Ind. J. Pharm. Edu. Res., 2025; 59(4):1312-1322.
https://www.ijper.org

Original Article

Formulation and Evaluation of Propolis Loaded Nanosponges Based Topical Anti-Inflammatory Gel

Mukesh P. Ratnaparkhi[1], Swati Dattatray Kshirsagar[2,*]

[1]Department of Pharmaceutics, Marathwada Mitra Mandal's College of Pharmacy, (Research Center Affiliated to Savitribai Phule Pune University), Thergoan, Pune, Maharashtra, INDIA.
[2]Department of Pharmaceutics, JSPM's Rajarshi Shahu College of Pharmacy and Research, Tathawade, Pune, Maharashtra, INDIA.

ABSTRACT

Objectives: To develop and evaluate a Propolis-Loaded Nanosponge (PLN)-based topical gel with potential anti-inflammatory, antifungal and antibacterial activities. **Materials and Methods:** PLNs were prepared using the emulsion solvent diffusion method with Ethyl Cellulose (EC) and Polyvinyl Alcohol (PVA) as key components. Various parameters, including particle size, Polydispersity Index (PDI), zeta potential, entrapment efficiency, production yield and drug release profile, were analyzed to determine the optimal formulation. Physicochemical characteristics were analyzed using FTIR, DSC, SEM and XRD. The optimized PLN was incorporated into a 2% w/w topical gel and further characterized for pH, viscosity, spreadability, drug content, *in vitro* drug diffusion, *ex vivo* skin penetration, antifungal, antibacterial activity and stability. **Results:** The optimized PLN showed high drug encapsulation efficiency and compatibility with the polymer. The formulated 2% w/w PLN-based gel exhibited significant *in vitro* drug diffusion (90.43%) and *ex vivo* skin penetration (79.82%) within 4 hr. It demonstrated potent antifungal and antibacterial activities. *In vivo* anti-inflammatory studies revealed that the gel provided comparable anti-inflammatory efficacy to standard 2% Diclofenac gel. **Conclusion:** The Propolis-loaded nanosponge-based gel demonstrated promising results, including effective drug delivery, antimicrobial activity and comparable anti-inflammatory performance to standard treatments, making it a potential topical therapeutic agent.

Keywords: Emulsion Solvent Diffusion Method, *In vivo* Anti-Inflammatory Study, Nanosponges, Propolis, Topical Gel.

Correspondence:
Ms. Swati Dattatray Kshirsagar
Department of Pharmaceutics, JSPM's Rajarshi Shahu College of Pharmacy and Research, Tathawade, Pune-411033, Maharashtra, INDIA.
Email: swatibhosale430@gmail.com

Received: 14-10-2024;
Revised: 09-01-2025;
Accepted: 22-05-2025.

INTRODUCTION

The management of inflammatory skin conditions often relies on topical treatments that can deliver therapeutic agents directly to the affected area. However, the effectiveness of many anti-inflammatory agents is often hindered by challenges like poor solubility, limited stability and insufficient skin penetration. Enhancing drug distribution and increasing therapeutic outcomes, nanotechnology has emerged as a possible strategy to overcome these obstacles. One such innovative delivery system is the nanosponge, a porous, nanoscale structure capable of encapsulating and releasing active compounds in a controlled manner. Honey bees naturally generate a resinous material called Propolis, is renowned for its potent anti-inflammatory, antioxidant and antimicrobial properties.[1-9] despite its therapeutic potential, Propolis has limited bioavailability due to its hydrophobic nature and instability in conventional formulations. Incorporating Propolis into nanosponges offers a novel approach to enhance its stability,[10] control its release and improves its skin penetration for effective topical application.[11,12] Nanomaterials are used in the quickly expanding field of nanomedicine to diagnose, treat and prevent a wide range of illnesses, including cancer.[2,13] This study focused on the formulation of Propolis nanosponges and their integration into a topical gel formulated for anti-inflammatory use. The goal of the study was to evaluate the formed gel's physical, chemical and biological characteristics, such as its anti-inflammatory efficacy, skin penetration and release profile.[14-16] This work not only explains the potential of nanosponges as a remarkably effective delivery for Propolis but also aids in the creation of more effective remedies for inflammatory skin diseases.[17] Through this research, we aim to address the limitations of current topical anti-inflammatory therapies and explore the benefits of integrating natural bioactive compounds with advanced nanotechnology. Also, in comparison to other nanoparticles, Nanosponges are stable even at high temperatures, porous and non-toxic.[14,18-20]

DOI: 10.5530/ijper.20251073

Copyright Information :
Copyright Author (s) 2025 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

MATERIALS AND METHODS

Materials

Bee Propolis extract powder purchased from Bio-molecules Pvt. Ltd., (India). Ethyl cellulose was obtained from Sigma-Aldrich, (India). PVA and Xanthan gum were obtained from Research-Lab Fine Chem Industries (Mumbai, India). All other chemical used were of analytical grade.

Methods

Drug-excipient compatibility study

Using an Attenuated Total Reflectance (ATR) accessory, the FTIR spectra of pure Propolis and a physical mixture of excipients (1:1) were obtained using FTIR equipment (ATR alpha Brucker, Germany). Single spectra in the wavelength range of 4000 to 400 cm^{-1} were produced by averaging 10 scans at a resolution of 4 cm^{-1}.[21]

Propolis Loaded Nanosponges (PLN) Preparation

Propolis Loaded Nanosponges (PLN) were prepared by the emulsion solvent diffusion method.[2] First, ethyl cellulose was allowed to dissolve in 15 mL of dichloromethane and then Propolis was added into it, which was stirred using a mechanical stirrer until completely soluble. This mixture was considered as the organic internal phase. The internal phase (organic) was transferred drop wise to the aqueous external phase gradually, comprising PVA in 100 mL of purified water and stirring continued for 3 hr at 2500 rpm using a mechanical stirrer. The resulting dispersion was filtered and the collected filtrate then dried in an oven at 40°C for 24 hr, yielding a fine powder which was used for characterization. The formulation process was optimized using Central Composite Design (DESIGN EXPERT®, Version 13, State-Ease, Corporation, USA) was used to prepared different formulation. Ethyl cellulose (X1) and PVA (X2) were picked as the independent factors while production yield (Y1) and *in vitro* drug release (Y2) were designated as the dependent variables. Tables 1,2 representing Formulation batches were prepared and the responses were analyzed by different experimental models (linear, 2F, quadratic and cubic) to determine impact of independent variables. To assess the influence of external factors on answers, polynomial equations, 3D models and counterplots were utilized. Further optimized nanosponges formulation was selected and characterized.[22]

Preparation of Nanosponges gel

A gel basis with 2% w/v xanthan gum which is one of the most significant microbial polysaccharides that is commercially available.[23] has combined with the optimized batch of nanosponges. The procedure involved dispersing xanthan gum in 100 mL of distilled water and allowing it to swell over night at room temperature. The gel base was then mixed with nanosponges (equal to 10 mg of the medication) and used a magnetic stirrer (Bio Technics, India) to continuously whirl for 20 min until a uniform gel developed. For later analysis, the produced nanosponges gel was kept at 4°C. The same process was used to generate a pure drug-loaded gel. Sodium benzoate (0.1%) and glycerin (0.1%) were added to each formulation, respectively, as humectant and preservative.

Evaluation of Propolis loaded nanosponges

Production yield

Formula used for calculation of production yield

$$\text{Production yield} = \frac{\text{Practical yield of nanosponges}}{\text{Theoretical yield of nanosponges}} \times 100$$

Weighing the nanosponges after drying allowed us to determine the percentage yield of the various batches.[24]

In vitro Drug Release (DR)

In vitro studies (drug release) of the nanosponges were conducted using a USP Type II dissolution tester (Paddle Method). The nanosponges were placed inside dialysis membrane pouches, which were thereafter kept at 37±0.5°C in 250 mL of pH 6.8 phosphate buffers. At particular time intervals over a 4-hr period, 1 mL aliquots were withdrawn while maintaining a paddle speed of 50 rpm. A new batch of dissolving medium in an equivalent volume was used to replace each sample. At a wavelength of 356 nm, spectrophotometric analysis was performed on the obtained samples.[25]

Selection of optimized formulation

An overlay plot of % PY and DR was constructed by analyzing the response by ANOVA with the aim to achieve maximum %PY and DR. According to overlay plot, amount of EC and PVA was 484 mg and 183 mg. The optimized formulation was further prepared and evaluated.

Drug release kinetics from nanosponges porous matrix

Regression analysis was done after drug release result was added into 4 different kinetic models: zero-order, first-order, Higuchi and Korsmeyer-Peppas kinetics models.

$$Q_t = Q_0 + k_0 t \ (\text{zero} - \text{order}) \ \ldots\ldots\ldots (1)$$

$$\log Q_t = \log Q_0 - -\frac{k_1 t}{2.303} \ (\text{first} - \text{order}) \ldots\ldots\ldots (2)$$

$$Q_t = k_{H^{\frac{t1}{2}}} \ (\text{Higuchi}) \ldots\ldots\ldots (3)$$

$$\frac{M_t}{M_\infty} = kt^n \ (\text{Korsmeyer} - \text{peppas}) \ldots\ldots\ldots (4)$$

Where, k0 is the zero-order kinetics constant, k1 is the first-order rate constant and kHt1/2 is the constant for the Higuchi model. Qt, amount of drug dissolved over time t and Q0 is the initial amount of drug dissolved in the diffusion medium (which is equal to zero). The release period is denoted by time t; the cumulative release of drugs at time t and infinite time is represented by Mt

and M∞, respectively; and the diffusional exponent illustrating the release mechanism is indicated by 'n'.[26]

Entrapment Efficiency (EE)

10 mL of volumetric flask was filled with precisely 10 mg of Nanosponges that had been dissolved in ethanol. The efficiency of trapping was then assessed by agitating the liquid for 5 min. The capacity was increased to 10 mL by adding ethanol. Following the filtering and diluting of the solution, the concentration of nanosponges was then assess using UV Spectrophotometer at 368 nm.[21]

$$EE\ (\%) = \frac{\text{Initial amount of drug added} - \text{Drug amount in supernatant}}{\text{Initial amount of drug added}} \times 100$$

Particle size, Zeta Potential and Polydispersity Index (PDI)

The improved batch of formulation's size of particle, ZP and PDI were measured using the Horiba SZ-100 for Windows (Z type) Ver2.40. The stability indicator is the ZP. The PDI is measured to determine the homogeneity or heterogeneity of particle sizes within the formulation.[21]

Powder X-Ray Diffraction (PXRD) studies

Bruker AXS D-8 diffractometer (Berlin, Germany), equipped with a spinning target thermionic tube and a camera lens direction finder, to analyze the PXRD patterns of both the optimized batch of nanosponges and pure Propolis. The X-ray source consisted of Kα radiation generated from a copper target, utilizing a black lead monochromater. The thermionic tube was run at 150 mA of current and 40 kW of potential. The scans' range (2θ) was 5° to 70°, with increments of 0.1° and a speed of 2° per minute.[27-29]

Scanning Electron Microscopy (SEM)

Sample surfaces were examined using Scanning Electron Microscopy (SEM). When the specimen is exposed to a stream of fine electrons, secondary electrons are emitted from its surface. These emitted signals provide valuable information about the surface topography and composition. The analysis was conducted with a FEI Nova NanoSEM 450 equipped with a Bruker X Flash 6130, operating at a 10-kV accelerating voltage, to assess the surface morphology of the optimized batch of nanosponges.[28]

Differential Scanning Calorimetry (DSC)

We determined the thermal characteristics of the generated nanosponges using differential scanning calorimetry. (Toledo, Mettler) DSC experiments are conducted on a formulation containing pure medication, excipients and nanosponges. The sample (1-2 mg) was placed into aluminum pans (standard), heated and nitrogen was constantly purged at a rate of 80 mL/min. For 2 min while the sample was scanned at a rate of 10°C (from 0°C-300°C). The reference pan was a sealed, empty pan. Each measurement was made 3 times.[28]

Characterization of Propolis loaded nanosponges gel

pH, viscosity and spreadability

The pH of the pure drug incorporated in the gel base and the nanosponges gel was assessed in triplicate using a digital pH meter (Labman LMPH-9, India). The nanosponges gel and the marketed 0.5% gel (Acnesol, Systopic Laboratories Pvt. Ltd.,) was evaluated for viscosity by using Brookfield's Viscometer (LVDV, Brookfield, USA). Spindle no. 64 was used to measure viscosity and speed of rotation was 30, 50 and 100 rpm. Spreadability of nanosponges gel and marketed gel was shown in terms of work of adhesion Formula to calculate work of adhesion as follows.[30]

$$\text{Work of adhesion}\left(\frac{g}{sec}\right) = \frac{\text{Area under curve of tackiness}}{\text{Area under curve of firmness}}$$

Spreadability of the gel formulation was carried out using Brookfield texture analyzer CT3-100 (Brookfield Engineering Labs, Inc. USA). Formulation was inserted into the bottom cone; a 2 g trigger force was applied to force the conical analytical probe 45 down to a target of 10.0 mm at a speed maintained at 0.5 mM/s. Load Vs time graph were generated by the software. Various characteristics of gel formulation including tackiness, firmness, stringiness and work of adhesion were studied and compared with marketed gel.[31-33]

Drug content

1 g of formulation gel was thoroughly dissolved in ethanol to ascertain the drug concentration of the gel. It was sonicated for 30 min so that drug is completely dissolved in ethanol. Using ethanol as a blank, a 1 mL aliquot was collected and subjected to analysis using a UV visible spectrophotometer operating at 368 nm.[21]

$$\text{Drug content\%} = \frac{\text{Actual drug content}}{\text{Theorotical drug content}} \times 100$$

Drug diffusion *ex vivo* and *in vitro*

The Franz diffusion cell apparatus was employed to conduct drug diffusion *ex vivo* and *in vitro* studies using nanosponges and pure Propolis-loaded gel. The temperature kept constant at 37±0.5°C during the experiments. Cellophane and goat skin membrane were used to perform out *in vitro* and *ex vivo* drug diffusion studies respectively, by transferring gel equivalent to 10 mg of Propolis. Study was carried out for 4 hr by maintaining similar experimental conditions as mentioned under the drug diffusion studies of nanosponges formulation.[34,35]

Antimicrobial activity

Using the agar well diffusion method, the antimicrobial activities of Propolis-Loaded Nanosponges (PLN) and Ethanolic Propolis Extract (PEE), Nanosponges (PLN) gel+Plain gel+Marketed gel were compared. The zone of inhibition against *Staphylococcus aureus* (Gram+ve), *E. coli* (Gram-ve) and *Candida albicans* was

determined.[36] The culture of bacteria was developed in nutritive broth and incubated at 37°C for 24 hr, while the culture of fungus was generated in sabouraud dextrose broth and then incubated at 37°C for 24 hr. A 250 mL conical flask containing 100 mL of deionized water was filled with precisely weighed agar. It was cooked until all of the lumps were gone. The resulting solution was autoclaved for 30 min at 15 lb pressure and 1210°C to sterilize it. Sterile agar solution was mixed with the chilled mixture. The resultant medium was placed onto a sterile petri dish and allowed to harden. On a plate, strains of *Candida albicans, E. coli* and *S. aureus* were streaked. Bore wells (6 mm in diameter) on agar plates were filled with varying concentrations of PLN and PEE, as well as nanosponges gel, plain gel and commercial gel. The wells incubated for 24 hr at 37°C. Millimeters were used to measure and record the inhibition zones. We next compared the zones of inhibition for the gel that was commercialized and the gel made using nanosponges.[37]

In vivo Animal Study

Male Wistar rats weighing between 210 and 250 g were used in an *in vivo* animal investigation. The rats were divided into three groups, each with six rats. Inflammation was induced using 2.5% formalin, in all the study groups and the subsequent swelling was measured using Vernier calipers at multiple time points. Three groups were included in the study and inflammation was induced in all of them. Positive Control Group**:** This group serving as a baseline for the inflammatory response. Test Group: This group was treated with the test formulation PLN based topical gel (2%w/w) to evaluate its potential anti-inflammatory effect. Standard Group**:** This group was treated with a standard anti-inflammatory drug (Diclofenac gel: 2%w/w) to provide a comparison of efficacy. The time points for measuring the swelling and scoring edema were set at 0 min, 3 hr, 4 hr, 5 hr, 6 hr, 20 hr, 24 hr, Day 3 and Day 7. Vernier caliper readings were taken in millimeters to quantify the swelling, while the severity of edema was scored at each of these time points using a predefined scoring system.[18,38]

Histopathological study

After the treatment period, animals were humanely euthanized in accordance with ethical guidelines and the affected tissue samples, typically from the skin where inflammation was induced, were collected. 10% neutral-buffered formalin was used to fix the tissues, to preserve their morphology for subsequent histological analysis. Following fixation, the tissues were embedded in paraffin blocks to maintain structural integrity during sectioning. Using a microtome, thin tissue sections (5-10 microns thick) were cut to prepare samples for microscopic examination. The slices were stained using Hematoxylin and Eosin (H&E), a typical histology technique. Hematoxylin dyes cell nuclei blue or purple, while eosin dyes the cytoplasm, extracellular matrix and other tissue components pink or red. These stained sections were mounted on microscope slides for further examination.[39] Histopathological analysis involved examining the stained tissue sections under a microscope at various magnifications, such as 10x. In the Positive Control Group, signs of inflammation, particularly Mononuclear Cell (MNC) infiltration in the dermis, were expected. In the Standard Group, no inflammation or abnormalities were anticipated. The Test Group was evaluated for the effects of the anti-inflammatory treatment by comparing the degree of MNC infiltration with that in the Positive Control Group. Microscopic images for each group were captured at the designated magnification and important features, such as MNC infiltration, were marked with arrows for clarity.[18]

RESULTS

Drug excipient compatibility study by using FTIR

The Figure 1 presents FTIR spectroscopy-based compatibility study comparing pure Propolis and a physical admixture. The FTIR spectrum for pure Propolis shows peaks for key functional groups, including O-H stretching at 3585.49 cm^{-1}, C-H stretching at 2973.69 cm^{-1}, C-H deformation at 1455.18 cm^{-1}, C-O-C stretching at 823.18 cm^{-1} and another C-H vibration at 763.74 cm^{-1}. In contrast, the physical admixture's FTIR spectrum reveals similar functional groups but with slight shifts: O-H stretching at 3052.67 cm^{-1}, C-H stretching at 2869.65 cm^{-1}, C-H deformation at 1475.01 cm^{-1} and C-O-C stretching at 827.74 cm^{-1}. These shifts suggest possible interactions between Propolis and the components of the admixture, indicating some molecular changes and potential compatibility between the substances. The analysis demonstrated how FTIR spectroscopy was used to observe these interactions by identifying changes in the functional group absorption peaks.

Production yield

Nanosponges production yield was calculated which was found in the range of 12 to 48.85%. All batches of nanosponges' percent yields were computed using the theoretical and empirical yield data. The study evaluates the production yield (%) of nine formulations (F1 to F9), highlighting significant variability in manufacturing efficiency. Formulation F9 achieved the highest yield at 48.28%±0.08**,** followed by F4 (38%±0.07), F5 (33%±0.08) and F3 (30%±0.14), indicating well-optimized processes. Similarly, F1 and F7 also showed moderate yields of 30%±0.08**,** suggesting consistent but slightly less efficient production compared to the top performers. In contrast, F2 (12.07%±0.06) and F8 (14.85%±0.1) demonstrated the lowest yields, reflecting inefficiencies such as material losses or suboptimal process conditions. F6 (17%±0.06) and F1 (19%±0.08) showed moderate yields but remained significantly lower than F9. These results highlight the need for optimization in low-yield formulations (F2 and F8) and further investigation into the reproducibility and scalability of high-yield formulations (F9 and F4) to ensure consistent manufacturing performance. The responses of all the

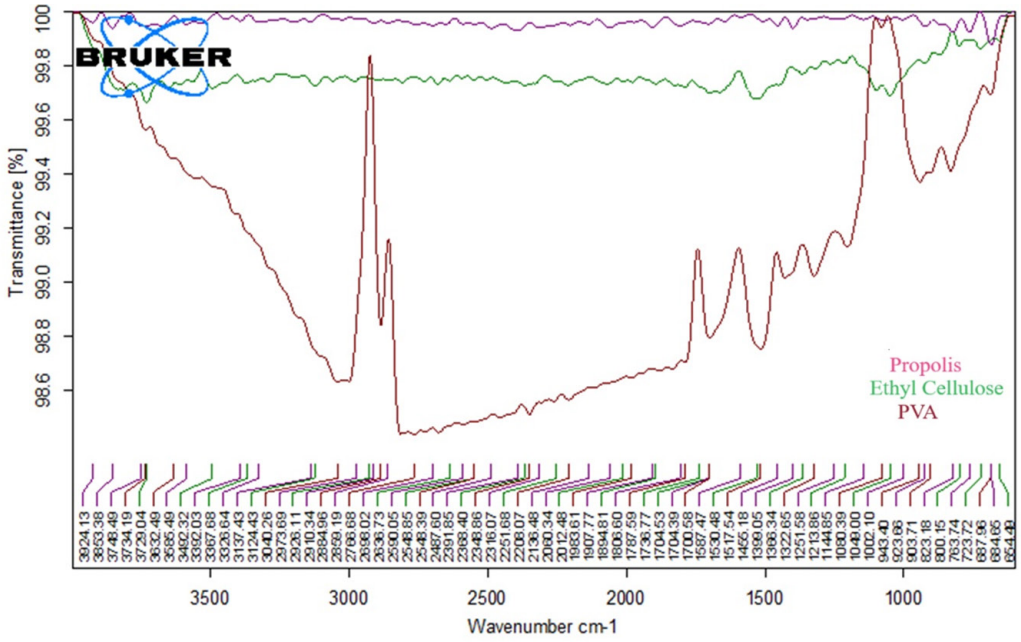

Figure 1: FTIR Propolis, Ethyl cellulose, PVA (Overlay).

formulation batches were entered into different experimental models (linear, 2F accomplished, quadratic and cubic). For production yield, the quadratic model was determined to be a significant model. R^2 and *p* value was 0.9241 and 0.0009 ($p<0.05$).[19]

In vitro drug release of PLN

For each of the four formulations, *in vitro* drug release experiments were conducted using the USP Type II paddle method. Each formulation showed evidence of drug release over four hr at 37°C in a 6.8 pH phosphate buffer. It was discovered that the medication release from the nanosponges varied from 64% to 85%. Order of release of drug was B2>B7>B6>B9>B1> B8>B4>B3> B5 respectively Figure 2. Difference in drug release pattern from nanosponges was due to difference concentration of polymer i.e. Ethyl cellulose and Polyvinyl alcohol. Batch B2 and B7 showed greater drug release of 96.62±0.02% and 94.02±0.04% respectively. This may be due hydrophilic nature of PVA and because of low concentration of Ethyl cellulose and high concentration of PVA. Conversely, the release kinetics of Propolis over a 4 hr period were significantly impacted by the hydrophobic properties of EC and phase transition. Slow drug release was noticed in batch B4, B3, B5 and B8 of 82.55±0.016%, 80.17±0.020%, 77.14±0.061%, 83.63±0.04% respectively which contains higher amount of ethyl cellulose. The linear model was determined to be the most significant model for drug release and 7 R^2, F and *p* value were 0.9880, 115.30 and <0.0001 respectively. Model terms with a P value of less than 0.05 are deemed significant.[19]

Selection of optimized batch

Selection of optimized PLN batch was done on the basis of overlay response of PY and DR focusing on maximizing drug release. According to data generated by Design Expert® software, the optimized PLN batch contained 484 mg of ethyl cellulose and 183 mg of polyvinyl alcohol. The optimized batch's cumulative drug release study result was fitted into four mathematical models of release kinetics using regression analysis. The R^2 values obtained were 0.9165 for zero order, 0.2737 for first order, 0.936 for Korsmeyer-Peppas and 0.9976 for Higuchi kinetics. The results indicated that the release profile closely aligned with the Higuchi diffusion kinetic model, as evidenced by the coefficient of correlation (R^2=0.9976).

Characterization of optimized formulation

Production yield and in vitro drug release

The predicted values for responses were production yield (56.31%) and drug release (87.59%) respectively. Actual experimental results for PY and DR were 56.06±0.06% and 87.09±0.06% respectively. The predicted value of PY and DR was in agreement with experimental results.

Entrapment Efficiency

The Entrapment Efficiency (EE) values of various formulation batches (F1 to F9 and OB), highlighting their effectiveness in encapsulating the drug. All formulations demonstrate high EE, with values ranging from 93.65% (F8) to 99.9% (OB). The Optimized Batch (OB) achieves the highest EE of 99.9%, signifying its superior capability for drug incorporation, which could translate into enhanced therapeutic efficacy. Among the

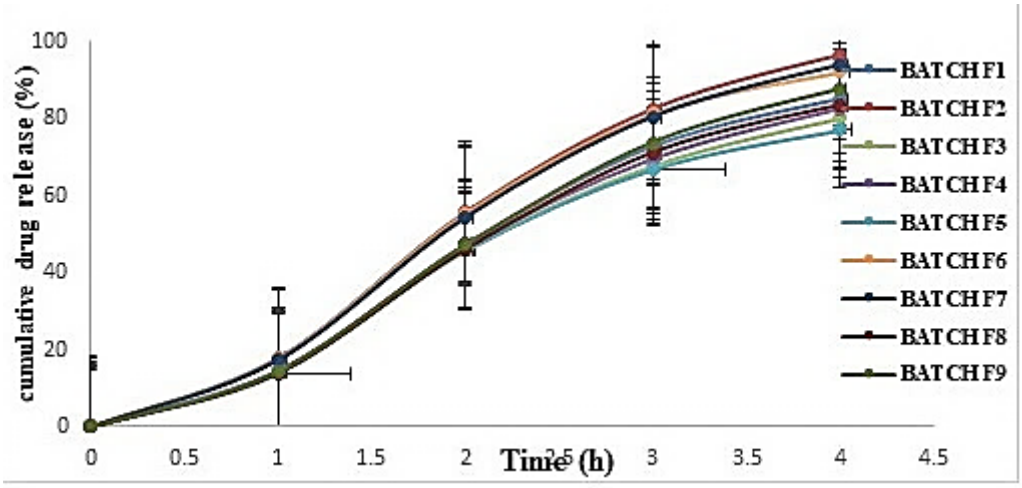

Figure 2: *In vitro* drug release of nanosponges.

other formulations, F3 stands out with an EE of 98.08%, suggesting it as a strong alternative to OB. Similarly, F5 (97.76%) and F9 (97.83%) also exhibit excellent efficiencies, confirming their robustness in drug entrapment. On the other hand, F6, F7 and F8 show slightly lower but still acceptable EE values in the range of 93.65% to 93.77%, maintaining a consistent performance. The minimal standard deviations across all formulations underline the reliability and reproducibility of the formulation process, ensuring consistent quality.

Particle size, Zeta potential and Polydispersity index

The average size of the PLN particles was found to be 231.9 nm. The result of the polydispersity index was 0.457 confirming moderate polydispersity particles with better homogeneity. The zeta potential of the prepared PLN was -35.4 mV, indicating colloidal stability. The developed Propolis Loaded Nanosponges (PLN) displayed a size in the 231.9 nm range, PDI (0.457) and, ZP (-35.4 mv), It has been demonstrated that. Particle size, ZP and PDI increases in response to an increase in the concentration of EC polymer. Increased EC polymer concentration impacts the formulation process by enhancing polymer deposition, altering surface properties and affecting dispersion stability. These changes collectively contribute to larger particle size, modified ZP and higher PDI values.

Powder X-Ray Diffraction (PXRD) studies

Pure Propolis does not have the structural benefits required for controlled release and could lead to faster degradation or uneven release in a formulation due to less uniform particles. The optimized nanosponges offer a controlled and sustained release of Propolis due to their structural uniformity, smoother surface and smaller particle size, which is critical for maintaining a steady therapeutic effect over time.

Scanning Electron Microscopy (SEM)

The provided images in Figure 3 are Scanning Electron Microscopy (SEM) images of particles, possibly nanosponges, shown at different magnifications. The uniformity in size and shape suggests a well-controlled synthesis process. The PLN with small grooves texture of the larger spheres indicate a consistent polymerization or encapsulation process.

Differential Scanning Calorimetry (DSC)

DSC Thermograms overlay of pure Propolis, EC, PVA and Formulation were displayed in Figure 3. The melting point of Propolis, which is represented by the exothermic DSC curve of a Propolis, is 87.07°C. Ethyl cellulose and PVA showed endothermic peak at 233.59°C and 195.03°C specific at its melting point.[21]

Formulation of gel

By using 2% xanthan gum as a gelling agent. The nanosponges gel was formulated and evaluated.

Evaluation of nanosponges gel

pH, drug content, viscosity and spreadability

The pH of the nanosponges gel was measured at 6.8 and its drug content was found to be 99.8%±0.43%, indicating consistent distribution throughout the gel.

A comparison of viscosity between the nanosponges gel and a marketed gel at different rotational speeds shows that the nanosponges gel has a lower viscosity across all speeds tested, suggesting easier spreadability. At 30 rpm, the nanosponges gel's viscosity was 8060 cp, compared to the marketed gel's 9140 cp. This difference persists at 60 rpm (5040 cp vs. 6150 cp) and 100 rpm (2639 cp vs. 3617 cp). Both gels exhibited shear-thinning behavior, with viscosity decreasing as speed increased, typical of non-Newtonian fluids like gels.

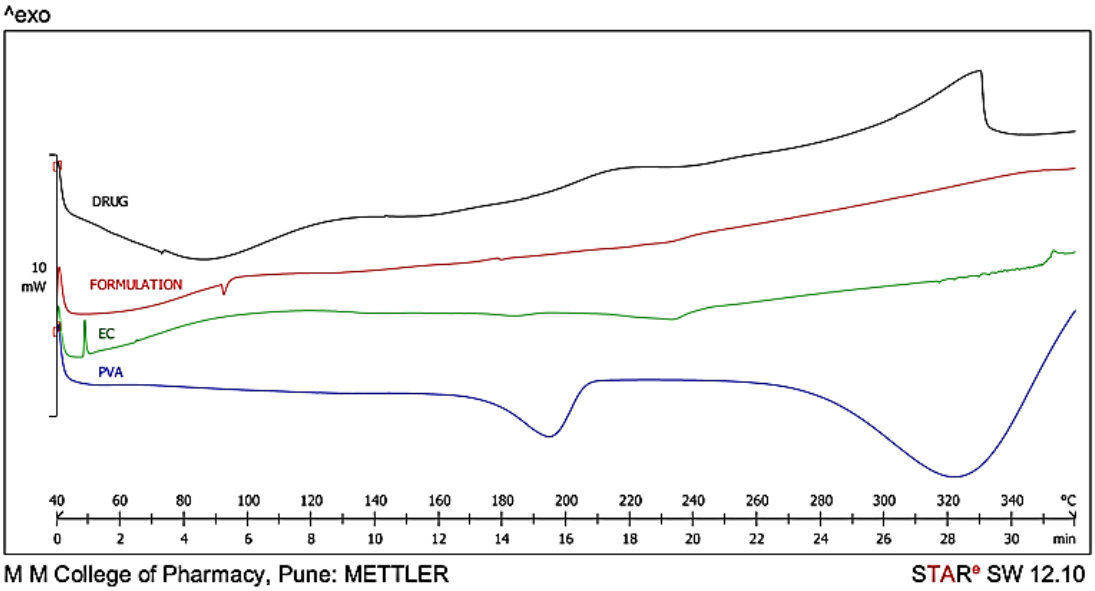

Figure 3: DSC of Propolis, EC, PVA formulation (overlay).

Physical property comparisons revealed that the formulation gel has slightly lower firmness (650 g vs. 715 g) but higher tackiness (455 g vs. 445 g) than the marketed gel, suggesting it may adhere more strongly to surfaces. The work of adhesion for the formulation gel (0.7 g/sec) was also higher, indicating stronger surface interaction, while the marketed gel showed slightly more spreadability with a stringiness of 0.3 mm compared to 0.2 mm for the formulation gel. Overall, the nanosponges gel offers a balance of easier application due to lower viscosity and a strong adhesive profile, while the marketed gel shows slightly higher firmness and spreadability.

In vitro drug diffusion study of gel

The graph presents the cumulative drug diffusion (%) of three gel formulations-Plain Gel, Nanosponge Gel and Marketed Gel-over a 4-hr period Figure 4. The Plain Gel exhibited the slowest drug release, with only 20% diffusion at 1 hr and increasing gradually to approximately 50% at 4 hr, indicating a limited drug delivery efficiency. In contrast, the Nanosponge Gel showed a significantly enhanced release profile, achieving 40% diffusion at 1 hr and reaching nearly 100% at 4 hr, showcasing its ability to provide controlled and sustained drug release due to the nanosponge particles. The Marketed Gel demonstrated an intermediate release pattern, with 30% drug diffusion at 1 hr and approximately 85% at 4 hr, suggesting moderate efficiency compared to the Nanosponge Gel. These results highlight the superior performance of the Nanosponge Gel in achieving faster and more complete drug release, making it a promising alternative to conventional and marketed formulations.

Ex vivo permeation study of gel

On the abdomen skin of goats, an *ex vivo* permeation research was done. It was found that nanosponges gel (79.82±0.04%) had high permeation as compared to plain gel (33.72±0.04%) and marketed gel (74.19±0.04%). The drug's penetration and solubility were improved by being entrapped within the porous, three-dimensional network structure of the nanosponge formulation.

Table 1: Formulation factors and their responses.

Variables	Levels	
Independent Factors	**Low**	**High**
X1=Ethyl Cellulose(mg)	300	600
X2=PVA (mg)	300	600
Dependent Factors		
Y1=Production yield (%)		
Y2=Drug release (%)		

Zone of Inhibition Study

The zone of inhibition analysis Table 3 reveals varying levels of antibacterial activity among the different formulations tested. In the top row, pure drugs demonstrate significant inhibitory effects on all tested bacteria, with zones of inhibition measuring 38 mm for *C. albicans*, 35 mm for *S. aureus* and 35 mm for *E. coli*. Notably, *C. albicans* exhibits the largest inhibition zone, indicating the strongest antibacterial effect among the pure drugs. In the middle row, plain gels also display antibacterial properties, albeit with reduced effectiveness compared to the pure drugs. The zones of inhibition for the plain gels are 44 mm for *C. albicans*, 42 mm for *S. aureus* and 46 mm for *E. coli*, suggesting that these formulations retain substantial antibacterial activity, particularly against *E. coli*, which shows the largest inhibition zone among the gel formulations. The bottom row presents the nanosponges

gels, which demonstrate varied inhibition zones of 38 mm for *C. albicans*, 36 mm for *S. aureus* and 41 mm for *E. coli*. These results indicate that while the nanosponges maintain good antibacterial activity, their effectiveness is slightly lower compared to plain gels for *S. aureus* and similar for *E. coli*, although they still show considerable efficacy, especially against *C. albicans*. Finally, the marketed gels display lower zones of inhibition compared to both the plain gels and nanosponges, measuring 34 mm for *C. albicans*, 32 mm for *S. aureus* and 37 mm for *E. coli*. This suggests that although the marketed gels are effective, they do not offer the same level of antibacterial activity as the experimental formulations, particularly against *C. albicans* and *S. aureus*. Overall, the analysis indicates the potential of the experimental formulations in delivering enhanced antibacterial effects.

In vivo Animal Study evaluation

The *In vivo* Anti-inflammatory study report aimed to assess the anti-inflammatory efficacy of a test compound in comparison to a positive control and a standard reference drug, utilizing Vernier caliper readings Table 4 and edema scoring across various time points. Inflammation was induced in all groups, with swelling monitored through Vernier calipers at intervals including 0 min, 3 hr, 4 hr, 5 hr, 6 hr, 20 hr, 24 hr, Day 3 and Day 7. The groups included a Positive Control, receiving an inflammation-inducing agent; a Test Group, administered the test compound; and a Standard Group, treated with a known anti-inflammatory drug. Swelling was assessed via caliper measurements and edema severity was scored at designated time intervals. The outcomes demonstrated that the Positive Control group exhibited a peak in swelling at 5 hr, which gradually decreased, while the Test Group, displayed significant swelling at 3 hr with a steady reduction over time. The Standard Group had the highest initial swelling but demonstrated the most effective reduction in inflammation by Day 7. Edema scoring mirrored these findings, with the Positive Control Initially showing severe edema that resolved by Day 7, while the Test Group also exhibited similar trends but maintained

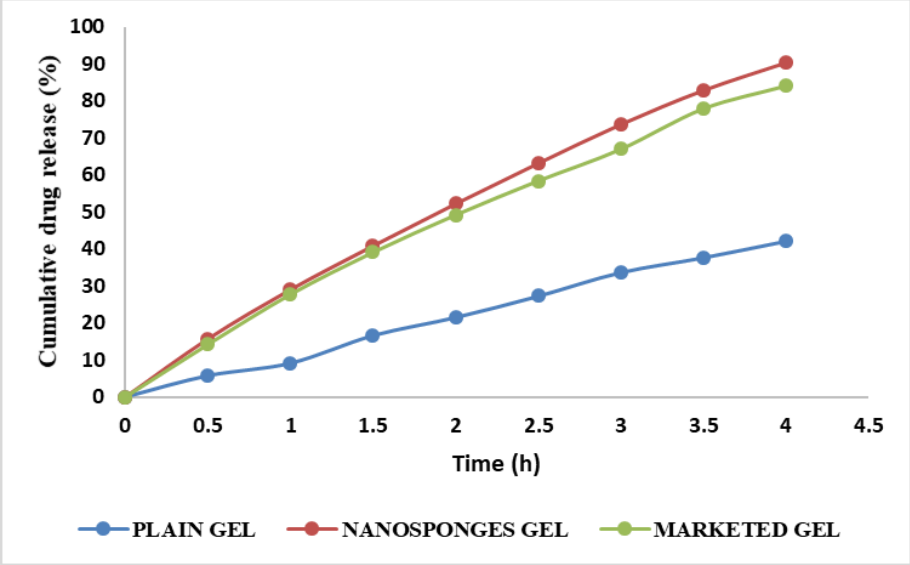

Figure 4: *In vitro* drug diffusion of plain gel, nanosponges gel and marketed gel.

Table 2: Formulation table of nanosponges.

Formulation	Drug (mg)	Ethyl Cellulose (mg)	Polyvinyl Alcohol (mg)	Dichloromethane (mL)	Distilled Water (mL)	RPM	Time
F1	100	450	450	15	50	2500	3 hr
F2	100	237.868	450	15	50	2500	3 hr
F3	100	600	600	15	50	2500	3 hr
F4	100	600	300	15	50	2500	3 hr
F5	100	662.132	450	15	50	2500	3 hr
F6	100	300	600	15	50	2500	3 hr
F7	100	300	300	15	50	2500	3 hr
F8	100	450	662.132	15	50	2500	3 hr
F9	100	450	237.868	15	50	2500	3 hr

Table 3: Zone of inhibition study.

Zone of Inhibition	*S. aureus*	*Candida albicans*	*E. coli*
Drug+Ethanol	35-10=25 mm	38-10=28 mm	35-10=25 mm
Nanosponges	42-10=32 mm	44-10=34 mm	46-10=36 mm
Plain gel	24 mm	30 mm	28 mm
Nanosponges gel	36 mm	38 mm	41 mm
Marketed gel	32 mm	34 mm	37 mm

Table 4: Anti-inflammatory model [Vernier Caliper reading (mm)].

Group	0 min	3 hr	4 hr	5 hr	6 hr	20 hr	24 hr	Day 3	Day 7
Positive Control	4.84	5.74	5.92	6.18	6.1	5.96	5.68	5.02	4.97
Test	4.98	7.84	7.7	7.4	7.3	7.02	6.52	5.15	4.99
Standard	5.87	8.32	8.18	7.47	7.13	6.92	6.92	5.98	5.91

a minimal presence of edema. Overall, the test compound displayed moderate anti-inflammatory efficacy, effectively reducing inflammation over time, though the standard treatment was more effective in achieving early resolution. Additionally, skin reactions were evaluated for erythema and edema using a standardized scoring system, where the Positive Control was expected to show moderate to severe erythema, while the test and standard groups were anticipated to exhibit minimal to slight erythema, indicating irritation but not toxicity. The outcomes suggest that both the test compound and standard treatment have anti-inflammatory potential, with the standard performing better in terms of early resolution of inflammation.

Histopathological evaluation

The histopathological analysis revealed distinct differences in the levels of Mononuclear Cell (MNC) infiltration across the three groups examined. In the Positive Control Group, notable signs of inflammation were evident, characterized by significant MNC infiltration in the dermis, indicating an inflammatory response. Conversely, the Standard Group exhibited no signs of inflammation or abnormalities, suggesting a healthy baseline tissue state. The Test Group, which received the anti-inflammatory treatment, demonstrated a marked reduction in MNC infiltration compared to the Positive Control Group, indicating the efficacy of the treatment in mitigating inflammatory responses. Microscopic images captured at 10x magnification, effectively illustrated these differences, with clear markings of MNC infiltration in the Positive Control Group, while the Test Group images showed reduced MNC presence. These findings support the conclusion that the anti-inflammatory compound tested possesses significant properties that reduce inflammatory markers, as evidenced by the reduction in MNC infiltration. Overall, this research offers compelling evidence for the anti-inflammatory efficacy of the test treatment.

DISCUSSION

The study evaluated various aspects of nanosponges; formulations for Propolis, focusing on production yield, drug release, optimization, characterization and therapeutic efficacy. Production yield ranged from 12% to 48.85%, with a quadratic model yielding significant results (R^2=0.9241, p=0.0009). *In vitro* drug release for different formulations, assessed using the USP Type II paddle method, demonstrated drug release between 64% to 85%, with Batch B2 showing the highest release at 96.62%, influenced by polymer concentration. A linear model best described the drug release kinetics (R^2=0.9880, p<0.0001). The optimized batch of Propolis-Loaded Nanosponges (PLN) was determined using overlay responses, achieving a release profile aligned with the Higuchi diffusion model (R^2=0.9976). Characterization included high entrapment efficiency (up to 99.9% in optimized batch), particle size (231.9 nm), moderate Polydispersity (PDI=0.457) and colloidal stability (zeta potential=-35.4 mV). Analytical techniques like PXRD, SEM, DSC and FTIR confirmed structural integrity, stability and drug-polymer compatibility. A nanosponge gel was formulated with 2% xanthan gum, demonstrating superior spreadability, lower viscosity and higher drug diffusion (90.43%) compared to marketed gels. *Ex vivo* studies showed enhanced skin permeation (79.82%) for the nanosponge gel. Antibacterial activity was maintained across formulations, with nanosponges showing notable inhibition zones against *C. albicans* and *E. coli*. The *in vivo* anti-inflammatory study demonstrated moderate efficacy of the nanosponge formulation, with significant reduction in swelling and edema over time, though slightly less effective than a standard treatment. Histopathological evaluation further supported the anti-inflammatory potential, showing reduced mononuclear cell infiltration in treated tissues. Overall, the study suggests that nanosponge formulations of Propolis offer a promising approach for enhanced drug delivery and therapeutic outcomes.

CONCLUSION

Nanosponge is one of the innovative drug deliveries, which is especially used for the successful topical delivery of the drug, the goal of creating a novel topical anti-inflammatory gel formulation was successfully accomplished. The Propolis nanosponges were successfully delivered since all the parameters produced results within the required range and Propolis has been used to treat topical skin inflammation due to its strong anti-inflammatory properties.

The outcome demonstrated the combination's potential as an anti-inflammatory agent for topical gel formulation. Based on the evaluation parameters, the formulation of the optimized nanosponges produced the desired positive outcomes when compared to the topical gel formulation.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to JSPM's Rajarshi Shahu College of Pharmacy and Research, Tathawade, Pune, India and the Research Center Marathwada Mitra Mandal's College of Pharmacy, Thergoan, Pune, India for providing the essential infrastructure, laboratory facilities and technical support necessary for the successful completion of this research. We are also profoundly thankful to Savitribai Phule Pune University, Pune for its unwavering academic support and guidance throughout the research process. Furthermore, we extend our appreciation to Bio-molecules Pvt. Ltd., (India) for supplying Propolis and Sigma-Aldrich (India) for providing Ethyl Cellulose.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Conc: Concentration; **ºC:** Degree Centigrade; **FTIR:** Fourier Transform Infrared Spectroscopy; **G:** Gram, **hr:** Hour; **Mg:** Microgram; **mg:** Milligram; **min:** Minute; **mL:** Milliliter; **nm:** Nanometer; **No:** Number; **rpm:** Revolutions per minute; **PY:** Production Yield; **DR:** Drug Release; **SD:** Standard Deviation; **Sr. No:** Serial Number; **UV:** Ultraviolet; **w/w:** Weight by weight; **mm:** Micrometer; **%:** Percentage; **β:** Beta.

ETHICAL STATEMENT

This research study involving the formulation and evaluation of Propolis-loaded nanosponges-based topical anti-inflammatory gel was conducted following the approval of the Institutional Animal Ethics Committee (IAEC) of Preclinical Research and Development Organization (PRADO), Pvt. Ltd., Pune in compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Approval no. IAEC-24-037). All procedures were performed with efforts to minimize animal discomfort, distress and pain, adhering to the principles of Replacement, Reduction and Refinement. No human subjects were involved in this study.

SUMMARY

The study aimed to prepare and assess a Propolis-Loaded Nanosponges (PLN) based topical gel with anti-inflammatory, antifungal and antibacterial properties. PLNs were prepared using the emulsion solvent diffusion process with polyvinyl alcohol and ethyl cellulose. Various parameters, including particle size, drug release and entrapment efficiency, were analyzed to optimize the formulation. The physicochemical characteristics were evaluated using FTIR, DSC, SEM and XRD. The optimized PLN was incorporated into a 2% w/w gel and further tested for properties such as pH, viscosity, drug diffusion, skin penetration and antimicrobial activity. Results showed that the gel had high drug encapsulation efficiency, with 90.43% *in vitro* drug diffusion and 79.82% *ex vivo* skin penetration within 4 hr. It exhibited strong antifungal and antibacterial effects and demonstrated comparable anti-inflammatory efficacy to a standard Diclofenac gel. Overall, the Propolis-loaded nanosponges gel showed potential as an effective topical treatment for skin inflammation, with promising drug delivery and antimicrobial properties.

REFERENCES

1. Bankova V. Chemical diversity of Propolis and the problem of standardization. J Ethnopharmacol. 2005; 100(1-2): 114.
2. Damasco JA, Ravi S, Perez JD, Hagaman DE, Melancon MP. Understanding nanoparticle toxicity to direct a safe-by-design approach in cancer nanomedicine. Nanomaterials. 2020;10:2186.
3. Zulhendri F, Lesmana R, Tandean S, Christoper A, Chandrasekaran K, Irsyam I, *et al*. Recent update on the anti-inflammatory activities of Propolis. Molecules. 2022; 27(23):8473. doi: 10.3390/molecules27238473.
4. Sforcin JM. Biological properties and therapeutic applications of Propolis. Phytother Res. 2016;30:894-905.
5. Sforcin JM. Propolis and the immune system: a review. J Ethnopharmacol. 2007;113(1):1-14.
6. Toreti VC, Sato HH, Pastore GM, Park YK. Recent progress of Propolis for its biological and chemical compositions and its botanical origin. Evid Based Complement Alternat Med. 2013;2013:697390.
7. Veiga RS, De Mendonça S, Mendes PB, Paulino N, Mimica MJ, Lagareiro Netto AA, *et al*. Artepillin C and phenolic compounds responsible for antimicrobial and antioxidant activity of green Propolis and *Baccharis dracunculifolia* DC. J Appl Microbiol. 2017;122:911-20.
8. Sihorkar V, Jain NK. Nanocarriers in the management of topical psoriasis: advanced drug delivery systems in topical dermatology. Nanomedicine. 2008;4(1):60-9.
9. Pasupuleti VR, Sammugam L, Ramesh N, Gan SH. Honey, Propolis and royal jelly: A comprehensive review of their biological actions and health benefits. Oxid Med Cell Longev. 2017;2017:1259510.
10. Appleton SL, Tannous M, Argenziano M, Muntoni E, Rosa AC, Rossi D, *et al*. Nanosponges as protein delivery systems: Insulin, a case study. Int J Pharm. 2020;590:119888.
11. Ansari KA, Torne SJ. Cyclodextrin-based nanosponges for drug delivery. J Incl Phenom Macrocycl Chem. 2011;69(3):371-83.
12. Swaminathan S, Cavalli R, Trotta F. Cyclodextrin-based nanosponges: a versatile platform for drug delivery. Int J Pharm. 2010;398(1-2):190-5.
13. Oryan A, Alemzadeh E, Moshiri A. Potential role of Propolis in wound healing: Biological properties and therapeutic activities. Biomed Pharmacother. 2018;98:469-83.
14. Silva MV, de Moura Jr NG, Motoyama AB, Ferreira VM. A review of the potential therapeutic and cosmetic use of Propolis in topical formulations. J Pharm Biomed Sci. 2023;15(3):123-34.
15. Mura S, Manconi M, Sinico C, *et al*. Penetration enhancer-containing vesicles as carriers for dermal delivery of minoxidil. Int J Pharm. 2009;380(1-2):72-9.
16. Gupta M, Vyas SP, Singh V. Development and characterization of a liposomal system for topical delivery of celecoxib. J Liposome Res. 2010; 20(1):68-75.

17. Elkhenany H, El-Badri N, Dhar M. Green Propolis extract promotes *in vitro* proliferation, differentiation and migration of bone marrow stromal cells. Biomed Pharmacother. 2019;115:108861.
18. Balderas-Cordero D, Canales-Alvarez O, Sánchez-Sánchez R, Cabrera-Wrooman A, Canales-Martinez MM, Rodriguez-Monroy MA. Anti-inflammatory and histological analysis of skin wound healing through topical application of Mexican Propolis. Int J Mol Sci. 2023; 24(14):11831. doi: 10.3390/ijms241411831.
19. Patel S, Pandya K, Shah N, Gohil D, Aundhia C. Development of risedronate sodium loaded nanosponges by experimental design optimization and *in vitro* characterization. Indian J Pharm Sci. 2019;81:309-18.
20. Kaur IP, Kakkar V. Nanoscience in dermal and transdermal drug delivery. Skin Pharmacol Physiol. 2010;23(4):193-208.
21. Ahmed MM, Fatima F, Anwer MK, Ibnouf EO, Kalam MA, Alshamsan A, *et al*. Formulation and *in vitro* evaluation of topical nanosponge-based gel containing butenafine for the treatment of fungal skin infection. Saudi Pharm J. 2021;29:467-77.
22. Praveen K, Balamurugan K. Targeted drug delivery through nanosponges and its approach. Res J Pharm Technol. 2020; 13:3524-31.
23. Jadav M, Pooja D, Adams DJ, Kulhari H. Advances in xanthan gum-based systems for the delivery of therapeutic agents. Pharmaceutics. 2023;15(2):402. doi: 10.3390/pharmaceutics15020402.
24. Ghurghure S, Kamalapurkar K, Thorat Y, Pathan M, Sana M, Pathan A. Preparation and *in vitro* evaluation of itraconazole-loaded nanosponges for topical drug delivery. Indo Am J Pharm Res 2019;9(4):1-8. DOI: 10.5281/zenodo.2659719.
25. Manyam N, Budideti KKR, Mogili S. Formulation and *in vitro* evaluation of nanosponge loaded extended release tablets of trimethoprim. UPI J Pharm Med Health Sci. 2021; 1:78-86.
26. Pudjiastuti P, Wafiroh S, Hendradi E, Darmokoesoemo H, Harsini M, Fauzi M, *et al*. Disintegration, *in vitro* dissolution and drug release kinetics profiles of k-carrageenan-based nutraceutical hard-shell capsules containing salicylamide. Open Chem. 2020; 18:226-31.
27. Alshehri S, Shakeel F, Ibrahim M, Elzayat E, Altamimi M, Shazly G, *et al*. Influence of the microwave technology on solid dispersions of mefenamic acid and flufenamic acid. PLoS One. 2017;12.
28. Memon S, Bhise K. Microwave irradiation technique, a green chemistry approach for dissolution enhancement of lopinavir. J Pharm Res Int. 2022;34(13):13-24.
29. Sharma K, Kadian V, Kumar A, Mahant S, Rao R. Evaluation of solubility, photostability and antioxidant activity of ellagic acid cyclodextrin nanosponges fabricated by melt method and microwave-assisted synthesis. J Food Sci Technol. 2022;59:898-907.
30. Tonchev M, Atanasov T, Todorova A, Atanasova Ts, Shtrankova N, Momchilova M, Zsivanovits G. Sensory and instrumental texture analysis of Bulgarian commercial pates. Agric Sci Technol. 2017;9:251-6.
31. Hurler J, Engesland A, Kermany BP, Škalko-Basnet N. Improved texture analysis for hydrogel characterization: Gel cohesiveness, adhesiveness and hardness. J Appl Polym Sci. 2012;125:180-8.
32. Sarah Y, Muzib YI. Formulation and optimization of quercetin loaded nanosponges topical gel: *Ex vivo*, pharmacodynamic and pharmacokinetic studies. Int J Appl Pharm. 2019;11:156-65.
33. Serri A, Mahboubi A, Zarghi A, Moghimi HR. PAMAM-dendrimer enhanced antibacterial effect of vancomycin hydrochloride against gram-negative bacteria. J Pharm Pharm Sci. 2018;22:10-21.
34. Chauhan V, Malviya N. Design, development and evaluation of nanosponges loaded topical gel for rejuvenation of skin. Int J Drug Deliv Technol. 2023;13:1277-82.
35. Nagoba S, Shaikh S, Swami A. Formulation and evaluation of nanosponges hydrogel for topical drug delivery containing griseofulvin. Int J Med Pharm Sci 2020;57-70.
36. Przybyłek I, Karpiński TM. Antibacterial properties of Propolis. Molecules. 2019; 24:2047.
37. Patel J, Ketkar S, Patil S, Fearnley J, Mahadik KR, Paradkar AR. Potentiating antimicrobial efficacy of Propolis through niosomal-based system for administration. Integr Med Res. 2015;4:94-101.
38. Tikhonov O, Bobro S. Studying "Propolis gel" pharmacological activity. Pharma Innov J. 2016;5(6):26-30.
39. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and Eosin staining of tissue and cell sections. Cold Spring Harb Protoc. 2008. doi: 10.1101/pdb. prot4986.

Cite this article: Ratnaparkhi M, Kshirsagar SD. Formulation and Evaluation of Propolis Loaded Nanosponges Based Topical Anti-Inflammatory Gel . Indian J of Pharmaceutical Education and Research. 2025;59(4):1312-22.