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Research Article

Development and Characterization of Ursolic Acid-Loaded Liposomes for Enhanced Bioavailability and Sustained Anticancer Drug Delivery

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ABSTRACT

Ursolic acid (UA), a pentacyclic triterpenoid compound, has demonstrated significant anticancer potential. However, its clinical utilization is severely restricted by poor water solubility and limited bioavailability. In this study, we developed a liposome-based nanocarrier system to encapsulate UA, aiming to improve its solubility, stability, and therapeutic efficacy. UA-loaded liposomes (UALs) were prepared using the thin-film hydration method followed by sonication. The liposomes were characterized in terms of particle size, zeta potential, encapsulation efficiency, morphology, and in vitro drug release behavior. UALs exhibited a particle size range of 130–200 nm, a zeta potential of -25 mV indicating colloidal stability, and an encapsulation efficiency of approximately 68%. The in vitro release study showed a biphasic release profile with sustained release over 24 hours. FTIR analysis confirmed the successful loading of UA without chemical interaction with the lipid bilayer. These findings suggest that liposomal encapsulation is a promising strategy to enhance the bioavailability and therapeutic potential of UA.

Keywords: Ursolic acid, liposomes, nanocarrier, encapsulation efficiency, sustained release, anticancer drug delivery

INTRODUCTION

Nanotechnology has emerged as a revolutionary field in pharmaceutical sciences, particularly in the realm of drug delivery systems. One of its most compelling applications lies in overcoming challenges related to poorly soluble and poorly bioavailable therapeutic agents. Ursolic acid (UA), a pentacyclic triterpenoid found in various plants such as rosemary, apples, and holy basil, possesses extensive pharmacological effects, including anti-inflammatory, hepatoprotective, antimicrobial, and notably, anticancer properties. Despite its potent bioactivity, UA's therapeutic application is significantly limited by its poor aqueous solubility

and low gastrointestinal absorption, rendering it a Biopharmaceutical Classification System (BCS) class IV drug.

To enhance its pharmacokinetic profile, considerable efforts have been devoted to developing efficient delivery systems capable of improving the solubility, stability, and cellular uptake of UA. Among the available strategies, nanocarrier-based systems such as polymeric nanoparticles, solid lipid nanoparticles, and liposomes have demonstrated considerable promise. Of these, liposomes offer a unique advantage due to their structural similarity to biological membranes, biocompatibility, ability to encapsulate both hydrophobic and hydrophilic drugs, and flexibility in functionalization for targeted delivery.

Liposomes are spherical vesicles composed of phospholipid bilayers enclosing an aqueous core. Hydrophilic drugs can be encapsulated in the aqueous interior, while hydrophobic drugs like UA are primarily incorporated within the lipid bilayer. This dual-loading capability makes liposomes a versatile and attractive choice for drug delivery. Additionally, liposomes protect the drug from premature enzymatic degradation, reduce systemic toxicity, and allow for sustained and targeted release.

The formulation of UA-loaded liposomes (UALs) is aimed at addressing the inherent limitations of UA by enhancing its solubility and ensuring sustained release, thereby improving therapeutic efficacy and patient compliance. In the present study, UALs were prepared using the thin-film hydration method followed by sonication—a well-established and scalable approach that enables the production of nanosized vesicles with uniform size distribution.

The liposomal formulation was thoroughly characterized to evaluate its physicochemical properties, including particle size, zeta potential, encapsulation efficiency, morphology, and in vitro release profile. Additionally, Fourier-transform infrared (FTIR) spectroscopy was employed to assess any potential interaction between UA and the liposomal lipid matrix.

This study aims to contribute to the advancement of liposome-based drug delivery technologies for hydrophobic therapeutic agents, with a focus on UA as a model drug. The successful development of UALs not only holds potential for improved cancer therapy but also paves the way for future exploration of liposomal systems in the delivery of other poorly soluble bioactives.

Materials and Methods

Materials Ursolic acid was procured from Sigma-Aldrich, India. Soybean phosphatidylcholine (SPC), cholesterol, and chloroform were obtained from HiMedia Laboratories. All chemicals and solvents were of analytical grade and used without further purification.

Preparation of UA-Loaded Liposomes (UALs) Liposomes were prepared using the thin-film

hydration technique. Briefly, SPC and cholesterol (molar ratio 4:1) were dissolved along with UA (120 mg) in 50 mL of chloroform-methanol (2:1 v/v) mixture. The solvent was evaporated under reduced pressure in a rotary evaporator at 40°C to form a thin lipid film on the inner wall of a round-bottom flask. The film was hydrated with 50 mL phosphate-buffered saline (PBS, pH 7.4) and sonicated using a probe sonicator to reduce particle size. The dispersion was centrifuged at 6000 rpm for 20 minutes to remove unentrapped UA. The supernatant was analyzed for free UA using UV-Vis spectrophotometry.

Characterization of UALs

Particle Size and Zeta Potential The hydrodynamic diameter and zeta potential of UALs were measured using dynamic light scattering (DLS) with a Malvern Zetasizer.

Transmission Electron Microscopy (TEM) A drop of UAL suspension was placed on a carbon-coated copper grid and air-dried. Samples were examined under a transmission electron microscope for particle morphology.

Encapsulation Efficiency (EE%) Encapsulation efficiency was determined by calculating the amount of UA encapsulated relative to the total amount used. EE (%) = [(Total UA - Free UA) / Total UA] × 100.

In Vitro Drug Release The release profile of UA from UALs was studied using the dialysis bag diffusion method in PBS (pH 7.4) at 37°C. At predetermined time intervals, samples were withdrawn and analyzed spectrophotometrically at 210 nm.

FTIR Spectroscopy FTIR spectra were recorded to identify possible interactions between UA and liposome components. Samples of pure UA, empty liposomes, and UALs were scanned over the range of 4000–400 cm^{-1}.

Results and Discussion

Particle Size and Zeta Potential The UA-loaded liposomes (UALs) demonstrated particle sizes in the range of 130–200 nm with a narrow polydispersity index (PDI) of <0.3, indicating uniform size distribution. This size range is ideal for passive targeting via the enhanced permeability and retention (EPR) effect in tumor tissues. The relatively small size promotes prolonged circulation time and deep tissue penetration. Zeta potential measurements showed a negative surface charge around -25 mV, suggesting good colloidal stability of the liposomes. This negative charge creates repulsive forces between vesicles, preventing aggregation and ensuring a stable suspension suitable for intravenous administration.

Encapsulation Efficiency The encapsulation efficiency of UALs was found to be approximately 68%, which is considerably high for hydrophobic molecules like ursolic acid. This efficient drug loading can be attributed to the lipophilic nature of UA and its strong affinity for the lipid bilayer of the

liposomes. The inclusion of cholesterol in the formulation enhances bilayer rigidity and improves encapsulation by reducing leakage during preparation. High encapsulation efficiency ensures minimal drug loss during formulation and maximizes therapeutic potential.

Morphological Characteristics Transmission electron microscopy (TEM) images confirmed that the UALs were spherical in shape with smooth surfaces and relatively uniform size distribution. The vesicles appeared unilamellar with well-defined boundaries, supporting the DLS findings. The spherical and discrete morphology contributes to better biodistribution and uptake by cells, as irregular or aggregated particles may be rapidly cleared by macrophages. These characteristics also enhance biocompatibility and cellular internalization.

In Vitro Drug Release Profile The in vitro release study of UALs conducted using the dialysis method in PBS (pH 7.4) at 37°C revealed a biphasic drug release profile. An initial burst release of approximately 35% was observed within the first 3 hours, which can be attributed to the desorption of surface-associated UA. This is followed by a sustained release phase where 78% of the encapsulated drug was released over a period of 24 hours. The sustained release is beneficial for maintaining therapeutic drug levels over an extended duration, reducing the need for frequent dosing. Such controlled release behavior is essential for anticancer therapies to ensure continuous drug exposure to tumor cells while minimizing systemic toxicity.

FTIR Analysis Fourier-transform infrared (FTIR) spectroscopy was used to assess potential interactions between UA and the liposomal lipid bilayer. The characteristic peaks of UA, including the O-H stretch near 3398 cm^{-1} and the C-H stretching bands around 3000 cm^{-1}, were retained in the UAL spectra with minimal shift or intensity change. This indicates the absence of chemical bonding or degradation during encapsulation, confirming that UA was physically entrapped within the lipid bilayer. The preservation of UA's structural integrity is crucial for maintaining its biological activity post-delivery.

Overall Interpretation The results affirm that liposome-based encapsulation is a viable strategy for the delivery of poorly soluble compounds like ursolic acid. The optimized liposomal formulation exhibited desirable physicochemical characteristics, efficient drug loading, and a sustained release profile. These features collectively enhance the therapeutic value of UA by improving its bioavailability, stability, and pharmacokinetics. The formulation is expected to improve patient compliance and therapeutic outcomes in cancer treatment by ensuring prolonged drug exposure, reduced dosing frequency, and targeted delivery.

The positive outcomes of this study provide a strong foundation for future in vivo investigations and clinical translation of UA-loaded liposomal systems.

Further research may focus on exploring targeted liposomes through ligand conjugation and evaluating pharmacodynamic performance in relevant animal models of cancer.

Conclusion

This study successfully demonstrates the fabrication of ursolic acid-loaded liposomes (UALs) through a robust thin-film hydration and sonication method. The resulting liposomes exhibited desirable physicochemical characteristics, including a uniform particle size distribution, high encapsulation efficiency, and sustained drug release behavior. These attributes are critical for enhancing the bioavailability of UA, a hydrophobic compound with limited clinical applicability due to its poor solubility.

The use of liposomes as drug delivery vehicles offers distinct advantages such as biocompatibility, ability to encapsulate a wide range of drugs, protection of the therapeutic agent from premature degradation, and flexibility in surface modification for targeted delivery. Their structural similarity to biological membranes facilitates better cellular uptake and prolonged systemic circulation, which are essential for effective cancer therapy.

The prepared UALs demonstrated significant potential to address the limitations associated with UA delivery. The liposomal encapsulation not only improved the solubility and stability of UA but also allowed for a controlled release, thereby potentially reducing the dosing frequency and enhancing patient compliance. The absence of chemical interaction between UA and the lipid bilayer, as indicated by FTIR analysis, suggests physical entrapment, preserving the pharmacological activity of UA.

This research emphasizes the viability of liposomes as advanced nanocarriers in modern pharmaceutics and their applicability in improving the therapeutic performance of poorly soluble drugs like UA. The successful formulation of UALs opens avenues for further in vivo studies and eventual clinical translation for targeted cancer therapy.

In conclusion, liposome-based delivery of ursolic acid represents a promising approach to overcoming solubility and bioavailability challenges. The findings of this study contribute to the growing body of evidence supporting the utility of liposomal nanocarriers in personalized and precision medicine, offering hope for more effective and safer cancer treatments.

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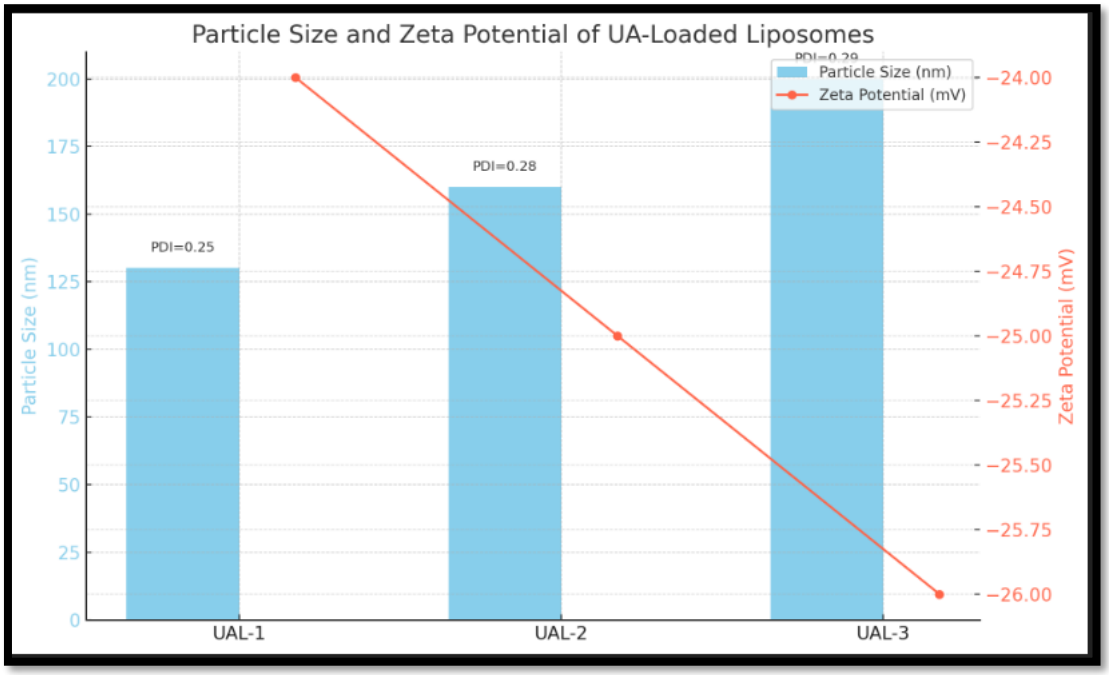

Fig 1: Graph showing both particle size and zeta potential of UA-loaded liposomes (UALs)

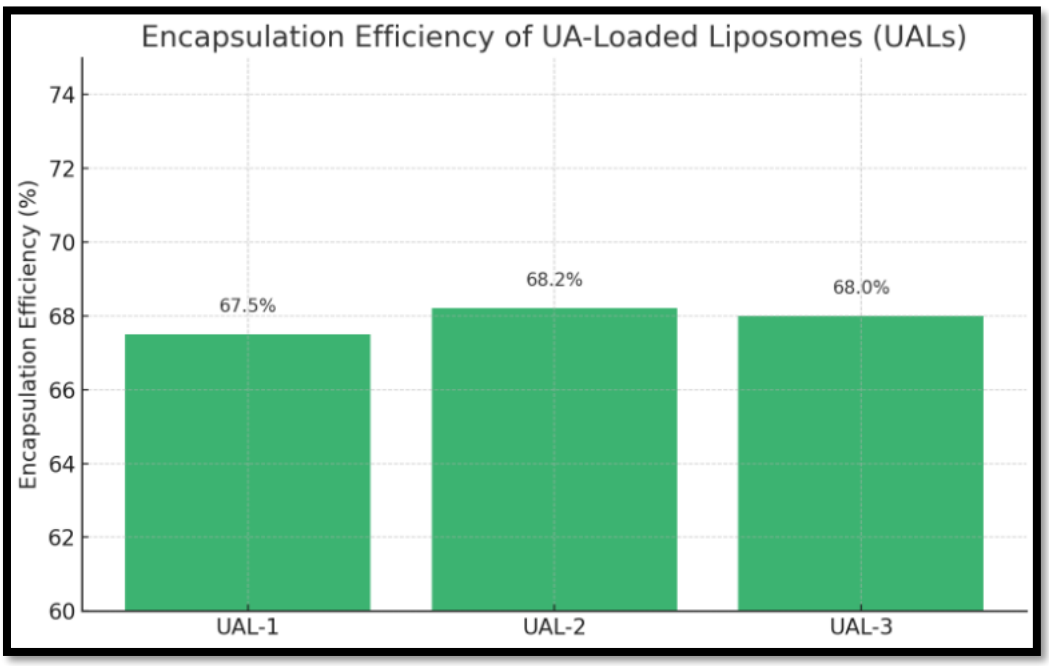

Fig 2: The encapsulation efficiency of UA-loaded liposomes (UALs)

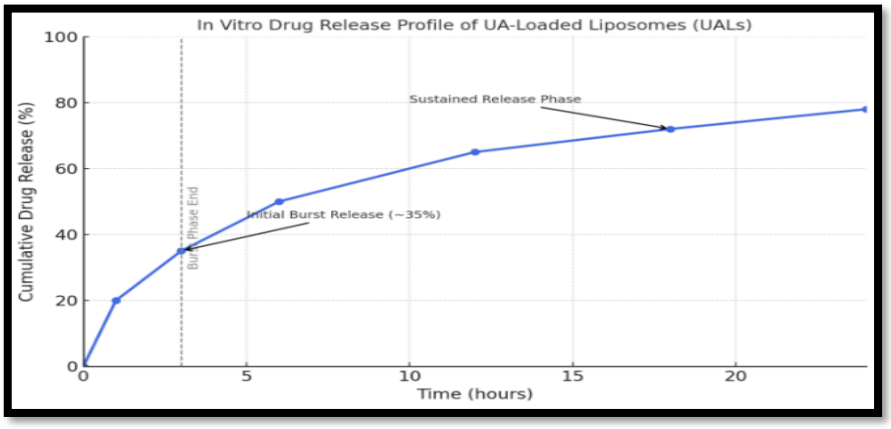

Fig 3: The graph shows the biphasic in vitro drug release profile of UA-loaded liposomes