-Emulsion Solvent Diffusion method. Preparation Procedure consisted of 2 stages: Preparation of the organic phase and the aqueous phase. For The Preparation of the organic phase, 100 mg of ethyl cellulose, along with 50mg of Gallic Acid, was mixed with 20 ml of methanol. In the Aqueous Phase, sufficient amounts of PVA along with the ß-Cylodextrin was mixed with 100 ml of Distilled water. The subsequent stage included slowly introducing the dispersed phase into the aqueous phase using a dropwise addition, all the while maintaining stirring with a magnetic stirrer. The mixture was further homogenised at 1000 RPM for 2 hours. The resultant nanosponges were gathered using filtration and subsequently subjected to drying in a hot air oven at a temperature of 40°C for 24 hours. To eliminate any remaining solvent, the nanosponges were placed in a vacuum desiccator. The same technique was used for all the batches, with only the drug-to-polymer ratios varying among them[22-23]. Different concentrations of gallic acid nanosponges are shown in Table 1.

b) Preparation of Gallic acid Nanosponge-Loaded Hydrogel

Hydrogel was prepared by soaking the various concentrations (0.25%, 0.5%, 0.75%) of Carbopol in distilled water. The Carbopol was soaked in the distilled water and left overnight. The soaked carbopol was stirred in the morning until it was mixed uniformly. To this one drop of triethanolamine was added along with methyl

paraben as preservatives to ensure the formation of the gel.

Table no.1 Formulation table of Gallic acid-Loaded Nanosponges

Ingredients	B1	B2	B3	B4	B5
Organic Phase					
Ethyl Cellulose (mg)	250	200	150	100	50
Gallic Acid (mg)	50	50	50	50	50
Methanol (ml)	20	20	20	20	20
Aqueous Phase					
Polyvinyl chloride (% w/v)	0.5	0.5	0.5	0.5	0.5
ß-Cylodextrin (% w/v)	0.5	0.5	0.5	0.5	0.5
Distilled water (ml)	100	100	100	100	100

3) EVALUATION PARAMETERS OF GALLIC ACID -LOADED NANOSPONGES

a) Morphology

Transmission Electron Microscopy (TEM): The characteristics, such as the form and shape, of the optimized Gallic acid-loaded nanosponges were analyzed using transmission electron microscopy (TEM) testing. TEM photos were obtained at various magnifications using nanosponges, which were produced and carefully dried to minimize moisture content

b) Average Particle size and zeta potential analysis

At 25°C, the light scattering technique is used to ascertain the average diameter and size distribution of the loaded nanosponges. To generate the light distribution intensity needed for the Tacrolimus nanosponges, dried nanosponges are first dispersed in water [24].

The zeta potential is determined based on electrophoretic mobility. Zeta potential plays a pivotal role in assessing the surface charge and the nanosponge's stability. Zeta potential, also known as 'electrophoretic mobility', provides valuable information about the surface charge of nanoparticles. Its significance lies in understanding the nanosponge's stability.

c) Actual drug content

For one hour, an accurately measured amount of drug-loaded nano sponges was held in a 100-ml volume of phosphate buffer at pH 6.8. The solution was constantly stirred during this time to ensure uniform mixing. Subsequently, the solution was examined using a UV-Vis Spectrophotometer. Here; where: Nact – Actual drug content of nanosponges, Nms – Weight of nanosponges[25].

d) **% Drug Loading (DL) & % Encapsulation Efficiency (EE):** (%) Drug loading evaluation is critical for calculating the amount of gallic acid incorporated into a nanosponge to its total weight was determined using the mini-column centrifugation method with Sephadex G-50 [18].Briefly, Sephadex G-50 was hydrated overnight in a phosphate buffer (pH 7.4) and packed into mini-columns. One milliliter of nanosponge dispersion was carefully applied to the column and centrifuged at 3000 rpm for 10 minutes. The eluted fraction containing unentrapped drug was collected and analyzed spectrophotometrically at λmax 260 **nm**. Entrapment efficiency and drug loading were calculated using the following equations:

% EE= Entrapped drug/ Total drug added ×100

% Drug Loading= Entrapped drug/Total weight of liposomes ×100

3.1. EVALUATION PARAMETERS OF NANOSPONGE HYDROGEL

a) Organoleptic properties

The hydrogel of Gallic-loaded nanosponges was evaluated to ascertain their Organoleptic properties, including physical, visual, and sensory characteristics such as colour, odour, texture, consistency, and homogeneity.

b) pH

pH of the hydrogel was measured using the digital pH meter (Swastika Electric & Scientific Works, Ambala, Haryana); the electrode was dipped into the hydrogel, and the pH was recorded successfully

c) Spreadability

The spreadability of the formulated hydrogel was assessed. The procedure involved placing the gel between two glass slides in the spreadability apparatus and attaching a weight to the upper glass slide

[26]. Subsequently, the gel was allowed to slide over both slides. To compute the spreadability of the gel, the subsequent formula was employed:

$S = M \cdot L/T$

where: S = Spreadability
M = weight tied to upper slide
L = length of the glass slide
T = Time taken to separate the 2 slides (sec.)

d) Actual Drug Content

Using a UV-visible spectrophotometer (Shimadzu, UV2600), we measured the absorbance at 260 nm of a 5 mL sample extracted from a solution containing 10mg of gel dissolved in 100 mL phosphate buffer at pH 6.8 and further diluted to 10 mL. The purpose of this process was to ascertain the drug content.

e) Washability

Formulations were applied to the skin, and the ease of washing with water was checked manually

f) Viscosity

The viscosity of the gel was checked with the help of the Brookfield viscometer (Brookfield Engineering Laboratories, Massachusetts).

g) Extrudability: To calculate the extrudability of gel, 20g of gel placed in a closed collapsible tube was pressed at the curvy end firmly and a clamp was applied to ensure that it did not roll back. The tube cap was taken off and the gel was extruded. The quantity of the gel that was extruded was measured and weighed

h) *In-Vitro* Drug Permeation Study

In the in vitro diffusion study, a hydrogel loaded with nanosponge was tested using the Franz diffusion cell apparatus. A cellophane membrane separated the donor compartment (gel side) from the receptor compartment. The receiving compartment was filled with a pH 6.8 phosphate buffer, and a consistent temperature of 37±0.5°C was maintained using a magnetic stirrer. To begin the experiment, 0.1 g of the hydrogel was put on the cellophane membrane. Over a time interval of 8 hours, a 1 ml sample was taken out of the receptor compartment and adequately diluted. To maintain the experimental conditions, at regular intervals, a sample was removed and replaced with an equal amount of pH 6.8 phosphate buffer. The diluted sample was analyzed for the concentration of TAC (Gallic Acid) using a UV-visible spectrophotometer at a wavelength of 293 nm [25].

i) Stability Study

Stability studies of the gel were carried out at different times, that is, on the 0th, 30th, and 90th days. At these time intervals, all the evaluation studies were performed to check the stability of the formulation.

4 RESULTS AND DISCUSSION

4.1) Evaluation Parameters of Gallic acid-Loaded Nanosponges

a) Fourier- Transform Infrared Spectroscopic Studies

The FT-IR Spectrum studies were used to analyze the drug purity and drug: polymer interaction studies. The recorded results are presented in Figure 1 and Table 2; Figure 2 and Table 3 respectively.

Fourier transform infrared (FTIR) spectroscopy was used to study the molecular interactions and compatibility of gallic acid with excipients of the nanosponge gel.

Gallic acid showed distinct peaks in its FTIR spectrum. The strong and broad band at ~3418 cm^{-1} is due to the stretching vibration of phenolic O-H groups, suggesting strong intermolecular hydrogen bonding. A strong and sharp band observed at ~1704 cm^{-1} is assigned to the C=O stretching of the carboxylic acid group. The bands observed at ~1612 cm^{-1} and ~1534 cm^{-1} are due to the aromatic C=C stretching. Moreover, bands at ~1315-1210 cm-1 were attributed to C-O stretching of the phenolic and carboxylic groups [27]..

The FTIR spectrum of the gallic acid-loaded nanosponge gel also showed all the major bands of gallic acid at similar wavenumbers with a marginal shift and decrease in intensity. Significant broadening and a slight shift of the O-H stretching band to ~3390 cm^{-1} was observed, which can be attributed to increased hydrogen bonding between gallic acid and the hydroxyl-rich region of β-cyclodextrin, and possibly with residual hydroxyl groups of polyvinyl alcohol (PVA). The C=O stretching band of gallic acid was present at ~1700 cm^{-1} with little shift, indicating preservation of the drug's carbonyl group and no degradation.

Slight shifts in aromatic C=C stretching vibrations (e.g., from 1612 to ~1605 cm^{-1}) and reduced peak intensity were observed, which may be due to dispersion and entrapment of gallic acid within the nanosponge. The ether vibrations of ethyl cellulose (~1100-1060 cm^{-1}) also overlapped with the C-O stretching band, confirming the formation of a polymer network.

Majorly, no new bands and/or loss of primary peaks were observed, suggesting no covalent chemical interaction between gallic acid and other components in the formulation. Thus, the changes in the spectrum reflect non-covalent interactions (mostly hydrogen bonding and van der Waals) and

efficient physical entrapment of the drug in the nanosponge structure.

Hence, FTIR data demonstrate good compatibility, stability of drug, and efficient entrapment into the nanosponge gel system without significant chemical interactions.

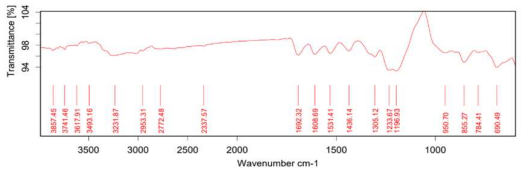

Figure 1 FTIR Spectra of Gallic Acid

Table 2: Gallic Acid FTIR Spectra Analysis

Functional Group	Observed Peak (cm^{-1})	Standard Region (cm^{-1})
O-H/ N-H Stretching	~3350	3200-3600
Aromatic C-H Stretch	~3065	3000-3100
C=O stretching	~1682	1680-1750
C–O–C stretching	~1319	1000-1150

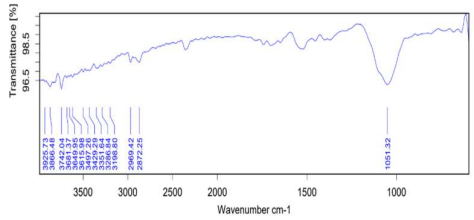

Figure 2 FTIR Spectra of Drug and Polymer Mixture

Table 3 Drug and Polymer Mixture FTIR Spectra Analysis

Functional Group	**Observed Peak (cm^{-1})**	**Standard Region (cm^{-1})**
O-H Stretching	~3350	3200-3600
Free O-H Stretching	~3065	3000-3100
C=O stretching	~1682	1680-1750
C–O–C stretching	~1051	1000-1150

b) Morphology

Transmission Electron Microscopy (TEM) analysis was employed to evaluate the morphology and particle size of the prepared nanosponges.The nanosponge particles exhibited a discrete, well-dispersed, and predominantly spherical in shape, with a uniform appearance and clearly defined boundaries. TEM Results are shown in figure 3. Lack of aggregation suggests that the system is well-stabilized, probably attributed to the steric effect of polyvinyl alcohol. The size of the particles was in the range of 130-150 nm, which suggests the successful formation of nanosponges in the nanoscale. The spherical shape and smooth surface morphology suggest successful incorporation of gallic acid into the polymer matrix, confirming the stability of the nanosponge system [28]. These nanoscale sizes are desirable for improving surface area, drug entrapment and diffusion, which can impact drug release and other performance parameters.

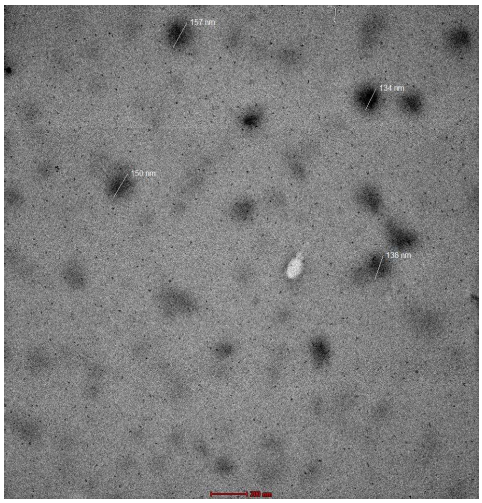

Fig 3 TEM analysis of Nanosponges

c) Average Particle size and Zeta Potential analysis

Particle size analysis is one of the most important characteristics of nanosponges; the mean particle size was found to be 407.2 nm, as illustrated in the figure 4, indicating that the formulation falls within the submicron to nanoscale range. confirming the successful formation of nanosponges. The low polydispersity index indicates a controlled nucleation and growth during the quasi-emulsion solvent diffusion method, leading to formulation uniformity.

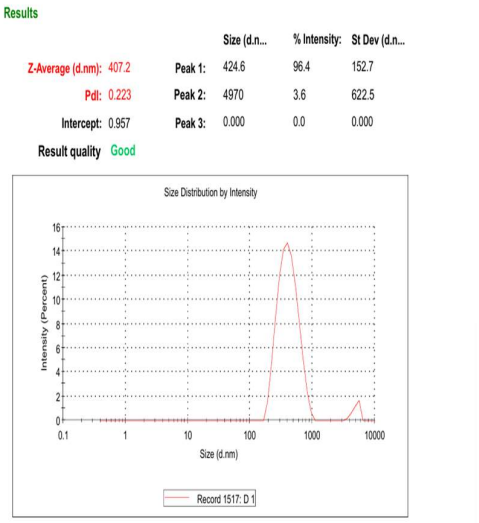

Fig 4 Particle size measurement of Nanoponges

The zeta potential of the nanosponge formulation was found to be –22.1 mV, indicating a moderately stable colloidal system as shown in Figure 5. The negative surface charge can be attributed to the presence of ionizable functional groups of gallic acid and the polymeric components, particularly β-cyclodextrin and residual hydroxyl groups. This level of zeta potential suggests sufficient electrostatic repulsion between particles, which helps prevent aggregation and contributes to physical stability of the nanosponge dispersion [29].

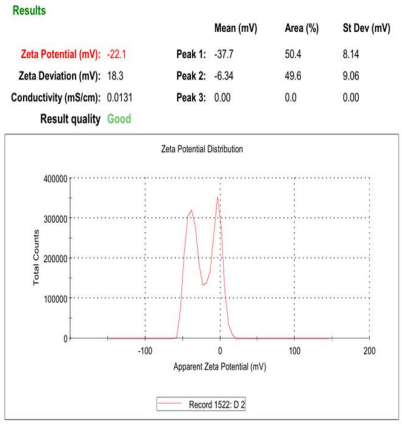

Fig 5 Zeta potential of nanosponge

d) % Drug Loading and % Encapsulation Efficiency (EE)

The entrapment efficiency (EE%) and drug loading (DL%) of nanosponges containing gallic acid were successfully assessed. Table 4 summarizes the results of the preparation of different formulations that showed entrapment efficiency (EE%) between 66- 81% and % DL 15.8-24.6 %. The result indicates efficient drug incorporation and suitability of the quasi-emulsion solvent diffusion method.

Table No 4: Entrapment Efficiency and Drug Loading of gallic loaded nanosponges Formulations

Formulation Code	Entrapment Efficiency (EE%)	Drug Loading (DL%)
B1	66.4 ± 0.2	15.8 ± 1.5
B2	69.6 ± 1.3	18.3 ± 0.9
B3	73.8 ± 0.3	22.1 ± 0.7
B4	81.6 ± 0.9	24.6 ± 1.6
B 5	79.3 ± 1.7	23.2 ± 0.5

4.2) Evaluation of Nanosponge-Loaded Hydrogel

a) **Physical appearance:** Each formulation was found to be homogenous without any colour intensity changes and aggregates.

b) **Physical Properties of Gel:** The viscosity of gel formulations usually reflects its consistency. Viscosity of all the formulations were studied and the viscosity of optimized formulation (B 4) was found to be 13,453± 209 cP. The pH values of all the prepared liposome gel formulations ranged from 5.0 -5.5, which is considered to be suitable for avoiding the risk of irritation upon application to the skin. The spread ability value indicated that the gel was easily spreadable by a small amount of shear. All the formulations showed good extrudability in the range 80% -85%. The percentage of the extruded gel was calculated and the result is reported in table 5.

c) **Drug Content :** The drug content for the Nanosponge gel was found to be 97.38% at 260nm

Table 5: Evaluation Parameters for Hydrogel

Hydrogel	Color	Odor	pH	Spreadability (g·cm/s)	Viscosity (cP)	Extrudability (g/cm²)
B1	Colorless	Odurless	5.3	10.23	9344± 215	81.6 ±26
B2	Col	Odo	5	8.25	11,2	80.9

	orless	urless	.4		30 ± 211	±14
B3	Colorless	Odourless	5.1	7.96	12,980 ± 210	82.3 ±33
B4	Colorless	Odourless	5.2	9.09	13,453± 209	85.3 ±75
B5	Colorless	Odourless	5.3	7.44	10,238± 214	82.3 ±83

d) *In-Vitro* Drug Permeation Study

In vitro drug release profile of the nanosponge loaded hydrogel with gallic acid exhibits a controlled and sustained pattern of release within 12 hours as shown in Table 6 and Figure 6. The release during the first phase (0.5-2 hours) was relatively slower (6.77% to 22.02%), which could be explained by the slow diffusion of the drug through the surface of the nanosponges embedded in the hydrogel matrix. This low starting dose is beneficial since it prevents dose dumping and minimizes the chances of irritation in case of topical application.

There was an increased release between 2-6hours where the drug release ranged between 22.02 %-56.99 %. The stage is probably the diffusion of gallic acid of the porous structure of the nanosponges into the surrounding hydrogel and consequently through the membrane. The nanosponge architecture with its high surface area and porosity is critical in the facilitation of this controlled diffusion. Moreover, the hydrogel matrix helps in controlling the release since it provides a viscous barrier to maintain a constant supply of the drug [30].

Between 6 and 12 hours, the release profile was sustained with 82.94 percent being attained after 12 hours. It means that the formulation can retain long-term levels of available drug at the site of application that is especially important in chronic diseases such as seborrheic dermatitis that require a long-term course of treatment. The lack of a peak release also proves the stability and effectiveness of the nanosponge system to regulate drug delivery.

Comprehensively, the release kinetics indicate that the formulated nanosponge-loaded hydrogel adheres to a controlled diffusion mechanism, which may be consistent with the behavior of matrix-based releases. Nanosponges combined with hydrogel are effective to overcome the constraints of traditional formulations by improving drug retention and sustaining release. The results justify the appropriateness of the developed system as a potential topical delivery system of gallic acid that may enhance the treatment effect and patient adherence.

Table 6: **% Drug Release of nanosponge-loaded hydrogel**

Time (in hrs)	% Cumulative Drug Release
0.5	6.77
1	12.32
2	22.02
4	47.65
6	56.99
8	67.51
10	73.66
12	82.94

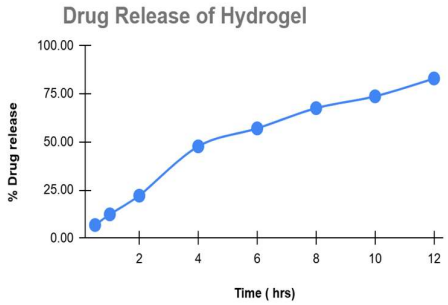

Fig 6: % Drug Release of nanosponge-loaded hydrogel

e) Stability Studies

The evaluation of the hydrogel's physical characteristics under accelerated conditions indicates that the hydrogel displayed a remarkable capacity to uphold its physical attributes. As shown in Table 7. The absence of significant alterations further reinforces the formulation's potential to retain its efficacy. This stability-focused assessment reinforces the notion that the hydrogel maintains its inherent properties, thereby bolstering its suitability for sustained application and therapeutic benefit

Table 7: Stability Studies Observation

	Observation at different temperature and humidity conditions		
Parameters	**250C and 60 % RH**	**Room temperature 300C and 65 % RH**	**Accelerated condition 400C and 75 % RH**

Clarity	Clear	Clear	Clear
Color	off-white	No Change	No change
Odor	Odourless	Odorless	Odorless
pH	5.4	5.32	5.31
Spreadability (g·cm/s)	8.25 cm	8.90	9.1
Viscosity (cP)	13,400± 202	13,551± 211	13,543± 205
Extrudability (g/cm²)	84.6 ±45	87.9±93	88.6±33

CONCLUSION

Using the quasi-emulsion solvent diffusion approach, Gallic Acid was successfully integrated into nanosponges in the current study. The developed nanosponges underwent comprehensive examination across multiple parameters, including physical characteristics, production yield, Zeta potential, entrapment efficiency, and particle size. The findings demonstrated that all formulations displayed favourable attributes. Particle size results showed the lowest particle size, establishing it as the optimized batch owing to the synergistic utilization of ethyl cellulose polymer and beta-cyclodextrin. The high drug content confirmed uniform drug distribution, while the in vitro release profile demonstrated a controlled and sustained release pattern over 12 hours, governed primarily by diffusion through the nanosponge matrix and hydrogel network. This release behavior is advantageous in minimizing dose dumping and maintaining prolonged drug availability at the site of application, which is critical in the management of chronic conditions such as seborrheic dermatitis.Gallic Acid nanosponges were incorporated into a hydrogel, which was then tested for pH, spreadability, and in-vitro drug diffusion. Furthermore, the formulation showed excellent physical stability under accelerated and room temperature conditions, with no significant changes in key parameters, confirming its robustness and shelf stability.

Ethical statement: This study did not involve human participants, human data, or animal experimentation. Therefore, ethical approval from an institutional review board or ethics committee was not required.

Declaration : This manuscript is original, unpublished, and not under consideration elsewhere. All authors approved its submission and declared no conflict of interest

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