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FORMULATION, DEVELOPMENT AND OPTIMIZATION OF NANOSPONGES CONTAINING ANTI-INFLAMMATORY DRUG

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ABSTRACT

The study aimed to develop and optimize ursolic acid-loaded nanosponges for anti-inflammatory applications. Ursolic acid was first characterized for its organoleptic and physicochemical properties, appearing as a white to pale cream crystalline solid with a slight herbal odor. Its melting point (282 °C) corresponded with reported values, confirming purity. The compound exhibited a slightly acidic pH (5.9), showed high solubility in PBS buffer and several organic solvents, but was sparingly soluble in water and DMSO, guiding excipient and solvent selection for formulation. Calibration using UV spectroscopy displayed excellent linearity (R^2 = 0.9993), while FTIR confirmed the structural integrity of the compound through characteristic functional groups. Nanosponges prepared with ursolic acid exhibited a stable solid white appearance with characteristic odor. Among five formulations (NS 1–NS 5), NS 2 demonstrated superior performance, with the smallest particle size (53.03 nm), highest zeta potential (-30.2 mV), and maximum entrapment efficiency (93.47%), indicating enhanced stability and drug-loading capacity. SEM analysis confirmed spherical and porous morphology suitable for controlled release. Conversely, NS 5 showed the least favorable characteristics. Overall, NS 2 was identified as the optimized formulation, highlighting the potential of nanosponge technology to improve ursolic acid delivery, stability, and therapeutic efficacy in inflammation management.

KEYWORDS: Ursolic acid, nanosponges, formulation optimization, entrapment efficiency, particle size, anti-inflammatory.

1. INTRODUCTION

Inflammation is a complex biological response to tissue injury, infection, or chemical insult, often characterized by pain, swelling, and redness. While it is a protective mechanism, persistent or uncontrolled inflammation is associated with the progression of several chronic disorders, including arthritis, cardiovascular disease, and cancer **(Harvanová and Duranková 2025).** Conventional anti-inflammatory agents, although effective, are frequently limited by poor solubility, low bioavailability, and undesirable side effects, necessitating the development of safer and more efficient delivery systems **(Placha and Jampilek 2021).**

Ursolic acid is a naturally occurring pentacyclic triterpenoid widely distributed in medicinal herbs, fruits, and vegetables. It has attracted significant attention in pharmaceutical research due to its broad spectrum of pharmacological properties, including anti-inflammatory, antioxidant, hepatoprotective, anticancer, and antimicrobial activities **(Sandhu *et al.*, 2023).** Despite its therapeutic potential, the clinical translation of ursolic acid remains limited because of its poor aqueous solubility, low permeability, and reduced oral bioavailability. These biopharmaceutical challenges necessitate the development of novel formulation strategies to enhance its therapeutic efficacy **(Namdeo *et***

***al.*, 2023).** Conventional dosage forms of ursolic acid have shown limited success in overcoming these drawbacks, often requiring high doses to achieve therapeutic plasma concentrations, which may increase the risk of side effects. Advanced drug delivery approaches such as nanoparticles, nanosponges, liposomes, and solid dispersions have been explored to address these challenges. Among them, nanosponge-based formulations have demonstrated particular promise due to their high drug-loading capacity, ability to improve solubility, and potential for controlled drug release **(Garg *et al.*, 2024).**

Nanosponges are typically composed of biodegradable polymers crosslinked to form a three-dimensional network with nano-sized cavities. These cavities act as reservoirs that can encapsulate both hydrophilic and lipophilic molecules, protecting them from degradation while allowing for sustained and targeted delivery **(Tiwari and Bhattacharya 2022).** Compared to conventional dosage forms, nanosponge formulations offer significant advantages such as increased bioavailability, reduced dosing frequency, and improved patient compliance. The formulation, development, and optimization of nanosponges involve careful selection of polymers, crosslinkers, and preparation techniques to achieve desirable physicochemical and biopharmaceutical properties **(Moin *et al.*, 2020).** Over the past decade, nanosponges have gained significant attention in pharmaceutical research due to their versatility, safety, and efficiency. With their potential applications in cancer therapy, antifungal treatments, and solubility enhancement of poorly soluble drugs, nanosponges represent a promising strategy in modern drug delivery systems **(Ghurghure *et al.*, 2018).**

The present study focuses on the formulation, development, and optimization of ursolic acid-loaded nanosponges.

2. MATERIAL AND METHODS

2.1 Chemicals

Propylene glycoland, Ethanol, Acetonitrile and Methyl paraben, were obtained from Merck. Loba provided the Triethanolamine. Sulab provided the Carbopol 934 while Sigma eldrich supplied Ursolic acid, PVA and Ethyl cellulose. Methanol was procured from Rankem, a well-known provider of high-quality laboratory chemicals.

2.2 Pre-formulation studies

Pre-formulation studies are the initial stage in the development of a new drug, where the physical, chemical, and biological properties of a drug substance are thoroughly evaluated. These studies help in understanding the drug's characteristics, such as solubility, stability, pH, particle size, and compatibility with other ingredients. The goal is to gather essential data that will guide the formulation of a safe, effective, and stable dosage form. Pre-formulation studies are crucial for identifying potential challenges early in the development process and for designing an optimal drug delivery system **(Singh *et al.*, 2024).**

2.2.1 Organoleptic Properties

Organoleptic properties of a drug refer to the aspects that can be perceived by the senses, including color, odor, taste, and texture **(Clapham, 2022).**

2.2.2 Solubility study

To perform a solubility study of ursolic acid by visual observation, add a fixed amount of ursolic acid to equal volumes of various polar (e.g., water, ethanol) and non-polar (e.g., hexane, chloroform) solvents in separate test tubes. Shake or stir the mixtures for a set period (e.g., 24 hours) at room temperature. After settling, observe each tube visually for clarity, presence of undissolved particles, or sediment. A clear solution indicates higher solubility, while turbidity or residue suggests poor solubility **(Alfei *et al.*, 2021).**

2.2.3 Melting Point

To determine the melting point of ursolic acid using a melting point apparatus **(Zhou *et al.*, 2015).**

2.2.4 Determination of Lambda max and calibration curve

2.2.4.1 Lambda (λ) max

A stock standard solution containing 1 mg/mL of ursolic acid was prepared in 80% PBS buffer. A working standard solution equivalent to 100 µg/mL of ursolic acid was prepared by appropriate dilution of the stock solution with the same solvent. The solution was scanned in the range of 200–400 nm UV spectrum using a Shimadzu 1700 double beam spectrophotometer **(Schneider *et al.*, 2009).**

2.2.4.2 Standard calibration curve

100 mg of ursolic acid was accurately weighed and transferred into a 100 mL volumetric flask. It was dissolved in 80% PBS buffer, and the volume was made up to the mark with the same solvent. From this solution, 1 mL was pipetted into a 10 mL volumetric flask and diluted to volume with PBS buffer to prepare the stock solution. The resulting solution was scanned in the wavelength range of 200–400 nm using a UV spectrophotometer to determine the absorption maximum (λ max).

2.2.5 Preparation of calibration curve

A stock solution of ursolic acid was prepared and subsequently diluted with the appropriate solvent to obtain working standard solutions with concentrations of 5, 10, 15, 20, and 25 µg/ml. These solutions were used to measure the absorbance values using the solvent as a blank. A calibration curve was then constructed by plotting the absorbance (Y-axis) against the concentration of ursolic acid (X-axis) over the range of 40 to 100 µg/ml, in accordance with Beer's law.

2.2.6 Fourier transmission Infra-Red Spectroscopy

The FT-IR spectra of pure ursolic acid and a mixture of ursolic acid with excipients were recorded in the range of 4000 to 400 cm^{-1} using the KBr pellet method on an FT-IR spectrophotometer. To prepare the pellets, 1 mg of the sample (either ursolic acid alone or with excipients) was thoroughly mixed with 100 mg of spectroscopic grade potassium bromide (KBr), which had been pre-dried under an infrared lamp. The mixture was then compressed under hydraulic pressure to form a transparent disc. This disc was placed in the sample holder of the FT-IR instrument, and the infrared spectra were obtained within the 4000–400 cm^{-1} range **(Maphanao *et al.*, 2020).**

2.3 Preparation of ursolic acid -loaded nanosponges

Ursolic acid loaded NS (NS) were prepared by the emulsion solvent evaporation technique using the drug (Ursolic acid) 100 mg and polyvinyl alcohol (PVA) 0.3%, w/v, compositions of formulations were tabulated in Table 1. Briefly, organic phase was prepared by dissolving ethyl cellulose (EC) (50–250 mg) and Ursolic acid in 25 mL dichloromethane (DCM). Separately, an aqueous phase was prepared composed of (0.3%, w/v) PVA in 100 mL of deionized water. Thereafter, the organic phase was emulsified dropwise into the aqueous phase by ultrasonication, at power for 3 min (on-off cycles). The formed NS was stabilized by PVA, which avoid particle agglomerations. Thereafter, the dispersion was kept on thermostatically controlled magnetic stirrer ‘‘(Hot Plate and Stirrer)” with continuous stirring at 1000 rpm under atmospheric pressure and temperature for 24 h. After complete evaporation of the organic solvent, the Urosolic acid nanosponges were washed three times with ultra-purified water to remove the adsorbed PVA, NSs were then collected by ultracentrifugation and 4^0 C for 30 min” and freezedried **(Ahmed et al., 2021).**

Table 1: Composition of Nanosponges formulation.

Ingredients	Nanosponges (F1)	Nanosponges (F2)	Nanosponges (F3)	Nanosponges (F4)	Nanosponges (F5)
Drug (mg)	100	100	100	100	100
Ethyl cellulose (mg)	50	100	150	200	250
PVA (%)	0.3	0.3	0.3	0.3	0.3
Dichloromethane (DCM) (ml)	25	25	25	25	25
Distilled water (ml)	100	100	100	100	100
Stirring time (Hrs.)	1-6	1-6	1-6	1-6	1-6
Sonication time (min.)	2-5	2-5	2-5	2-5	2-5

2.4 Characterization of Nanosponges

2.4.1 Physical Appearance

To assess the physical appearance of nanosponges, a small amount of the sample is placed on a clean surface and examined under adequate lighting. Key observations include color, texture, and flow properties. **(Pandey, 2019).**

2.4.2 Particle size

The particle size of ursolic acid loaded nanosponges can be determined using a Malvern Zetasizer, which operates on the principle of dynamic light scattering (DLS) **(Darandale & Vavia 2013).**

2.4.3 Zeta potential

The zeta potential of nanosponges is measured using a Malvern Zetasizer to assess their surface charge and stability in suspension. **(Allahyari *et al.*, 2020).**

2.4.4 Scanning Electron Microscopic (SEM)

The surface morphology of the ursolic acid-loaded nanosponges was analyzed using a scanning electron microscope (SEM). Prior to imaging, the samples were coated with a thin metallic layer (approximately 2–20 nm) of gold, palladium, or platinum using a sputter coater under vacuum conditions to enhance conductivity. Once prepared, the specimen was exposed to an electron beam, which interacted with the sample’s surface, generating secondary electrons, including Auger electrons. Electrons scattered at a 90° angle were selectively detected and processed according to principles based on Rutherford and Kramer’s Law, allowing detailed visualization of the surface topography **(Abbas *et al.*, 2018).**

2.4.5 Entrapment efficiency

To calculate the entrapment efficiency, a precisely measured quantity (10 ml) of ursolic acid- loaded nanosponges was transferred into a volumetric flask containing 5 ml of PBS buffer. The mixture was shaken thoroughly for 1 minute using a vortex mixer to ensure complete extraction of the drug. The volume was then adjusted to 10 ml with methanol. After proper mixing, the solution was filtered and suitably diluted. The concentration of entrapped ursolic acid was then determined using a UV-Visible spectrophotometer **(Salman *et al.*, 2021).**

%EE = Initial amount of drug added - Drug amount in supernatant / Initial amount of drug added × 100

2.4.6 *In-vitro* drug release study

The in-vitro drug release study of the ursolic acid-loaded nanosponges was carried out using the dialysis bag diffusion method. A measured amount of the formulation was placed inside a dialysis bag, which was then immersed in a beaker containing 100 ml of

phosphate buffer (pH 7.4). The setup was maintained at a constant temperature of 37 ± 2 °C and stirred continuously at 100 rpm using a magnetic stirrer. At predetermined time intervals, 2 ml of the release medium was withdrawn and immediately replaced with an equal volume of fresh phosphate buffer to maintain sink conditions. The collected samples were appropriately diluted and analyzed using a UV-Visible spectrophotometer to determine the amount of drug released. To understand the drug release behavior, the data were fitted into various kinetic models **(Manyam *et al.*, 2018).**

3. RESULTS AND DISCUSSION

3.1 Pre-formulation study of Ursolic acid

3.1.1 Organoleptic evaluation

Table 2: Organoleptic evaluation of Ursolic acid.

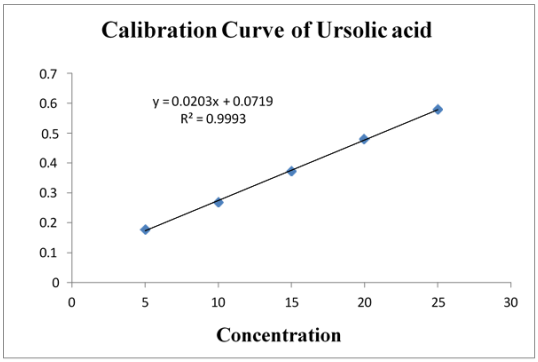
Physical parameter	Observation
Color	White to pale cream
Odor	Slight herbal odor
State	Solid
Appearance	Crystalline solid

3.1.2 Melting Point

Table 3: Melting Point of Ursolic acid.

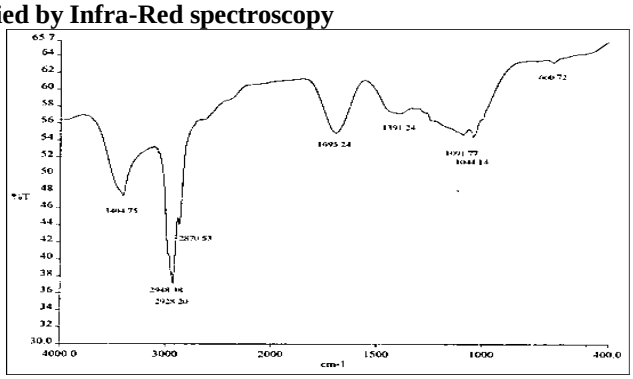
Drugs	Observed	Reference
Ursolic acid	282 °C	283-285 °C

3.2 Standard calibration curve



Graph 1: Calibration curve of Ursolic acid.

3.3 Functional group identified by Infra-Red spectroscopy



Graph 2: FTIR study of Ursolic acid.

Table 4: Interpretation of IR spectrum of Ursolic acid.

Peak obtained	Reference peak	Functional group	Name of functional group
3404.75	3500- 3400	N-H stretching	Primary amine
2928.20	3000-2840	C-H stretching	Alkanes
1659.24	1690-1640	C=N stretching	Imine / oxime
1391.24	1415-1380	S=O stretching	Sulfate
1044.14	1050-1040	CO-O-CO stretching	Anhydride
660.72	690-515	C-Br stretching	Halo compound

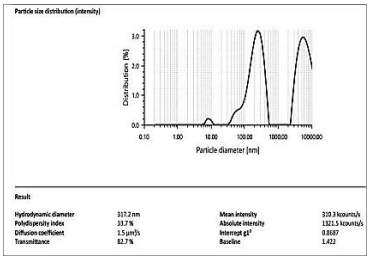
3.4 Characterization of ursolic acid loaded Nanosponges formulation

3.4.1 Physical Appearance of Nanosponges

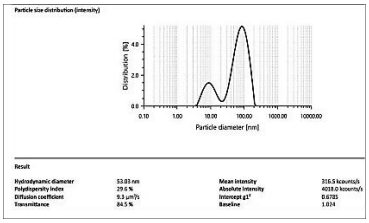
Table 5: Physical Appearance of Nanosponges.

Formulation	Parameters	Observation
Nanosponges	Colour	White
	Odour	Characteristic
	Appearance	Solid

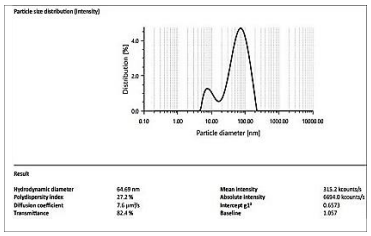
3.4.2 Particle Size



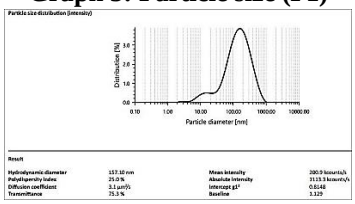
Graph 3: Particle size (F1)



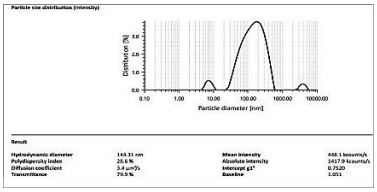
Graph 4: Particle size (F2)



Graph 5: Particle size (F3)



Graph 6: Particle size (F4)

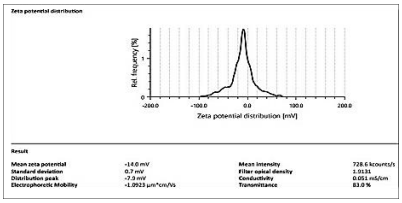


Graph 7: Particle size (F5)

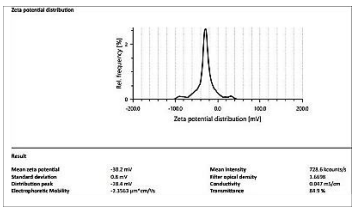
Table 6: Particle size.

Formulation code	Particle size (nm)	PI Value %
NS 1	317.2 nm	33.7
NS 2	**53.03 nm**	**29.6**
NS 3	64.69 nm	27.2
NS 4	157.10 nm	25.10
NS 5	144.31 nm	28.6

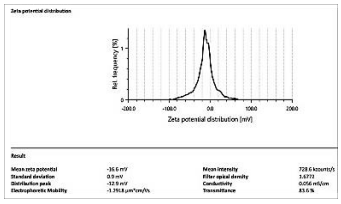
3.4.3 Zeta potential



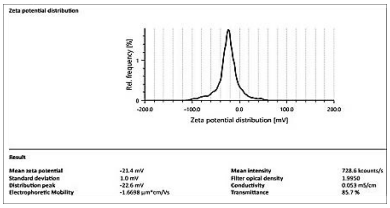
Graph 8: Zeta potential (NS 1)



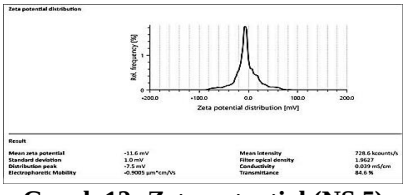
Graph 9: Zeta potential (NS 2)



Graph 10: Zeta potential (NS 3)



Graph 11: Zeta potential (NS 4)



Graph 12: Zeta potential (NS 5)

Table 7: Zeta potential.

Formulation Code	Zeta potential
NS 1	-14.0 mV
NS 2	**-30.2 mV**
NS 3	-16.6 mV
NS 4	-21.4 mV
NS 5	-11.6 mV

3.4.5 Scanning electron microscope (SEM)

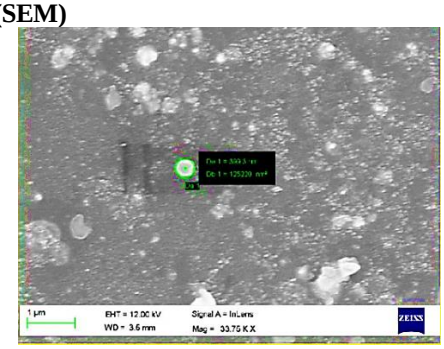


Figure 1: Scanning electron microscope (SEM).

3.4.6 Entrapment efficacy

Table 8: Entrapment efficacy.

Formulations (NS 1- NS 5)	Entrapment efficacy (%)
Nanosponges (NS 1)	87.56
Nanosponges (NS 2)	**93.47**
Nanosponges (NS 3)	77.10
Nanosponges (NS 4)	86.35
Nanosponges (NS 5)	81.79

DISCUSSION

The present investigation successfully developed and optimized ursolic acid-loaded nano sponges for anti-inflammatory applications. The initial characterization of ursolic acid confirmed its suitability for formulation, as its organoleptic properties, melting point, and pH were consistent with reported standards, thereby validating its purity and stability. Solubility studies further highlighted its limited aqueous solubility but good solubility in organic solvents, which directly influenced the choice of formulation excipients. These results align with existing literature, where ursolic acid is often classified as a poorly water-soluble compound requiring advanced delivery systems to improve its bioavailability.

Spectroscopic analyses provided additional confirmation of the compound's integrity. The UV calibration curve exhibited excellent linearity (R^2 = 0.9993), ensuring reliable quantitative estimation. Likewise, FTIR analysis revealed distinct functional groups characteristic of ursolic acid, suggesting that no major structural changes occurred during formulation.

Among the nanosponge formulations developed (NS 1–NS 5), substantial variations were observed in physicochemical properties. NS 2 was found to be the most effective formulation, demonstrating a small particle size (53.03 nm), high negative zeta potential (-30.2 mV), and superior entrapment efficiency (93.47 %). These parameters are critical in determining formulation stability and drug-loading capacity. The high zeta potential suggests that NS 2 possesses strong electrostatic repulsion, reducing the risk of particle aggregation and ensuring long-term dispersion stability. SEM imaging further confirmed that the nanosponges were spherical and porous, features that are advantageous for achieving sustained and controlled drug release. Conversely, NS 5 showed inferior performance, highlighting the importance of optimizing formulation parameters for enhanced therapeutic outcomes.

Overall, the findings indicate that nanosponge technology holds significant potential in addressing the solubility and stability challenges associated with ursolic acid. By improving entrapment efficiency and maintaining structural stability, nanosponges not only enhance drug delivery but may also contribute to better therapeutic efficacy in managing inflammation. These outcomes support the growing evidence that nanocarrier systems can serve as a promising strategy for delivering poorly soluble bioactive compounds.

4. CONCLUSION

In conclusion, the study successfully developed ursolic acid-loaded nanosponges, with NS 2 identified as the optimized formulation based on particle size, zeta potential, and entrapment efficiency. These findings suggest that nanosponge technology can be effectively utilized to enhance the delivery, stability, and therapeutic efficacy of ursolic acid for managing inflammation.

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