

Stability studies and characterization of nanoemulgel containing gallic acid

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Abstract: Background: Gallic acid is a natural phenolic compound with excellent antioxidant, antimicrobial and anti-inflammatory activity and good skin penetration. Gallic acid demonstrates both stability and solubility problems in topical formulations. A nanoemulgel combines an emulsion for enhanced solubility with a gel base for improved physical stability. **Objectives:** The main objective of this study is to characterize and assess the stability of a gallic acid-containing nanoemulgel. **Methods:** Various gallic acid nanoemulsions (GNE) were prepared using optimized ratios of oil, surfactants, gallic acid and water and evaluated for stability. Finally, stable GNE-19 was characterized for particle size and converted to nanoemulgel (GNEG-19). Nanoemulgel was subjected to short-term and accelerated stability studies and characterized for pH, viscosity, spreadability, occlusion effect, FTIR, *in-vitro* drug release and *in-vitro* pharmacological activities. **Results:** The results showed that formulation GNEG-19 showed optimum thermodynamic stability, having no phase separation and color change. The droplet size of freshly prepared GNE and after 28 days was 175.43nm and 183.23nm, respectively. The pH was slightly acidic but still within the human skin range (3.9–4.5). No interaction was found between the gallic acid and other components of nanoemulgel following FTIR analysis. The viscosity of freshly prepared GNEG-19 was 2127.1 cP and after 90 days, it was 2102.3 cP, which showed its stability. The percent drug release of gallic acid from nanoemulgel after 24 hrs was 93.12%. GNEG-19 also showed effective occlusion properties when compared with gel base and petroleum jelly. *In-vitro* pharmacological activities showed that GNEG-19 had antioxidant activity of 63.77% and a zone of inhibition (ZOI) of 11.4 mm compared to pure gallic acid (9.5 mm). **Conclusion:** In conclusion, nanoemulgel containing gallic acid showed good physical and chemical stability and was pharmacologically active.

Keywords: Characterization; Gallic acid; Nanoemulgel; Pharmacological activities; Stability studies

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INTRODUCTION

Gallic acid (3,4,5-trihydroxybenzoic acid) is a naturally occurring phenolic compound widely present in green tea, grapes, oak bark and *Phyllanthus emblica* (Amla). Gallic acid has exhibited a variety of biological interests, such as anti-inflammatory, anti-cancer, anti-microbial and antioxidant qualities (Wianowska and Olszowy, 2023). Gallic acid helps shield cellular constituents, including lipids, proteins and DNA, from oxidative damage by scavenging free radicals (Shaghghi *et al.*, 2026). Furthermore, gallic acid has shown antibacterial and antibiofilm activity against several harmful bacteria, such as *Propionibacterium acnes* and *Staphylococcus aureus*, both linked to acne vulgaris (Choinska *et al.*, 2021). This characteristic enables the use of gallic acid in acne formulations, where it reduces inflammation while combating microbial colonization.

Gallic acid has good pharmacological potential, but its clinical efficacy is limited by its poor permeability and low bioavailability when taken by traditional methods. Because of its poor solubility, instability in aqueous solutions and least possible skin penetration, its clinical merits are

limited, especially for topical treatments (Harwansh *et al.*, 2024). Adding gallic acid to a nanoemulgel system can improve skin penetration, stability and sustained release, helping overcome these delivery limitations (Sghier *et al.*, 2024).

The nanoemulgel, a hybrid delivery system that combines the benefits of hydrogels with nanoemulsions, is a cutting-edge strategy. In addition to improving skin penetration and controlled-release properties, this delivery system increases the solubility and stability of poorly water-soluble substances. Their nanoscopic droplets permit greater skin penetration, while the gel bases assure simpler administration and more stability (Donthi *et al.*, 2023).

For topical and transdermal delivery, nanoemulgel provides a thermodynamically stable system with a high drug-loading capacity and enhanced penetration. They are made up of nanosized emulsified droplets, usually in the range of 20 to 200 nm, distributed within a gelling matrix (Mandal *et al.*, 2023). Additionally, this formulation approach gets beyond the drawbacks of traditional gels and emulsions, such as their poor solubility and unpredictable drug release patterns.

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Stability is a main factor to consider while developing topical formulations. By ensuring that the formulation maintains its physical, chemical and microbiological properties for the duration of time, it shows the product's safety and efficacy (Pathak *et al.*, 2024). Typically, the formulation's performance is evaluated in both short- and long-term stability tests under various environmental conditions (Sengupta *et al.*, 2018). Under varied environmental circumstances, these tests evaluate a formulation's physicochemical and microbiological integrity throughout time. Stability testing guarantees that the product will continue to be as safe, effective and of the desired quality for the duration of its shelf life.

For short-term (accelerated) stability testing, the formulation is usually stored at high temperatures and humidity levels (e.g., $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH) for one to three months (Gonzalez-Gonzalez *et al.*, 2022). Because higher temperatures accelerate degradation, this method allows you to predict long-term stability in a comparatively short time. The following criteria are commonly tracked during short-term testing: globule size, zeta potential, microbial load, pH, viscosity, drug content and physical appearance (color, texture and phase separation). For instance, in a nanoemulgel system, phase separation or variation in droplet size may suggest instability or coalescence of the emulsion system, while pH changes may indicate excipient or active chemical degradation (Ansari *et al.*, 2025).

In contrast, long-term stability testing, as required by International Council for Harmonization (ICH) recommendations Q1A (R2), replicates real-time storage conditions, such as $25 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ RH, for up to 12 months. It offers information to determine a product's shelf life under expected usage and storage circumstances. Though they are examined over longer time periods, frequently at regular intervals like 0, 3, 6, 9 and 12 months, the parameters analyzed are essentially the same as those in accelerated trials. Data from long-term studies confirm the ability to sustain initial product standards over time (Huynh-Ba and Zahn, 2009).

Unlike long-term studies, accelerated stability testing occurs under heightened storage conditions, usually at 40°C and $75 \pm 5\%$ relative humidity (RH) for at least 3 to 6 months, following ICH Q1A (R2) guidelines. For sophisticated delivery systems such as nanoemulgel, these investigations are crucial to pinpoint potential "worst-case" instabilities, including phase separation, droplet merging, or quick oxidation of sensitive bioactive like gallic acid, within a condensed timeframe. Data from a 90-day accelerated study serve as an essential indicator of the product's potential stability under ambient conditions and support initial shelf-life estimates until 12-month real-time data are acquired (Kashinath *et al.*, 2024). The significance of such stability testing has been validated by numerous

research studies. According to researchers (Shakeel *et al.*, 2019), formulation robustness was demonstrated by the nanoemulgel formulations' ability to retain droplet size and viscosity with little pH fluctuation when stored under accelerated conditions for three months.

The main objective of this research was to create, optimize and assess a nanoemulgel containing gallic acid to improve the oxidative stability of the phytochemical (gallic acid). This research sought to develop an advanced cosmeceutical delivery system that combines the benefits of nanoemulsion, characterized by its small droplet size, with hydrogel bases that offer extended contact time, thereby addressing the conventional challenges associated with aqueous phenolic solutions. Accordingly, this study aimed to characterize and assess the stability of a nanoemulgel containing gallic acid. Gallic acid is well known for its rapid oxidation. This research offers a framework for safeguarding bioactive compounds within a lipid-based nanostructure, marking significant progress in dermatological treatment.

MATERIALS AND METHODS

Materials

Gallic acid was purchased from Mubychem Group. Paraffin oil, Tween80 and Span 80 were purchased from Merck (KGaA, Darmstadt, Germany). Carbopol 940 was purchased from Haihang Industry Co., Ltd. Methyl paraben was purchased from Foodchem International Corporation. All chemicals were of analytical grade. Distilled water was obtained from the university lab distillation plant.

Instruments

Magnetic Stirrer (Velp Scientifica, Italy), High pressure homogenizer (WiseTis® HG-15D, Daihan Scientific Co., Ltd, South Korea), Analytical Weighing balance (ATY224, Shimadzu Corporation, Japan), Zetasizer Nano ZS (Malvern Instruments, UK), pH meter (inoLab®, WTW, Germany), Centrifugation machine (model: 5810 R Eppendorf, Germany), Brookfield Digital Rheometer (Model DV-III, Brookfield Engineering Laboratories Middleborough, MA, USA) and Refrigerated Incubator (FTC-90E, Velp Scientifica, Italy)

Preparation of gallic acid-loaded nanoemulgel

Gallic acid-loaded nanoemulgel was formulated in three steps. In the first step, a nanoemulsion containing gallic acid was formulated following a previously reported method, with minor modifications (Khan *et al.*, 2018).

Step 1: Preparation of nanoemulsion containing gallic acid (GNE)

First, aqueous and oily phases were prepared individually for nanoemulsion formulation. *For the oily phase:* 1.2 g of gallic acid was added to paraffin oil and mixed by stirring.

For the aqueous phase: The surfactant mixture of Tween80 and Span80 was first dissolved in distilled water by stirring until a homogeneous mixture was formed. The oily phase was added dropwise to the aqueous phase under continuous magnetic stirring at 1,100 rpm. The emulsion was then homogenized by high-pressure homogenization at 15000 revolutions per minute for ten minutes. This was followed by two hours of stirring at room temperature.

Thirty formulations (GNE-1 to GNE-30) were made using different concentrations of surfactants, cosurfactants and paraffin oil (optimization parameters) using DOE Software and tested for stability in an incubator at 25°C for one month (Khan *et al.*, 2018). Formulations GNE-1 to GNE-18 were made keeping oil concentration constant, while formulations GNE-19 to GNE-23 were made keeping surfactant concentration constant. GNE-24 to GNE-30 were formulated using variable concentrations of both oil and surfactants. All formulations had the same gallic acid concentration (Table S1).

GNE-19 nanoemulsion (Table 1) was found to be stable during a preliminary stability study of one month and was further selected/processed for nanoemulgel formation.

Step 2: Preparation of gel

By thoroughly mixing purified water with a gelling agent, the gel phase was developed (Mwangi *et al.*, 2021). Carbopol-940 was dissolved in cold water with 0.4% methyl paraben and stirred using a magnetic stirrer (Velp Scientifica, Italy) at a controlled speed of 500 rpm for 60 minutes until a homogeneous mixture was obtained and then kept overnight with moderate stirring to form a swollen gel. The composition of the gel base is given in table 2.

Step 3: Preparation of gallic acid-loaded nanoemulgel (GNEG-19)

To create a homogeneous nanoemulgel, the gel base was added to the nanoemulsion (1:1) and stirred slowly for 20 minutes (Mwangi *et al.*, 2021).

Stability studies

Stability studies were conducted according to International Council for Harmonization (ICH) guidelines for stability of products under various storage conditions (Khan *et al.*, 2022).

Short-term stability study

A short-term stability study usually comprises testing the formulation under stress conditions during an incubation period. During the short-term stability investigations, GNEG-19 was kept for 28 days at accelerated storage conditions. The following tests were conducted.

Visual examination

Physical and visual examinations of GNEG-19 were carried out to confirm its homogeneity, color change and

odor (Khan *et al.*, 2022). Physical and visual examinations were carried out during 0hr (fresh), 24hrs, 48hrs, 72hrs, 7days, 14days, 21days and, 28 days at 40°C ± 2°C.

Tests of thermodynamic stability

In thermodynamic stability studies, heating-cooling, freeze-thaw cycles and centrifugations were performed.

Heating-cooling cycles

The nanoemulgel was heated and cooled at high and low temperatures (e.g., 4°C and 40°C) in six cycles, each lasting twenty-four hours.

Freeze-thaw cycles

Each time the nanoemulgel was frozen at -20°C and thawed at 25°C, phase changes and gel disintegration were examined (Alam *et al.*, 2020).

Centrifugation test

To check instability and search for indications of phase separation, the nanoemulgel was centrifuged after 24 hrs. Centrifugation was performed at 5000rpm for 10 mins in a centrifugation machine (model: 5810R Eppendorf, Germany) (Algahtani *et al.*, 2020).

Accelerated stability study

To evaluate the formulation's performance, integrity and safety over a prolonged length of time, an accelerated stability study of GNEG-19 was conducted at 4°C ± 2°C, 25°C ± 2°C, 40°C ± 2°C and 40°C ± 2°C with 75% RH (Arun *et al.*, 2023) for a period of 90 days.

Characterization

Visual inspection

Visual examination of the GNEG-19 was carried out to confirm its homogeneity, color change and odor (Khan *et al.*, 2022) for 90 days at predefined regular intervals.

Particle size analysis

Particle size analysis was carried out to assess changes in droplet size and size distribution in the gel's nanoemulsion (Khan *et al.*, 2022). The optimized nanoemulsion formulation, GNE-19, was characterized for mean droplet size and zeta potential using a Zetasizer Nano ZS (Malvern, UK). The mean droplet size and zeta potential of three GNE-19 samples were measured at T₀ and after T₂₈ days.

FTIR examination

Fourier transform infrared (FTIR) spectroscopy was used to investigate potential chemical interactions between gallic acid and the excipients within the nanoemulgel. (Shaikh and N Nagoba Shivappa, 2019). FTIR spectra were obtained for gallic acid, methyl paraben, Carbopol and their physical mixtures. A spectral range of 4000–400 cm⁻¹ at a resolution of 4 cm⁻¹ was used to measure % transmittance.

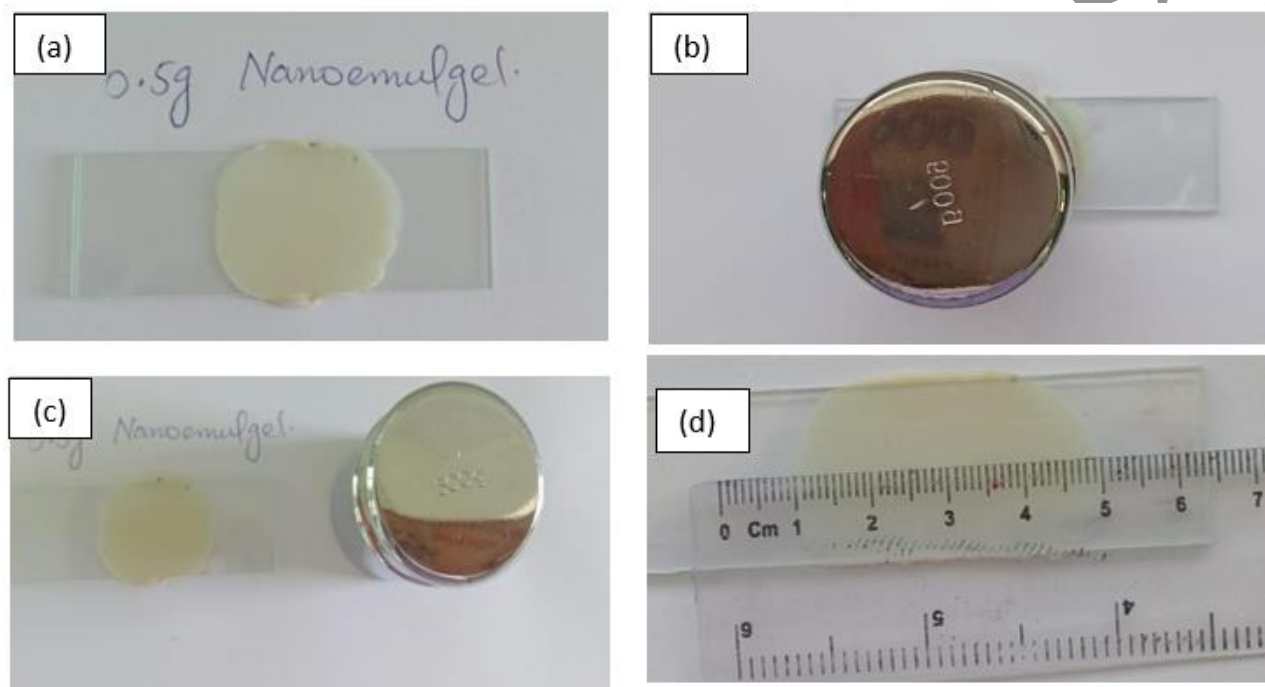
Table 1: Stable formulation of nanoemulsion containing gallic acid (GNE).

F S/No	Oil phase		Aqueous phase		S_{mix}	
	F code	Paraffin oil (%)	Gallic acid (%)	Water (%)	Tween 80 (%)	Span 80 (%)
1	GNE-19	8	1.2	82.8	4.26	3.74

Table 2: Composition of gel.

S/No	Carbopol-940	Water	Methyl paraben
Gel	4%	95.6%	0.4%

Carbopol gel was then tightly closed in a glass container and stored at room temperature (25°C) away from light to prevent from photodegradation.

**Fig. 1:** Steps involved in spreadability studies including (a) Nanoemulgel placed between two glass slides and (b) Known weight. (500g) was placed; (c) Upper slide removed; (d) Diameter of the spread circle was measured.

Measurement of pH

pH was measured to keep in view the formulation's chemical stability for time span of three months by recording pH variations over time (at regular intervals). A digital pH meter was used to measure the pH of GNEG-19 under various storage temperature conditions (Khan *et al.*, 2022). All readings were taken in triplicate and mean was taken.

Measurement of viscosity

Using rheometer, the viscosity of the nanoemulgel was evaluated at different times to confirm the gel keeps its spreadability, user acceptability and flow properties. The viscosity of Gallic acid loaded nanoemulgel GNEG-19 was measured at 100–200 rpm speed (with 50 increments) and spindle (CP41). All the readings were taken at 25°C on Brookfield Digital Rheometer (Model DV-III, Brookfield

Engineering Laboratories Middleborough, MA, USA). All measurements were taken in triplicate and mean was taken (Akhtar *et al.*, 2023).

Study of spreadability

The spreadability coefficient was determined using glass slabs and an electronic vernier caliper. Two standard-size glass slides were used. 0.5g of sample was placed in the center of the lower glass plate. Then a second glass plate was placed on top and ensured it made contact with the sample. A known weight (500g) was placed on the upper glass plate and remained in place for three minutes to allow it to spread under applied pressure. The upper plate was then removed and the diameter of the spread circle of nanoemulgel was measured (Fig. 1) and the average diameter was calculated. Spreadability was expressed qualitatively from the diameter of the spread area (Khan *et al.*, 2022; Trindade *et al.*, 2024).

Occlusion test *in-vitro* (hydration potential)

To find the emulgel's hydration potential through *in-vitro* occlusion test (Vaz *et al.*, 2019; Donthi *et al.*, 2023) was performed. Three beakers were filled with 10 mL of distilled water. The mouth of each beaker was covered with filter paper. 1g of nanoemulgel was uniformly applied onto the surface of the filter paper of the first beaker. The second beaker, covered with filter paper but without any sample, was taken as the positive control. A standard occlusive base (petrolatum jelly), applied in a similar fashion to the third beaker, was used as the reference control. All beakers were weighed (W_0) and placed in a stability chamber at 32°C for 48 hours. Each assembled beaker was weighed accurately. At predetermined time intervals (e.g., 6, 12, 24, 48 hours), beakers were removed and reweighed (W_t) to determine the water loss due to evaporation (Fig. 2).

Occlusion Factor (OF) was calculated using the formula:

$$A-B/A \times 100$$

Where A = water loss from the unoccluded control beaker and B = water loss from the beaker with the nanoemulgel. A higher percentage indicates a stronger occlusive effect.

Pharmacological activities *in-vitro*

Antioxidant activity

The DPPH method, which was modified, was used to assess the radical scavenging capacity of various gallic acid nanoemulgel fractions (Akhter *et al.*, 2024).

In detail, 20 μ L of DPPH solution (0.1mM) was mixed with 20 μ L of the nanoemulgel fraction at a concentration of 200 μ g/mL in a 96-well plate. The mixture was then incubated in the dark at 37°C for 30 minutes, after which the absorbance was measured at 490 nm using a microplate reader (BioTek, Model ELx800). Pure methanol, used as the solvent for gallic acid, served as the control. Ascorbic acid was employed as a reference standard. DPPH decolorization is an indication of radical scavenging ability, which was estimated by:

$$\text{DPPH scavenging activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

Evaluation of antibacterial activity

To assess the efficacy of the nanoemulgel containing gallic acid, an *in-vitro* antibacterial activity was conducted (Vartak *et al.*, 2020; Akhter *et al.*, 2024). The agar well diffusion assay is a standardized method for evaluating antimicrobial activity, commonly performed using Mueller-Hinton agar (MHA) as the culture medium. Media was prepared according to the manufacturer's instructions and sterilized in an autoclave. An overnight culture of *Staphylococcus aureus* (*S. aureus*) was prepared. A suspension of *S. aureus* was prepared in sterile normal saline and turbidity was matched with a 0.5 McFarland turbidity standard (107 CFU/mL) under a white and black

background. Bacterial suspension was inoculated on the surface of MHA plate with a sterile cotton swab, forming a uniform lawn. Inoculum was allowed to dry for 10–15 minutes. Afterward, an 8 mm-diameter well was punched into the agar using a sterile cork borer. An aliquot of 50 to 60 μ L test solutions were then pipetted into their respective wells and the plate was incubated at 37°C for 18–24 hours in incubator and their zones of inhibition were measured. Duplicate assays were performed against each formulation.

Investigations of drug release *in-vitro*

To ensure adequate drug penetration into the skin at an appropriate rate and in sufficient quantities, drug release studies are crucial when developing novel formulations. *In-vitro* diffusion study was conducted using a Franz diffusion cell with parafilm and cumulative drug release over time was assessed (Cetin *et al.*, 2022). The Franz diffusion cell was covered with three layers of Parafilm M. The dissolution medium (phosphate buffer pH 7.4) was added to the acceptor compartment and it was agitated at 300 rpm. A peristaltic pump circulated water, maintaining the temperature at $37 \pm 1^\circ\text{C}$. 1 g nanoemulgel was placed on stacked Parafilm and secured by clamping between the donor and receptor chambers. 1mL sample was collected from the sampling arm at predetermined time intervals (0, 0.5, 1, 2, 3, 4, 5, 8, 12, 24 and 48 hours) and replaced with fresh buffer. To ascertain cumulative drug release, the samples were diluted and then examined at 272.5nm using a UV spectrophotometer.

Statistical analysis

SPSS (Statistical Package for the Social Sciences) version 24 was used. Descriptive statistics (mean $\pm$ SEM) were used to present the data. One-way ANOVA was employed.

RESULTS

Results show that the nanoemulsion (GNE-19) was stable during the preliminary stability study of one month. Therefore, GNE-19 was selected for incorporation into the nanoemulgel system (GNEG-19). GNEG-19 was subjected to stability studies and characterization.

Short-term stability study

Visual examination

Gallic acid nanoemulgel (GNEG-19) was white and odorless immediately after preparation. Visual examinations at regular intervals showed that GNEG-19 was homogeneous, white and odorless at 40°C for the period of 28 days as shown in Table 3.

Thermodynamic stability

GNEG-19 was exposed to 6 heating-cooling cycles, 3 freeze-thaw cycles and 1 centrifugation test. Table 4 shows the thermodynamic stability results. GNEG-19 remained stable after all three cycles.

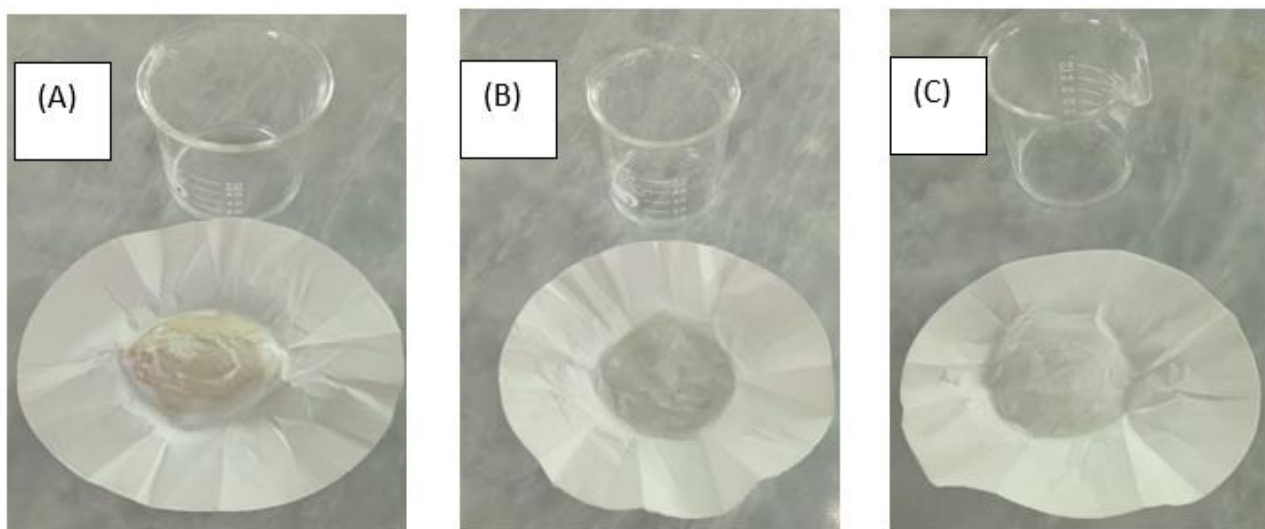


Fig. 2: Water loss at each time points of (A) Nanoemulgel, (B) Blank gel and (C) Petroleum jelly.

Table 3: Physical and visual examinations.

Time period	Effects on parameters at 40°C		
	Homogeneity	Color	Odor
Fresh	Homogenous	White	Odorless
24hrs	Homogenous	White	Odorless
48hrs	Homogenous	White	Odorless
72hrs	Homogenous	White	Odorless
7days	Homogenous	White	Odorless
14days	Homogenous	White	Odorless
21days	Homogenous	White	Odorless
28days	Homogenous	White	Odorless

Table 4: Results of thermodynamic stability.

Formulation code	Heating-cooling cycles						Freeze thaw cycles			Centrifugation	
	1	2	3	4	5	6	1	2	3	1	
GNEG-19	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable	

Table 5: Effects on stability at various storage conditions.

Time Period	Effects on stability at various storage conditions											
	4°C± 2 °C			25°C± 2 °C			40°C± 2 °C			40°C± 2 °C with 75%RH		
	Color	Odor	Homogeneity	Color	Odor	Homogeneity	Color	Odor	Homogeneity	Color	Odor	Homogeneity
24hrs	White	Odorless	Homogenous	White	Odorless	Homogenous	White	Odorless	Homogenous	White	Odorless	Homogenous
48hrs	-	-	-	-	-	-	-	-	-	-	-	-
72hrs	-	-	-	-	-	-	-	-	-	-	-	-
7days	-	-	-	-	-	-	-	-	-	-	-	-
14days	-	-	-	-	-	-	-	-	-	-	-	-
21days	-	-	-	-	-	-	-	-	-	-	-	-
28days	-	-	-	-	-	-	-	-	-	-	-	-
45days	-	-	-	-	-	-	-	-	-	Off white	-	-
60days	-	-	-	-	-	-	Off white	-	-	Off white	-	-
90days	-	-	-	-	-	-	Off white	-	-	Off white	-	-

(-) = no change; RH = relative humidity

Accelerated stability study

Visual examination

Visual examinations of the GNEG-19 showed that GNEG-19 was white, homogeneous and odorless at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with 75% RH for the period of 60 days, GNEG-19 showed a little change (off white) color after 90 days at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with 75% RH (Table 5).

Characterization

Particle size

The average droplet size and zeta potential recorded for three samples of GNE-19 at $T_{0\text{hr}}$ and T_{28} days are given in table 6. Particle size increased slightly ($p > 0.05$) after 28 days.

The zeta potential distribution of fresh GNE-19 and after 28 days is shown in figures 3A and 3B. The zeta potential of GNE-19 at 0 hr was -32.4 ± 1.5 mV. The zeta potential analysis shows that GNE-19 initially (0 hr) possessed strong colloidal stability, but its stability decreased slightly after 28 days, as evidenced by a decline in absolute zeta potential and possible peak broadening, as shown in figure 3B.

This trend is typical for nanoemulsion or nanogel formulations during storage and highlights the need to further optimize stabilizing agents to maintain long-term dispersion stability.

FTIR analysis

FTIR spectra of nanoemulgel and individual components are given in figures 4A-4E. The nanoemulgel shows a peak at 3357.40 cm^{-1} with a broad and strong absorption band, characteristic of O-H stretching vibrations due to the presence of hydroxyl groups from water, Carbopol and other formulation excipients. The broadness also suggests extensive hydrogen bonding. Peaks at 2923.28 cm^{-1} and 2853.57 cm^{-1} are associated with C-H stretching vibrations from aliphatic groups. These could originate from fatty acids, oils, or other hydrocarbon-based components found in this formulation. The peak at 1636.07 cm^{-1} can be attributed to the O-H bending vibration of water molecules. The FTIR spectra of the physical mixtures NE and NEG (Figures 4D and 4E) showed simple superimpositions of the pure-component spectra, with no significant differences from the individual components.

pH Measurements

The pH stability profile of GNEG-19 over time under various storage conditions is presented in table 7 and figure S1.

Measurement of viscosity

The formulation's viscosity has an impact on patient compliance, spreadability, flow properties and drug release.

The viscosities of freshly prepared gallic acid-loaded nanoemulgel (GNEG-19) and after 90 days are presented

in table 8. Each GNEG-19 rheogram showed a flow index of less than 1 (0.276), indicating shear-thinning behavior consistent with a pseudoplastic trend. These characteristics are essential for ideal cosmetic formulations. Formulations with pseudoplastic flow are key to creating a skin-protective layer. The main reason for pseudoplastic behavior may be the gradual breakdown of the formulation's internal structure under increasing shear, followed by reconstruction through Brownian movement.

Other tests

Study of spreadability

Spreadability of the nanoemulgel was checked using the two-glass plate method, where the spread diameter was measured. Gallic acid nanoemulgel showed a spreadability of 4.83 cm.

Occlusion test in-vitro (hydration potential)

Occlusion test graph for nanoemulgel (GNEG-19), gel base and petroleum jelly are given in figure 5.

Study of drug release in-vitro

To attain sustained distribution, the release study was carried out over a 48-hour period. The gallic acid release profile from nanoemulgel is displayed in figure 6.

Pharmacological activities

Antioxidant activity

The DPPH assay offers important information on antioxidant capacity. Using this technique, the gallic acid nanoemulgel capacity to scavenge free radicals was assessed. Gallic acid nanoemulgel showed 63.77% antioxidant activity at $200\text{ }\mu\text{g/mL}$, compared with ascorbic acid, used as the standard, which had 98.58% antioxidant activity.

Antibacterial activity

Results for antibacterial activity of Blank gel, GNEG-19, gallic acid and sterile distilled water are presented in figure 7 and table 9.

DISCUSSION

This study focused on formulation, stability studies and characterization of nanoemulgel containing gallic acid (GNEG-19). The evaluation included preliminary, visual and thermodynamic stability, along with physicochemical characterization (particle size, pH, viscosity and FTIR) and biological assays (antioxidant and antibacterial activity).

An initial stability study of various gallic acid nanoemulsion (GNE) formulations indicated that GNE-19 showed superior stability for one month at 25°C , whereas other formulations underwent phase separation or liquefaction within a few weeks. Phase separation is the visible separation of immiscible liquid phases, while liquefaction indicates a loss of consistency. Both are key signs of physical instability in emulsion-based systems (Karwal et al., 2016).

Table 6: Average droplet size and polydispersity index of nanoemulgel containing gallic acid.

Sample name	Droplet size (nm)	PdI
GNE-19. T _{0hr}	175.43	0.258
GNE-19. T _{28days}	183.23	0.306

T_{0hr} = fresh nanoemulgel; T_{28 days}= after 28 days

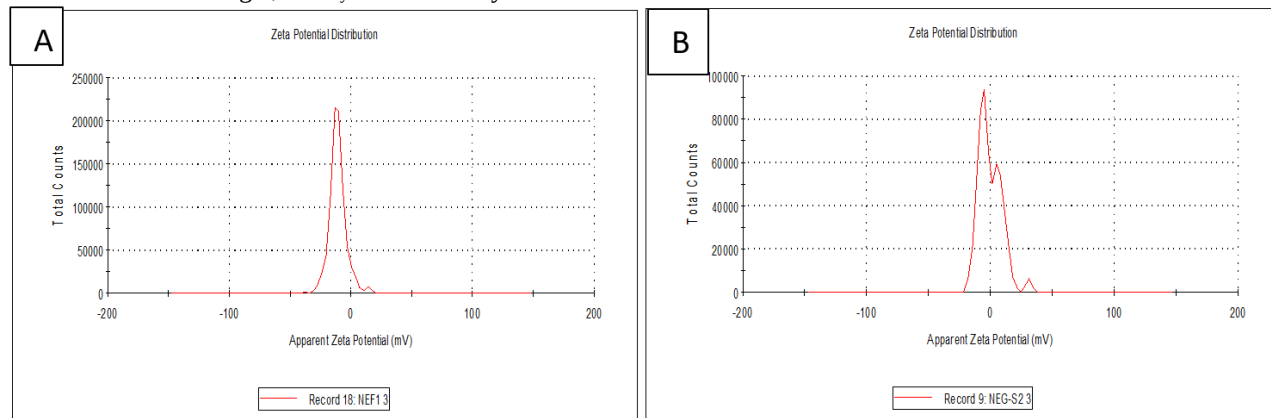


Fig. 3: Zeta potential distribution of GNE-19 (A) at 0hr and (B) after 28 days.

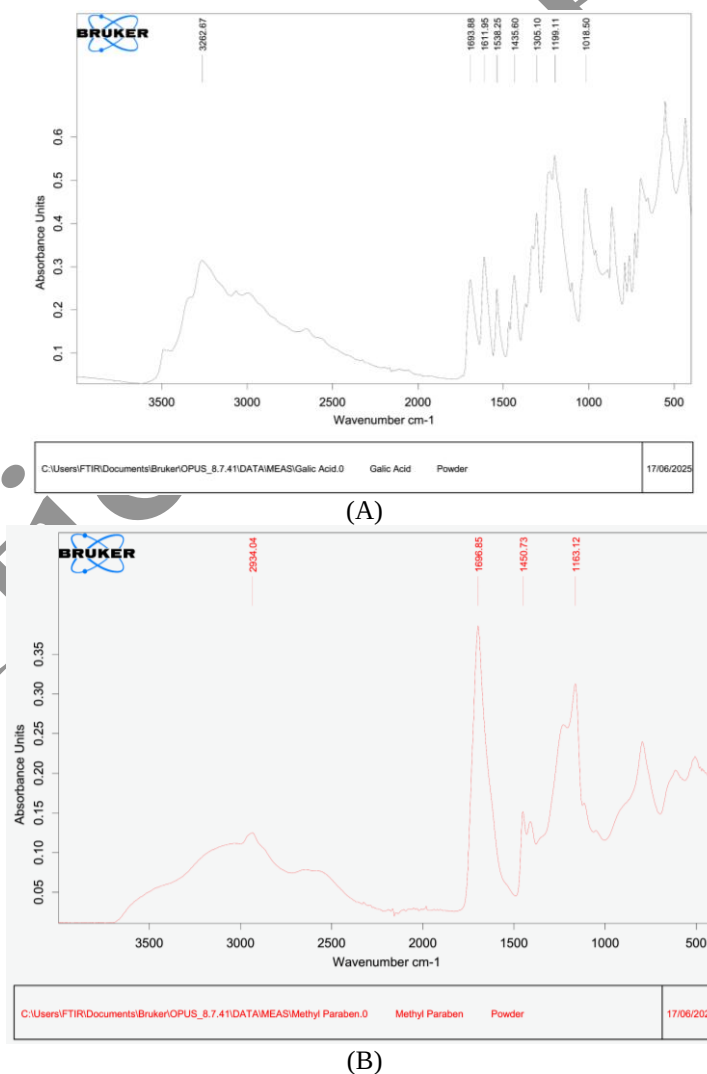
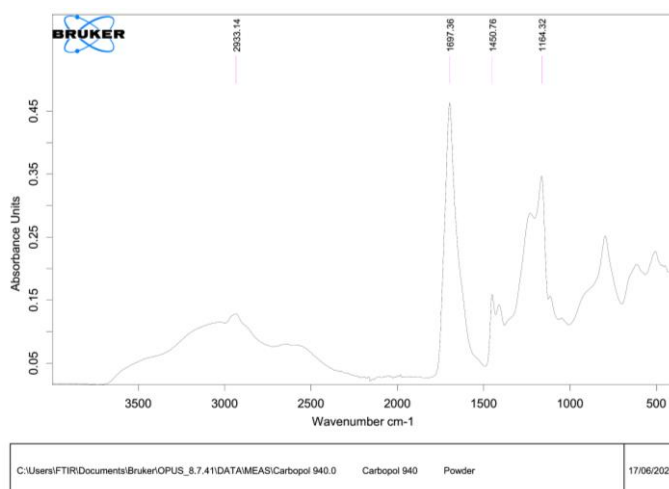
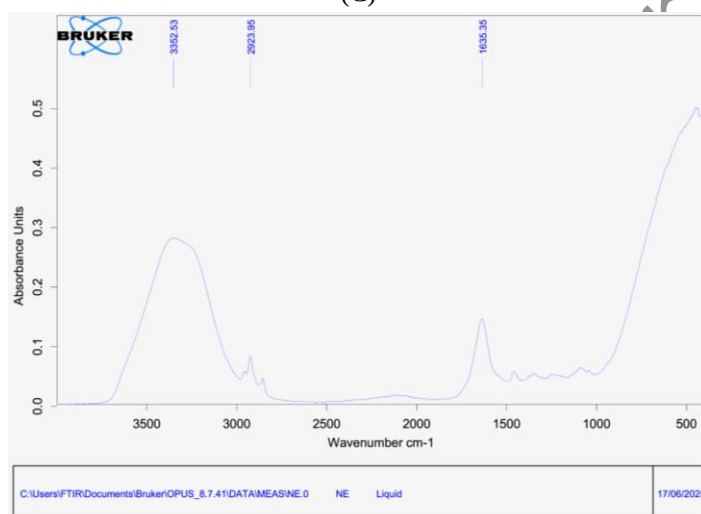


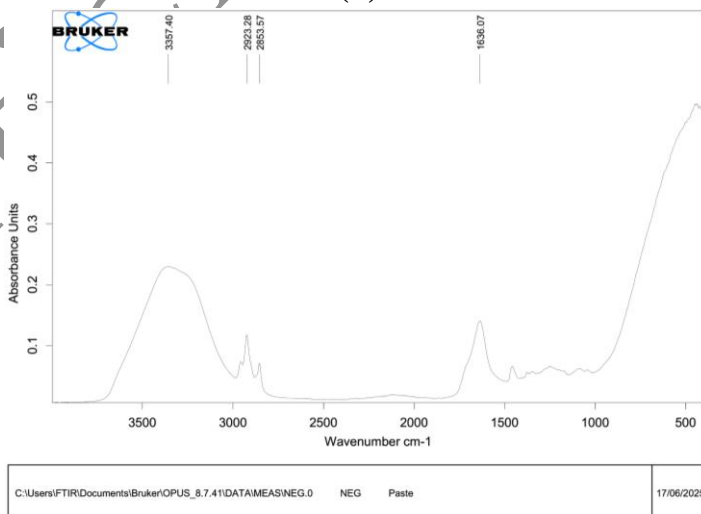
Fig 4 is continued.....



(C)



(D)



(E)

Fig. 4: FTIR studies of (A) Gallic acid, (B) Methyl paraben, (C) Carbopol 940, (D) Nanoemulsion and (E) Nanoemulgel (GNEG-19).

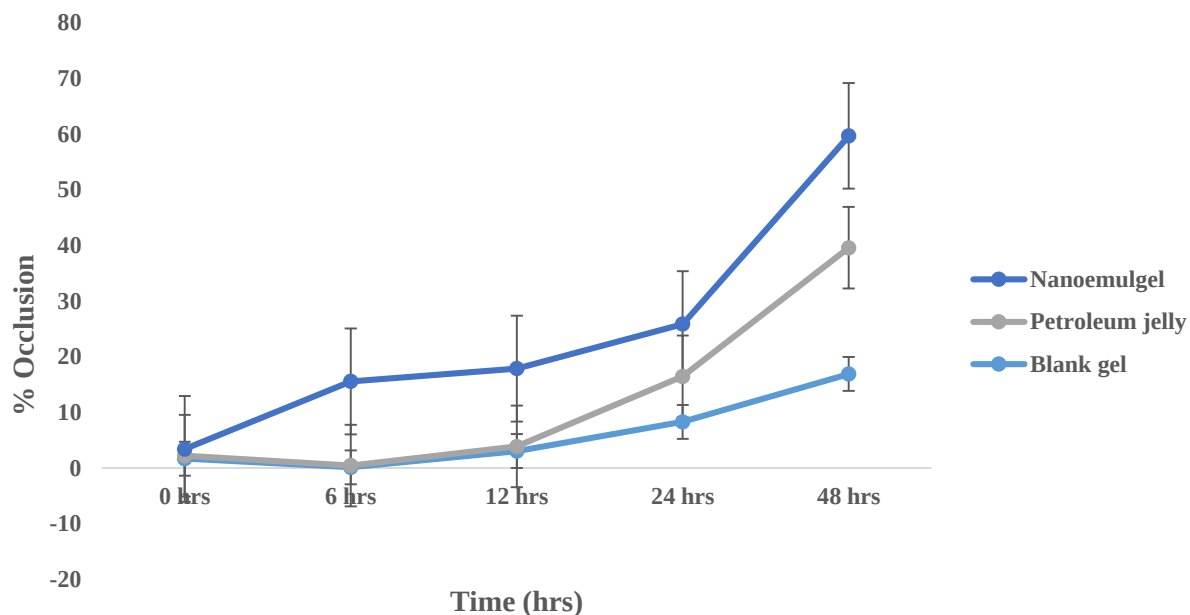
Table 7: Change in pH of GNEG-19 over time at various storage conditions.

Time period	4°C	25°C	40°C	40°C + 75% RH
Fresh	5.3	5.3	5.3	5.3
24 hrs	5.3	5.3	5.1	5.0
48 hrs	5.3	5.1	5.0	5.0
72 hrs	5.0	5.0	4.8	4.9
7days	4.9	4.9	5.0	4.9
14days	4.5	4.6	4.3	4.3
21days	4.0	4.1	4.2	4.0
28 days	3.9	3.9	3.9	3.7
45 days	3.9	3.7	3.9	3.7
60 days	3.9	3.9	3.9	3.7
90 days	3.7	3.7	3.7	3.6

RH = relative humidity

Table 8: Viscosity of freshly prepared GNEG-19 and after 90 days at 25°C.

Sr. No	Formulations	Viscosity (cps)	Consistency index(K)	Shear thinning factor (n)	R
1	GNEG-19 (fresh)	2127.1	0.834	0.434	0.943
2	GNEG-19 (after 90 days)	2102.3	0.802	0.411	0.933

**Fig. 5:** Hydration potential of nanoemulgel, petroleum jelly and blank gel.**Table 9:** Results of antibacterial activity.

S/N	Sample	Average ZOI (mm)
1	Sample 1 Blank gel	0
2	Sample 2 GNEG-19	11.4
3	Gallic acid	9.5
4	Sterile distilled water	0

ZOI = zone of inhibition

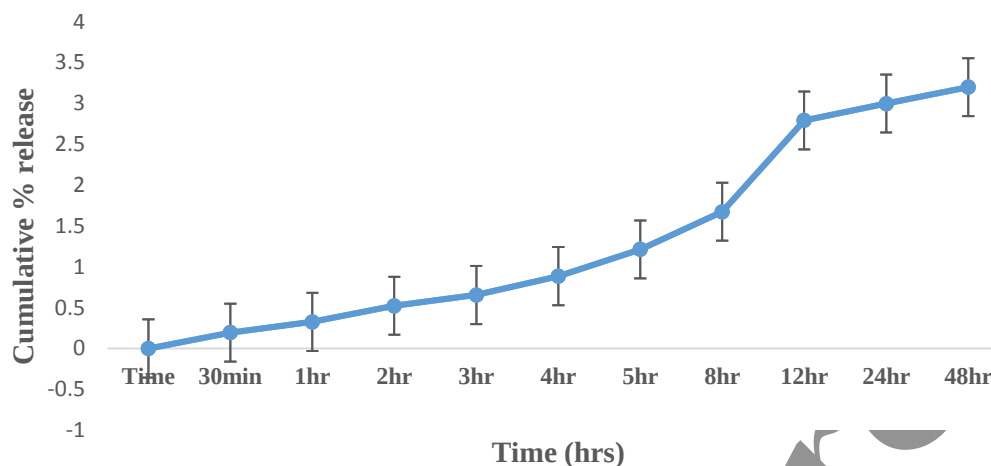


Fig. 6: Percentage cumulative drug release over 48 hours.



Fig. 7: Zone of inhibition (ZOI) of (1) blank gel, (2) GNEG-19, (3) Gallic acid and (4) Sterile distilled water.

The successful preliminary stability of GNE-19 highlighted its potential as a suitable base for the GNEG-19 nanoemulgel, providing a physically stable foundation for gallic acid.

The short-term stability assessment of GNEG-19 at 40°C showed excellent physical integrity. The nanoemulgel remained homogeneous, white and odorless throughout the 28-day observation period. Maintaining these organoleptic properties is crucial for patient acceptance and adherence,

as changes can signal product degradation or reduced efficacy (Dhandhi *et al.*, 2025). Thermodynamic stability tests, including heating-cooling, freeze-thaw cycles and centrifugation, are vital accelerated stress tests designed to predict a formulation's long-term physical stability. GNEG-19 passed these rigorous cycles, which indicates its high physical stability and resilience to environmental stresses like temperature fluctuations and gravitational forces (Jadhav *et al.*, 2024).

The long-term stability study provided insights into GNEG-19's performance under various storage conditions over an extended period. GNEG-19 generally maintained its physical attributes across various conditions. However, a slight color change to off-white was observed at 40°C and 40°C with 75% relative humidity after 90 days. This subtle discoloration at elevated temperatures and humidity indicates minor oxidative degradation of gallic acid, which is prone to oxidation, or other excipients (Badhani *et al.*, 2015). These changes require careful monitoring and may suggest optimized packaging or storage conditions to preserve the product's aesthetic and chemical integrity over long durations.

The initial average droplet size of GNE-19 was 175.43 nm with a PDI of 0.258. After 28 days, the average droplet size increased slightly to 183.23 nm, with a PDI of 0.306. For nanoemulsion, a small and narrow droplet size distribution (indicated by a low PDI, typically below 0.3) is crucial for enhanced drug permeation, improved bioavailability and long-term physical stability (Tabassum and Ahmad, 2018). The slight increase in droplet size and PDI, while still within acceptable limits for a nanoemulsion, suggests minor/negligible coalescence over time. However, the overall low PDI values confirm that the formulation maintained a relatively monodisperse and stable colloidal system. The zeta potential, which reflects the electrical charge on the particle surface, is also a key indicator of colloidal stability by preventing aggregation through electrostatic repulsion (Molaei *et al.*, 2024). The consistent zeta potential distribution over 28 days further supports the physical stability of GNEG-19.

The FTIR spectrum of pure gallic acid showed characteristic peaks corresponding to its hydroxyl, carboxylic acid and aromatic ring functional groups, consistent with its chemical structure as 3,4,5-trihydroxybenzoic acid. Similarly, methyl paraben exhibited its characteristic ester carbonyl and C-H stretching vibrations. Crucially, the FTIR spectrum of the nanoemulgel (physical mixture) primarily displayed an overlap of the individual components' characteristic peaks, with no significant shifts or new peaks indicating covalent bond formation or strong chemical interactions. This finding suggests excellent chemical compatibility between gallic acid and the excipients, indicating that the active ingredient is physically incorporated into the nanoemulgel matrix without chemical degradation or forming new undesired compounds (Alburyhi *et al.*, 2024). The broad O-H stretching band and C-H stretching vibrations in the nanoemulgel spectrum are consistent with the presence of water, oils, surfactants and Carbopol, which are common components in such formulations.

The pH of a topical formulation is critical for its stability, skin compatibility and the ionization state of the active drug, which influences its permeation (Hmingthansanga *et*

al., 2022). The pH of GNEG-19 remained relatively stable, showing no statistically significant variation ($p > 0.05$). The pH of GNEG-19 ranged from 3.6 to 4.2 across storage conditions over 90 days. This slightly acidic pH range is generally well-tolerated by the skin and is often beneficial for the stability of phenolic compounds like gallic acid, which can be prone to degradation at higher pH values. The consistent pH values throughout the study indicate good buffering capacity and chemical stability of the formulation (Martinengo *et al.*, 2021).

Viscosity is a key rheological property influencing the spreadability, residence time, patient compliance and drug release characteristics of topical formulations (Redkar *et al.*, 2019). The viscosity of freshly prepared GNEG-19 was 2127.1 cP, which remained remarkably stable at 2102.3 cP after 90 days. This minimal change in viscosity over time, within an overall range of 2100-4720 cP, demonstrates the excellent structural integrity and consistency of the nanoemulgel. Such stable viscosity ensures uniform application, adequate residence time on the skin and consistent drug release, all of which are vital for therapeutic efficacy.

The gallic acid nanoemulgel exhibited a spreadability of 4.83 cm, indicating good topical coverage and ease of application. This property is essential for topical formulations as it directly impacts ease of application and patient comfort, leading to better compliance (Dhandhi *et al.*, 2025).

The occlusion test assesses a formulation's ability to reduce trans epidermal water loss (TEWL) from the skin, thereby promoting skin hydration. The inclusion of this test highlights the consideration of the formulation's impact on skin barrier function. The Nanoemulgel shows the highest occlusion factor throughout the study (Fig. 5), reaching approximately 60% at 48 hours. Petroleum Jelly reached only 40%, while the blank Gel showed the lowest occlusion (18%), proving that the nanoemulsion droplets are responsible for forming the protective film, not exactly the gel base itself. Effective occlusion shown by GNEG-19 (Table S2) can enhance the hydration of the stratum corneum, which is beneficial for various dermatological conditions (Donthi *et al.*, 2023).

The DPPH assay is a widely used method to evaluate the free radical scavenging activity of compounds and formulations. The gallic acid nanoemulgel showed significant antioxidant activity, with 63.77% (88.88% at 200 µg/mL) radical scavenging, comparable to standard ascorbic acid (98.58%). This finding confirms that gallic acid retained its potent antioxidant properties within the nanoemulgel formulation, suggesting successful incorporation and stability of the active compound. Gallic acid is a well-documented natural antioxidant with various benefits for skin health, including protection against oxidative stress-induced damage (Gao *et al.*, 2019).

The antibacterial activity assessment showed that the gallic acid-loaded gel (GNEG-19) exhibited a larger zone of inhibition (11.4 mm) than pure gallic acid (9.5 mm). This enhancement suggests that the nanoemulgel formulation may improve the delivery or penetration of gallic acid into bacterial cells or the agar medium, increasing its antimicrobial effect (Gao *et al.*, 2019). The small droplet size of the nanoemulsion can facilitate better interaction with bacterial cell membranes, leading to enhanced uptake of the active compound and improved antibacterial action (Li *et al.*, 2023). This finding positions GNEG-19 as a promising candidate for topical applications requiring antimicrobial properties, potentially in wound healing or acne treatment.

When developing and accessing new pharmaceutical formulations, the *in-vitro* drug release study is essential, especially for topical applications where the drug must enter the target site at a controlled rate and in adequate amounts to produce a therapeutic effect (USP, 2017). The objective of this release study was to assess the release profile of gallic acid from the nanoemulgel (GNEG-19) over 24 hours. The release profile of gallic acid from the nanoemulgel demonstrated a sustained/gradual, continuous release. The nanoemulgel formulation appears to effectively encapsulate gallic acid and modulate its release, preventing rapid depletion and ensuring its availability over an extended period. This sustained release can be attributed to the polymeric matrix of the nanoemulgel, which acts as a barrier to drug diffusion (Pathak and Thassu, 2009).

The sustained release of a drug is highly desirable for many therapeutic applications, as it can lead to several advantages, including reduced dosing frequency, improved patient compliance and maintenance of drug concentration within the therapeutic window for an extended duration, thereby minimizing potential side effects associated with peak concentrations (Siepmann and Siepmann, 2011). For topical formulations like nanoemulgel, a sustained-release profile is particularly important to ensure a prolonged local effect and avoid rapid systemic absorption or premature clearance from the application site.

CONCLUSION

In conclusion, the nanoemulgel containing gallic acid (GNEG-19) showed excellent physical and chemical stability under various storage conditions, maintaining its physicochemical attributes throughout the study period. The formulation effectively preserved gallic acid's inherent antioxidant activity and notably enhanced its antibacterial efficacy, likely due to the nano-sized delivery system. These results collectively suggest that GNEG-19 is a stable, biologically active topical formulation with potential therapeutic applications in dermatology, particularly for conditions that benefit from antioxidant

and antimicrobial properties. Future studies should focus on *in-vivo* efficacy, safety and pharmacokinetic/pharmacodynamic evaluations to further validate its clinical potential.

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Author's contributions

Hina Awan: Performed this research and contributed to data curation and investigation; Hira Khan: Supervised this research and contributed to writing- original draft; Rizwan-ul Haq: Contributed to review and editing, formal analysis and validation; Nighat Fatima: Contributed to validation and visualization; Khalid Mehmood: Contributed to visualization and support; Atif Ali: Provided conceptualization, co-supervision and resources (lab facilities) to conduct this research work.

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Data availability statement

All data generated or analyzed during this study are included in this article [and its supplementary information files] and have been provided.

Ethical approval

The study was approved by the Research and Ethics Committee, Department of Pharmacy, Abbottabad University of Science and Technology (AUST/ Pharmacy/ 2024/373).

Conflict of interest

The authors declare no conflict of interest.

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