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FORMULATION DEVELOPMENT OF URSOLIC ACID–LOADED NANOSPONGES

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ABSTRACT

The present study focuses on the formulation, characterization, and optimization of nanosponges loaded with ursolic acid, a bioactive compound known for its anti-inflammatory properties. Pre-formulation studies were conducted to assess the physicochemical characteristics of ursolic acid, including organoleptic properties, melting point, pH, and solubility. UV-spectrophotometry established the drug's λmax at 208 nm, and a standard calibration curve confirmed linearity in PBS buffer. FTIR analysis confirmed the presence of key functional groups and absence of major interactions with excipients. Nanosponges were formulated using varying ratios of cholesterol and Span 60 via the emulsion-solvent diffusion technique. The prepared formulations were evaluated for physical appearance, particle size, zeta potential, morphology (SEM), entrapment efficiency, and in vitro drug release. Formulation F2 exhibited optimal physicochemical characteristics, including a particle size of 53.03 nm, zeta potential of -30.2 mV, and the highest entrapment efficiency (96.52%). In vitro drug release studies revealed sustained release behavior, and kinetic modeling demonstrated that the release followed a zero-order kinetic profile with $R^2 = 0.952$. These findings suggest that ursolic acid-loaded nanosponges offer a promising strategy for sustained and targeted drug delivery in the management of inflammatory conditions.

KEYWORDS: Ursolic acid, nanosponges, formulation development, inflammation, entrapment efficiency, particle size, zeta potential, SEM.

Concept of Drug Delivery

Drug delivery has evolved significantly with increasing complexity in disease management and therapeutic molecules. Traditional drug administration methods often fail to deliver drugs at the desired site in optimal concentration, leading to reduced efficacy and increased side effects. To overcome these limitations, modern drug delivery approaches aim to improve bioavailability, therapeutic index, patient compliance, and site specificity. Drug delivery can be defined as a therapeutic strategy in which a delivery device is coupled with a sensing or targeting mechanism to control drug release spatially and temporally.

The concept of controlled drug delivery was first demonstrated by Folkman and Long in 1964, who reported that hydrophobic drug molecules could diffuse through silicone tubing at a controlled rate. This discovery laid the foundation for the development of Drug Delivery Systems (DDS), which focus on modifying pharmacokinetics and pharmacodynamics to enhance therapeutic outcomes. Over time, DDS has become a crucial component of pharmaceutical research, particularly for poorly soluble, unstable, or highly potent drugs.

1.1.1 Early Research in Drug Delivery

Early drug delivery systems relied mainly on self-assembling carriers such as liposomes, niosomes, solid lipid nanoparticles (SLNs), and lipid-based vesicles. Liposomes gained attention nearly four decades ago as promising carriers capable of encapsulating both hydrophilic and lipophilic drugs. However, further research revealed several limitations including poor physical stability, limited drug loading capacity, sensitivity to environmental conditions, and high production costs.

Solid lipid nanoparticles also demonstrated drawbacks, particularly during storage. Drug expulsion occurs due to lipid polymorphic transitions from less ordered to more stable crystalline forms, which restrict drug

accommodation. Additionally, many particulate carriers are rapidly taken up by macrophages, leading to bioaccumulation in the liver and spleen, even after surface modification with polyethylene glycol (PEG). These challenges prompted researchers to explore advanced materials capable of overcoming instability, immunogenicity, non-specific toxicity, and limited targeting efficiency.

Polymers emerged as a promising alternative due to their structural stability, versatility, and tunable properties. Unlike self-assembled lipid systems, polymeric carriers provide better control over drug release, improved mechanical stability, and scalability for industrial production. Consequently, polymer-based drug delivery systems gained prominence for transporting therapeutic agents efficiently to target sites.

1.1.2 Drug Delivery Systems (DDS)

In recent decades, drug delivery and targeting have become major research priorities. DDS are designed based on the physiological and biochemical characteristics of the target site as well as the physicochemical properties of the drug. Drug carriers are broadly classified into particle-based carriers and soluble carriers.

Particle-based carriers include liposomes, lipid particles (LDL and HDL), microspheres, nanoparticles, polymeric micelles, and nanosponges. Soluble carriers include monoclonal antibodies, peptides, polysaccharides, modified proteins, and biodegradable polymers. Viral vectors and whole cells are also used for site-specific gene delivery. However, particle-based carriers are more extensively studied due to their versatility and controlled release capabilities.

1.2 Inflammation

Inflammation is a fundamental biological response in which white blood cells protect the body from infection, injury, and harmful stimuli. It is broadly classified into acute and chronic inflammation. Acute inflammation is short-term and generally resolves with appropriate treatment, whereas chronic inflammation persists over long periods and is associated with autoimmune disorders, metabolic diseases, and persistent infections.

Chronic inflammation is linked to several systemic conditions such as diabetes, cardiovascular disorders, depression, dementia, and gastrointestinal disturbances. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammatory conditions. Ibuprofen, one of the earliest NSAIDs, is widely prescribed but requires high and frequent dosing, leading to poor patient compliance and gastrointestinal side effects.

Dexibuprofen, the active S-enantiomer of ibuprofen, offers reduced dosage but still requires frequent administration due to its short half-life. Prolonged NSAID use increases the risk of adverse effects such as gastric irritation, nausea, and ulcers, highlighting the need for advanced drug delivery systems that provide sustained release and improved safety profiles.

1.3 Nanosponges: A Novel Drug Delivery Approach

Nanotechnology has revolutionized pharmaceutical research by enabling the development of nanoscale drug carriers that improve solubility, stability, and therapeutic efficiency. Nanosponges are porous, sponge-like nanoparticles with nanometric cavities capable of encapsulating a wide range of drug molecules.

Hydrophobic drugs present significant challenges in vivo due to poor aqueous solubility. Nanosponges enhance the solubility and bioavailability of such drugs by entrapping them within their porous network. An ideal drug delivery system should deliver the drug at the target site in optimal concentration for a prolonged duration while minimizing systemic toxicity. Nanosponges meet these criteria by offering controlled and site-specific drug release.

Nanosponges are classified based on their mode of drug association into encapsulating, conjugating, and complexing nanoparticles. Among these, encapsulating nanosponges are widely studied due to their high loading capacity and sustained release properties. They are solid, non-toxic, thermally stable, and compatible with multiple routes of administration including oral, parenteral, topical, and inhalational.

1.4 Nanosponges as Drug Carriers

Nanosponges are miniature mesh-like structures composed of cross-linked polymers. They are non-mutagenic, non-allergenic, non-irritating, and stable at temperatures up to 300°C. Initially developed for topical drug delivery, nanosponges are now widely explored for oral and intravenous applications.

Their porous structure allows encapsulation of lipophilic drugs, protection of unstable molecules, and controlled release over extended periods. Nanosponges improve formulation flexibility, enhance stability, and reduce side effects by limiting drug exposure to healthy tissues. Drug targeting can be achieved by surface modification, ensuring selective delivery to diseased cells.

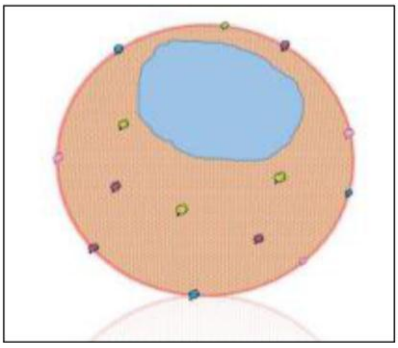


Figure 1: Structure of a nanosponge.

1.5 Drug Loading and Release Mechanism

Drug loading in nanosponges involves reducing particle size below 500 nm, dispersing them in aqueous media, and facilitating drug complexation through continuous stirring. Paracrystalline nanosponges exhibit higher drug loading compared to crystalline forms due to increased pore accessibility.

Drug release from nanosponges occurs through diffusion and equilibrium-based mechanisms. Upon application, the drug migrates from the nanosponge to the surrounding medium until equilibrium is achieved. This allows prolonged and targeted drug release, particularly beneficial for topical and localized therapies.

1.6 Classification and Types of Nanosponges

Nanosponges are classified as encapsulating, complexing, or conjugating nanoparticles. Various types include cyclodextrin nanosponges, polystyrene-based nanosponges, titanium dioxide nanosponges, silicon nanosponges, cellulose-based nanosponges, metal ion nanosponges, polymer nanosponges, and glycopolymer nanosponges.

Cyclodextrin nanosponges are extensively studied due to their biocompatibility and ability to encapsulate molecules matching their cavity size. Other nanosponges find applications not only in drug delivery but also in water treatment, catalysis, biosensing, and environmental remediation.

1.7 Methods of Preparation

Nanosponges can be prepared using various techniques including solvent method, ultrasound-assisted synthesis, emulsion solvent diffusion, melt method, polymerization, bubble electrospinning, and quasi-emulsion solvent diffusion. Among these, the emulsion solvent diffusion method is widely preferred due to its simplicity, scalability, and control over particle size and morphology.

1.8 Advantages and Limitations of Nanosponges

Nanosponges offer several advantages such as biodegradability, predictable drug release, site-specific targeting, improved solubility of hydrophobic drugs, reduced side effects, and formulation flexibility. Particle size and release behavior can be tailored by adjusting polymer-to-crosslinker ratios.

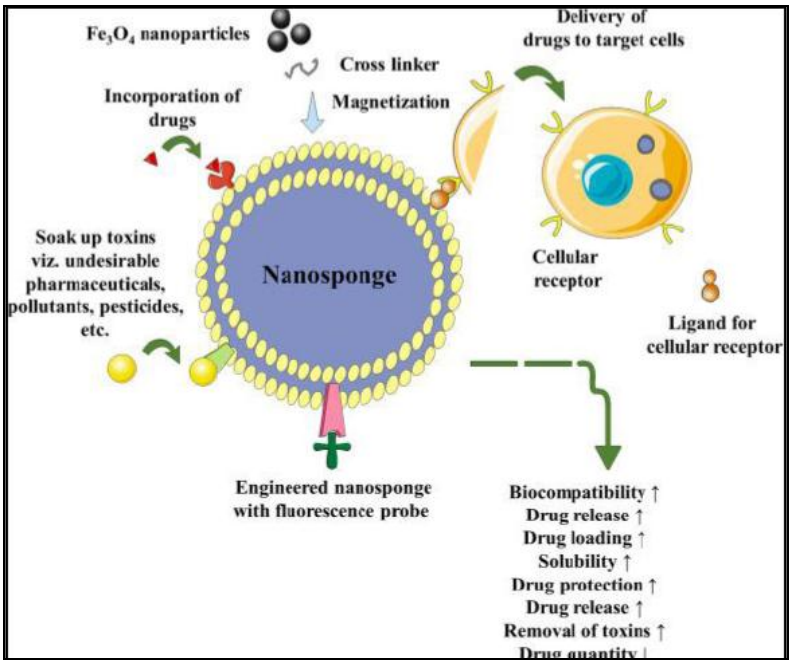


Figure 2: Mechanism of drug release from nanosponges.

However, limitations include restricted loading capacity for large molecules, risk of dose dumping, and dependence on polymer properties. Despite these challenges, nanosponges remain a promising platform for advanced drug delivery applications.

This study focused on the formulation and optimization of ursolic acid–loaded nanosponges for the treatment of inflammatory conditions. Ursolic acid was selected for its well-known anti-inflammatory and antioxidant properties, but its clinical use is limited by poor water solubility and low bioavailability. To overcome these limitations, a nanosponge-based drug delivery system was developed to enhance solubility, stability, and provide sustained drug release.

Multiple nanosponge formulations (F1–F5) were prepared using varying polymer concentrations and evaluated for particle size, zeta potential, entrapment efficiency, and morphology. Among them, formulation F2 showed optimal characteristics and superior drug delivery potential.

The findings suggest that ursolic acid nanosponges could serve as a promising platform for improved anti-inflammatory therapy and may be further explored for topical, oral, or parenteral applications. Additionally, this approach provides a basis for extending nanosponge technology to other poorly soluble bioactive compounds.

Ursolic Acid is a pentacyclic triterpenoid found in various fruits, vegetables and medicinal herbs, with a variety of potential pharmacologic activities including anti-inflammatory, antioxidative, antiviral, serum lipid-lowering, and antineoplastic activities. Upon administration, ursolic acid may promote apoptosis and inhibit cancer cell proliferation through multiple mechanisms. This may include the regulation of mitochondrial function through various pathways including the ROCK/PTEN and p53 pathways, the suppression of the nuclear factor-kappa B (NF-kB) pathways, and the increase in caspase-3, caspase-8 and caspase-9 activities.

6.2 Pre-Formulation Studies (Procedure)

1. Pre-formulation studies were carried out to evaluate the physical and chemical properties of ursolic acid.
2. Parameters such as organoleptic properties, solubility, melting point, λmax, calibration curve, and drug–excipient compatibility were assessed.
3. These studies were performed to ensure suitability of the drug for nanosponge formulation and to identify potential formulation challenges.

6.2.1 Evaluation of Organoleptic Properties

1. A small quantity of ursolic acid was taken on a clean surface.
2. The sample was visually examined under adequate lighting.
3. Color, odor, taste, and texture were recorded.

6.2.2 Solubility Study

1. A fixed amount of ursolic acid was added to equal volumes of different solvents (water, ethanol, chloroform, hexane) in separate test tubes.
2. The mixtures were shaken and kept undisturbed for 24 hours at room temperature.
3. Each test tube was visually observed for clarity or presence of undissolved particles.
4. Solubility was determined based on clarity or sediment formation.

6.2.3 Determination of Melting Point

1. Ursolic acid was finely powdered.
2. The powder was filled into a capillary tube.
3. The capillary tube was placed in a melting point apparatus.
4. The temperature at which melting started and completed was recorded.

6.2.5 Determination of λmax and Calibration Curve

6.2.5.1 Determination of λmax

1. A stock solution of ursolic acid (1 mg/mL) was prepared using 80% PBS buffer.
2. A working solution (100 µg/mL) was prepared by dilution.
3. The solution was scanned in the UV range of 200–400 nm using a UV spectrophotometer.
4. The wavelength showing maximum absorbance was recorded as λmax.

6.2.5.2 Preparation of Standard Calibration Curve

1. Accurately weighed ursolic acid (100 mg) was dissolved in 80% PBS buffer and volume made up to 100 mL.
2. Serial dilutions were prepared to obtain concentrations of 5, 10, 15, 20, and 25 µg/mL.
3. Absorbance of each solution was measured at λmax using a UV spectrophotometer.
4. A calibration curve was plotted between concentration and absorbance.

6.2.7 Fourier Transform Infrared (FT-IR) Spectroscopy

1. Pure ursolic acid and a physical mixture of ursolic acid with excipients were prepared.
2. About 1 mg of sample was mixed with 100 mg of dry KBr.
3. The mixture was compressed to form a transparent pellet.
4. FT-IR spectra were recorded in the range of 4000–400 cm^{-1}.
5. Spectra were analyzed for possible drug–excipient interactions.

6.3 Preparation of Ursolic Acid-Loaded Nanosponges

1. Ursolic acid (1.5 mg) was dissolved in 10 mL of chloroform to form the organic phase.
2. Cholesterol and Span 60 were added in varying ratios according to formulations F1–F5.
3. The mixture was stirred to obtain a homogeneous solution.
4. Distilled water (10 mL) was added dropwise under continuous stirring to form an emulsion.
5. The emulsion was sonicated for 3–5 minutes to reduce particle size.
6. The dispersion was cooled and washed to remove unentrapped drug.
7. The nanosponges were dried and stored for further evaluation.

6.4 Characterization of Nanosponges

6.4.1 Physical Appearance

1. A small amount of nanosponge powder was visually examined.
2. Color, texture, flow property, and presence of clumps were noted.

6.4.2 Particle Size Analysis

1. Nanosponges were dispersed in distilled water to form a clear suspension.
2. The suspension was transferred into a cuvette.
3. Particle size was measured using a Malvern Zetasizer based on DLS principle.

6.4.3 Zeta Potential Analysis

1. A nanosponge suspension was prepared in distilled water.
2. The suspension was placed in a zeta potential cell.
3. Surface charge was measured using a Malvern Zetasizer.
4. Zeta potential values were recorded to assess stability.

6.4.4 Scanning Electron Microscopy (SEM)

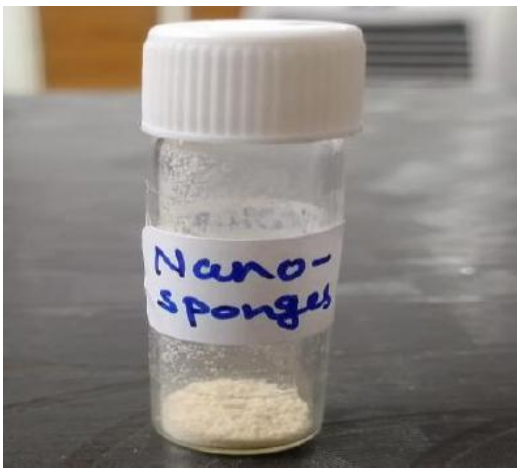
1. Dried nanosponge samples were mounted on a stub.
2. Samples were coated with a thin layer of gold or platinum using a sputter coater.
3. Surface morphology was examined using SEM.

6.4.5 Entrapment Efficiency (%EE)

1. 10 mL of nanosponge dispersion was mixed with 5 mL PBS buffer.
2. The mixture was vortexed for 1 minute.
3. Volume was adjusted to 10 mL with methanol.
4. The solution was filtered and analyzed using UV spectrophotometer.
5. Entrapment efficiency was calculated using: %EE=Initial drug−Free drugInitial drug×100\%EE = \frac{\text{Initial drug} - \text{Free drug}}{\text{Initial drug}} \times 100%EE=Initial drugInitial drug−Free drug×100

6.4.6 In-Vitro Drug Release Study

1. A measured amount of nanosponge formulation was placed in a dialysis bag.
2. The bag was immersed in 100 mL phosphate buffer (pH 7.4).
3. The system was maintained at 37 ± 2 °C with continuous stirring.
4. At fixed intervals, 2 mL of sample was withdrawn and replaced with fresh buffer.
5. Samples were analyzed using a UV spectrophotometer.
6. Release data were fitted to kinetic models:
   - Zero-order
   - First-order
   - Higuchi model
   - Korsmeyer–Peppas model



Summary of Results and Discussion

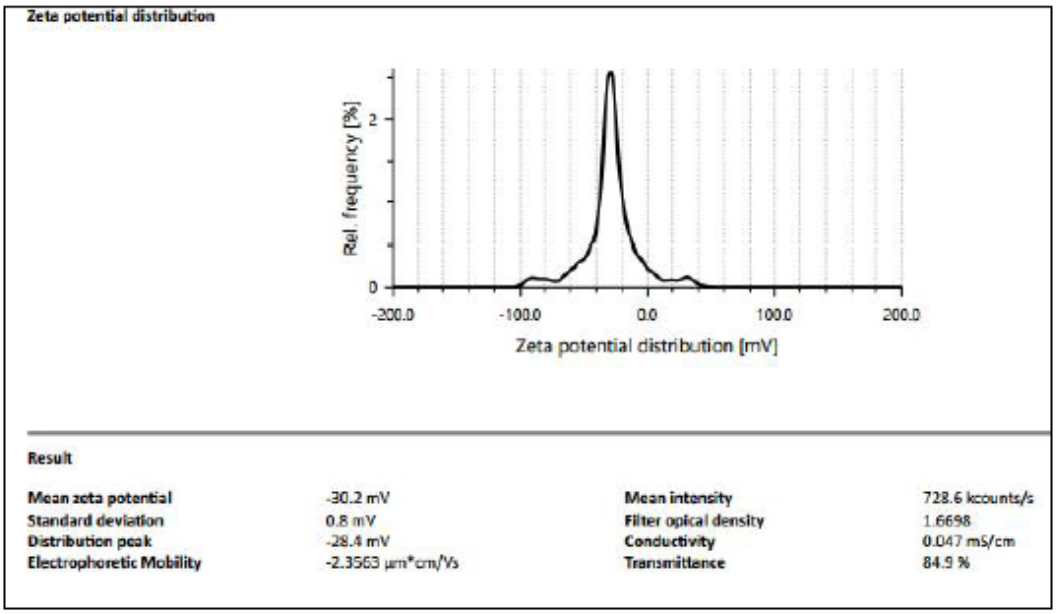
Preformulation studies of ursolic acid confirmed its suitability for pharmaceutical formulation. Organoleptic evaluation showed that ursolic acid is a white to pale cream, crystalline solid with a mild herbal odor, indicating good physical stability. The melting point was observed at 282 °C, closely matching the reported reference range, confirming the purity of the drug. The pH of ursolic acid was found to be 5.9, suggesting a slightly acidic nature. Solubility studies revealed that ursolic acid is sparingly soluble in water and DMSO, moderately soluble in acetone, and freely soluble in ethanol, methanol, and PBS buffer, which guided solvent selection for formulation and analysis.

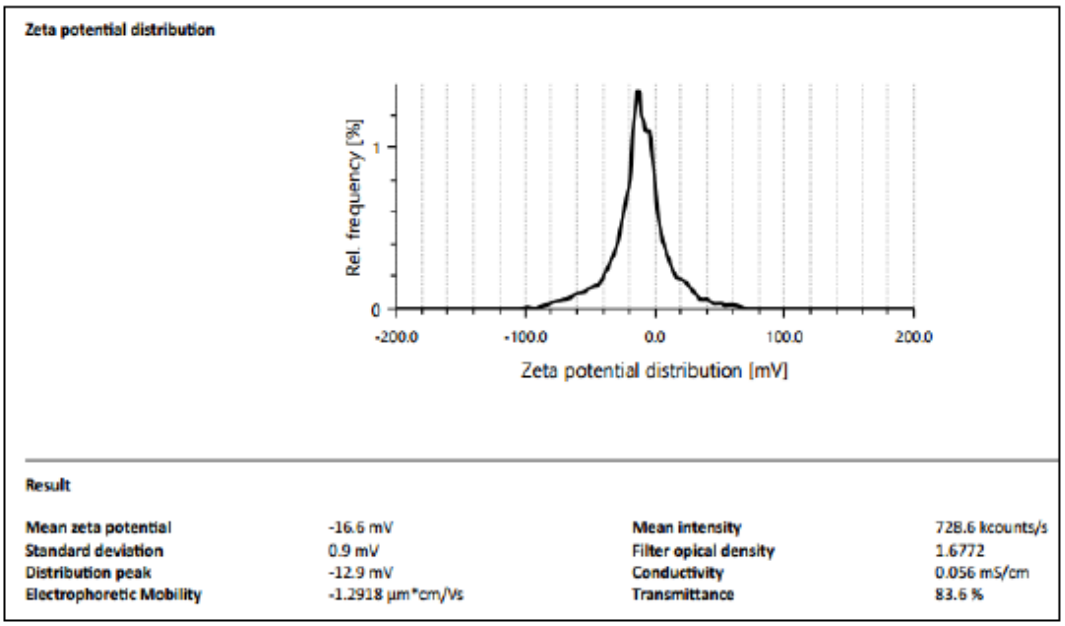
UV spectrophotometric analysis established the λmax of ursolic acid at 208 nm. The calibration curve constructed in PBS buffer showed excellent linearity (R^2 = 0.9993), confirming the reliability of the analytical method. FTIR analysis identified characteristic functional groups such as N–H, C=N, and S=O, confirming the chemical identity of ursolic acid and the absence of significant structural alteration.

Ursolic acid–loaded nanosponges (F1–F5) were successfully prepared using the emulsion solvent diffusion method. All formulations appeared white, solid, and uniform, indicating good physical stability. Particle size analysis showed nanoscale dimensions ranging from 53.03 nm to 317.2 nm, with formulation F2 exhibiting the smallest particle size, favoring enhanced drug delivery. Zeta potential values ranged from −11.6 mV to −30.2 mV, with F2 demonstrating the highest negative charge, indicating superior colloidal stability.

SEM studies revealed that the nanosponges were spherical, porous, and smooth-surfaced, which supports effective drug entrapment. Entrapment efficiency varied from 79.35% to 96.52%, with formulation F2 showing the highest entrapment, suggesting optimal polymer–drug interaction.

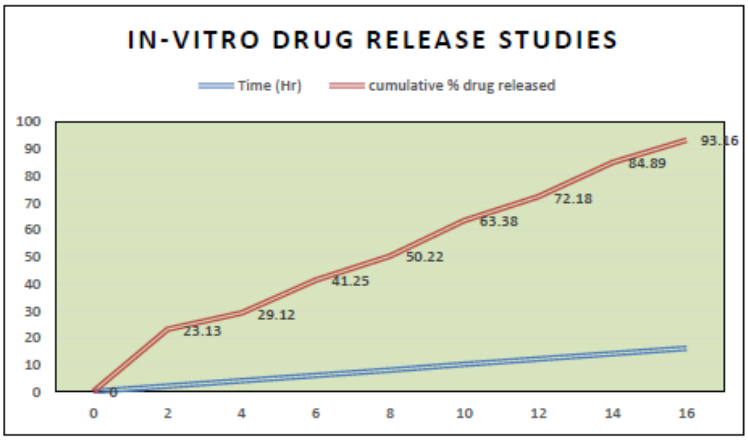
In vitro drug release studies showed a sustained release pattern, with more than 90% drug release achieved within 16 hours. Kinetic modeling indicated that the release data best fitted the zero-order model ($R^2 = 0.952$), confirming a controlled, concentration-independent drug release mechanism.





Zeta potential mese=urement

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
2	23.13	76.87	1.414	1.886	0.301	1.364
4	29.12	70.88	2.000	1.851	0.602	1.464
6	41.25	58.75	2.449	1.769	0.778	1.615
8	50.22	49.78	2.828	1.697	0.903	1.701
10	63.38	36.62	3.162	1.564	1.000	1.802
12	72.18	27.82	3.464	1.444	1.079	1.858
14	84.89	15.11	3.742	1.179	1.146	1.929
16	93.16	6.84	4.000	0.835	1.204	1.969



CONCLUSION

The study successfully demonstrated the formulation and optimization of ursolic acid–loaded nanosponges as an effective drug delivery system. Among all formulations, F2 emerged as the most promising due to its small particle size, high entrapment efficiency, and excellent stability, while exhibiting sustained drug release following zero-order kinetics. The nanosponge delivery system effectively addressed the limitations of ursolic acid related to poor solubility and bioavailability. Overall, these findings highlight the potential of nanosponges as a suitable platform for developing sustained and targeted anti-inflammatory drug delivery systems, warranting further preclinical and clinical investigation.

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