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Innovative Approaches in Liposomal Drug Delivery Systems: A Comprehensive Review of Novel Strategies and Advancements

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Abstract: Liposomal drug delivery systems (LDDS) offer significant potential for enhancing therapeutic efficacy by improving drug solubility, bioavailability, and targeting. However, their clinical translation requires overcoming challenges related to stability, encapsulation efficiency, and controlled release. This review focuses on recent advancements in LDDS formulation and optimization. Emerging preparation techniques, including dual asymmetric centrifugation (DAC), freeze-drying, microfluidic methods, and supercritical fluid processing, are discussed. DAC enables the production of highly uniform liposomes with high encapsulation efficiency, while freeze-drying improves long-term stability. Microfluidic techniques offer precise control over liposome size and uniformity, suitable for large-scale production. Supercritical fluid processing allows for the production of small, homogeneous liposomes with efficient encapsulation of hydrophobic drugs. Strategies for enhancing liposome stability, such as surface modification, incorporation of stabilizing agents, and optimized formulations, are also explored. The review highlights the potential of next-generation technologies, including stimuli-responsive liposomes and targeted delivery systems, to further improve the therapeutic outcomes of LDDS. This review provides a comprehensive overview of innovative approaches for improving LDDS formulation and optimization, paving the way for their successful clinical translation.

Keywords: Liposomes, Dual asymmetric centrifugation, Micro fluidic method, Freeze-drying, Supercritical fluid-based method

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Introduction

Liposomes, microscopic spheres formed by phospholipid bilayers, have garnered significant attention as drug delivery systems due to their ability to encapsulate both hydrophobic and hydrophilic agents. This structure mimics cell membranes, offering advantages like enhanced solubility, bioavailability, targeted delivery, and sustained drug release (Akbarzadeh *et al.*, 2013; Ahmad *et al.*, 2017). However, challenges such as storage stability and encapsulation efficiency remain areas of active research (Allen and Cullis, 2004; Barenholz, 2012).

Innovative preparation techniques, including dual asymmetric centrifugation (DAC), freeze-drying, microfluidic methods, and supercritical fluid processing, have demonstrated improved liposome size control and scalability (Deamer and Bangham, 1976; Cullis *et al.*, 1987). Stability enhancement strategies, like surface modifications and inclusion of stabilizing agents, show promise for clinical translation (Gregoriadis and Florence, 1993; Hua, 2014). Additionally, next-generation liposomes, such as stimuli-responsive and targeted systems, are being developed to enhance therapeutic outcomes (Liu *et al.*, 2013; Karimi *et al.*, 2017). These advancements highlight the transformative potential of liposomes in nanomedicine (Mozafari, 2005; Pattni *et al.*, 2015).

Further exploration of their applications in oncology and vaccine delivery underlines their versatility (Omri and Beaulac, 2003; Jaafari and Ghafelehbashi, 2018). This comprehensive review integrates insights from seminal and recent studies, providing a roadmap for the clinical success of liposomal drug delivery systems (Xu *et al.*, 2012; Sercombe *et al.*, 2015).

Novel Methods for Preparing Liposomes:

(i) *Dual Asymmetric Centrifugation*

Dual Asymmetric Centrifugation (DAC) employs a unique rotational mechanism (Figs. 1, 2). Sealed vials undergo simultaneous rotation on the main axis and their own axis. This dual rotation generates mechanical turbulence and cavitation within the sample, leads, formation of nano-sized liposomes with an ideal size distribution of approximately 60 nm.

Advantages: DAC offers several benefits, including compact equipment size, ease of operation, high reproducibility, and the production of small-sized liposomes.

Disadvantages: DAC requires a relatively high number of phospholipids to achieve sufficient viscosity for optimal liposome formation. Additionally, it is currently limited to batch-scale production.

(ii) *Freeze-Drying* (*Lyophilization*)

Freeze-Drying is a monophasic solution technique for preparing liposomes (Fig. 3). This method involves dissolving lipids and the drug in tert-butyl alcohol at 45°C and a lyoprotectant in water at 45°C. Both solutions are then mixed to form a third, homogenous solution, followed by filtration and subsequent freeze-drying. Freeze-drying comprises two stages: initial freezing at -40°C, followed by drying at room temperature. Upon hydration, these dried "proliposomes" readily reconstitute into liposomes with mean dimensions typically ranging from 100 to 300 nm.

Advantages: This method offers a single-step process for liposome preparation, for large production. Freeze-drying significantly enhances the shelf-life of liposomes by preventing physical degradation during storage.

Disadvantages: Freeze-drying is a time-consuming and energy-intensive process. It may induce minor physical alterations, changes in vesicle size. There is a potential for loss of material that was sealed during the freeze-drying procedure.

(iii) *Microfluidic Methods*

Microfluidic Methods utilize microchannels (5-500 μm in cross-section) to generate liposomes. A solution of lipids put in ethanol or isopropanol is introduced into the microchannel, where it is between two aqueous phases. This creates a hydrodynamic laminar flow and diffusive mixing

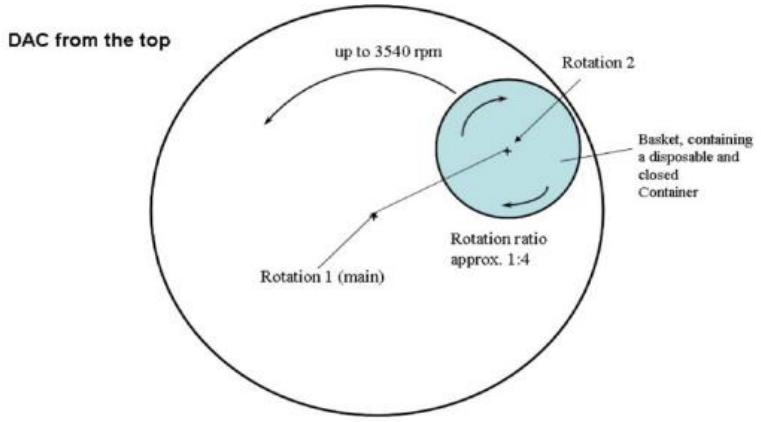

Fig. 1: DAC from top view.

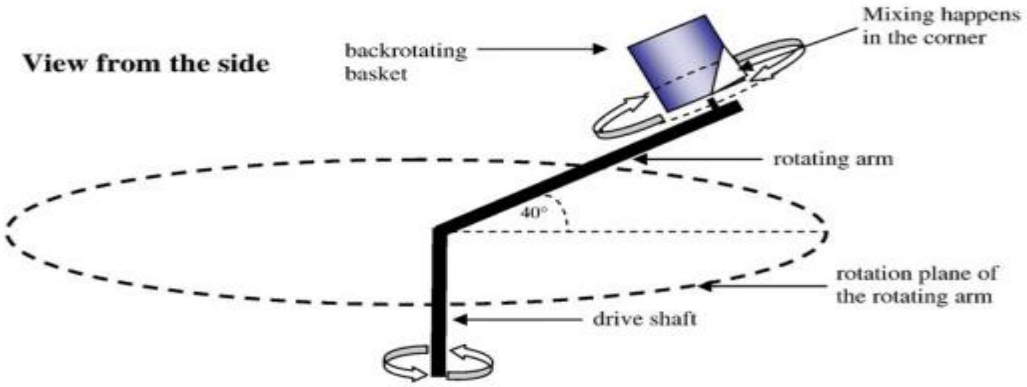

Fig. 2: DAC from side view.

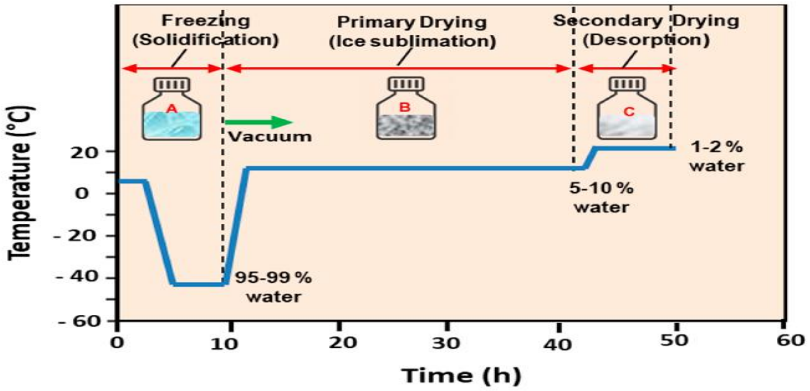

Fig. 3: Stages of Freeze Drying (Lyophilization) method.

at the liquid-liquid interfaces, facilitating the self-assembly of lipids into vesicles. Precise control over flow rates and mixing within the micro-channel enables the production of monodisperse liposomes with controlled size and size distribution. The use of low-toxicity solvents like ethanol is advantageous.

Advantages: Microfluidic methods offer a relatively simple approach with good control over particle size. These methods can be cost-effective compared to some traditional bulk methods.

Disadvantages: Removal of residual organic solvent from the final liposome product can be

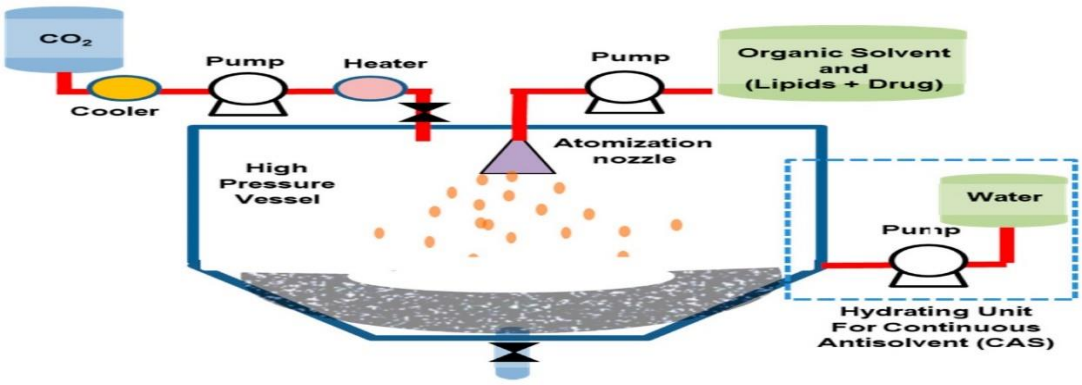

Fig. 4: SAS method for liposome formation.

challenging. Current microfluidic systems may not be readily scalable for large-scale, industrial-level production.

(iv) *Supercritical Fluid* (*SCF*) *Methods*

Supercritical Fluid (SCF) Methods have emerged as promising green alternatives to conventional liposome preparation techniques, addressing concerns related to solvent toxicity and environmental impact. Common SCF methods include (SAS), depressurization of an expanded liquid organic solution-suspension (DELOS), supercritical assisted atomization, supercritical assisted liposome synthesis (super Lip), supercritical fluid extraction of particles from gas-saturated solutions, and supercritical reverse phase evaporation (SCRPE).

(a) *Method of Supercritical Anti-Solvent* (*SAS*)

The SAS method is widely employed for the preparation of proliposomes (Fig. 4). It offers a relatively simple approach with minimal solvent residue, making it suitable for drugs with low solubility in supercritical fluids. In this process, a supercritical fluid, typically carbon dioxide (CO_2) – chosen for its inflammability, non-corrosivity, non-toxicity, and environmental friendliness – is passed through a high-pressure chamber. A solution containing the drug and lipids in an organic solvent is then sprayed into the chamber as fine droplets. The supercritical CO_2, which is immiscible with the solute but miscible with the organic solvent, induces rapid solvent precipitation, leading to the formation of liposomes. Upon hydration, these proliposomes reconstitute into liposomes. An upgraded version of this method, known as Continuous Anti-Solvent (CAS), has also been developed.

Advantages: Low solvent residue in the final product. Suitable for drugs with low solubility. Efficient and environmentally friendly process. Enables controlled and reproducible particle size.

Disadvantages: Potential for particle agglomeration and aggregation. Residual traces of toxic solvents may remain in the final product.

(b) *Supercritical Reverse Phase Evaporation* (*SCRPE*)

Developed by Otake *et al.* (2006) the SCRPE method involves combining lipids, an organic co-solvent, and compressed gas in a stirred variable-volume cell at a temperature exceeding the lipid phase transition temperature (typically around 60°C) and a pressure of 10-30 bar. An aqueous solution is then gradually introduced into the cell, followed by pressure reduction through controlled release of the compressed gas. This process leads to the formation of liposomes with a mean diameter ranging from 100 to 1200 nm, depending on the initial lipid concentration. The SCRPE

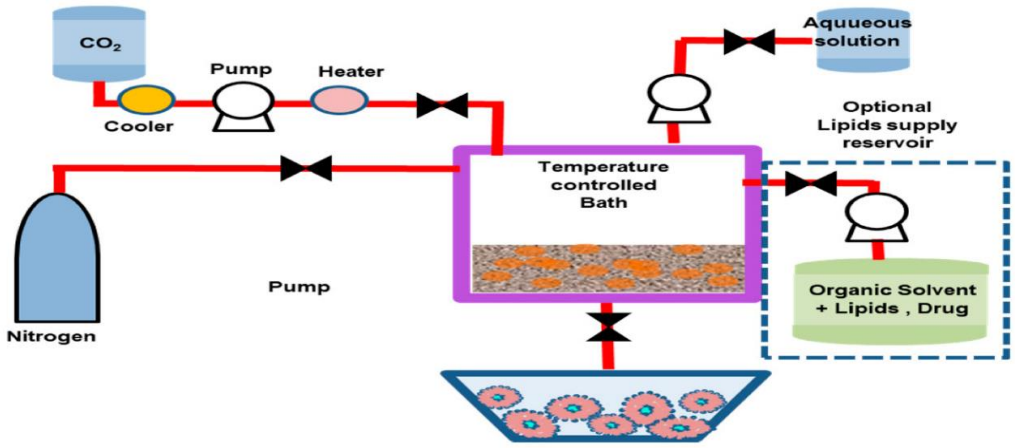

Fig. 5: DELOS method.

method shares similarities with the decompression method in its underlying principles.

A recent advancement by Otake *et al.* (2006) is the improved supercritical reverse phase evaporation (ISCRPE) technique. In this method, phospholipids and drugs are sealed in a viewing cell. The temperature is then raised to 60°C, followed by CO_2 introduction for 40 min. Finally, the pressure is released to obtain a liposome dispersion.

Advantages: Free from organic solvents in the final product. Capable of encapsulating both water-soluble and oil-soluble materials. Applicable for commercial-scale production. Relatively less time-consuming compared to some other SCF methods.

Disadvantages: Lower encapsulation efficiency compared to other methods. Reduced liposome stability.

(c) *Depressurization of an Expanded Liquid Organic Solution Suspension* (*DELOS*)

In the DELOS method, phospholipids are dissolved in an organic solvent at a predetermined temperature and pressure (Fig. 5). This solution is then introduced and mixed with supercritical CO_2 within a vessel, where the CO_2 acts as a co-solvent. Finally, the solution is depressurized using a nozzle to generate liposomes. A key advantage of DELOS over the PGSS method is its suitability for thermo-sensitive materials, as it does not require high temperatures and operates under milder pressure conditions.

Advantages: Simple methodology. Operates under moderate pressure and temperature conditions. Applicable for thermo-sensitive materials.

Disadvantages: Residual organic solvent may be present in the final product. Potential for nozzle blockage.

(d) *Supercritical-Assisted Liposome Formation* (*SuperLip*)

SuperLip involves several steps (Fig. 6):

1. Lipid dissolution in ethanol.
2. Mixing the lipid solution with pure CO_2 in a high-pressure saturator equipped with baffles to create an expanded fluid.
3. Heating thin bands within the saturator to generate a supercritical fluid (SCF).
4. Passing the mixture through a high-pressure formation vessel.
5. Atomization of an aqueous solution containing the drug.

The working temperature and pressure of the saturator and formation vessel are typically set to 40°C and 100 bar, respectively. The resulting liposome suspension is collected from the bottom

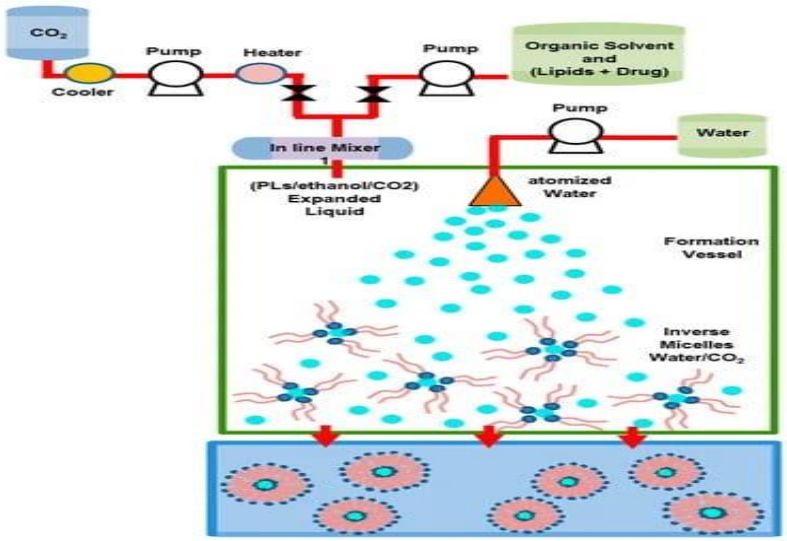

Fig. 6: Mechanism of liposome formation in superlip method.

of the vessel, and CO_2 and ethanol are separated using a stainless-steel separator maintained at 30°C and 10 bar pressure.

Advantages: Continuous and reproducible process. Applicable for encapsulating hydrophilic drugs. High entrapment efficiency. Low solvent residue.

Disadvantages: Time-consuming process. Requires high-pressure operation.

(e) *Rapid Expansion of a Supercritical Solution* (*RESS*)

The RESS technique is a two-step process (Fig. 7):

1. Dissolving lipids in a solution containing 5-10% (v/v) ethanol and supercritical CO_2 within an extractor.
2. Depressurizing the solution through a heated nozzle into a low-pressure chamber.

The rapid depressurization at supersonic speeds through the nozzle leads to a decrease in pressure and rapid evaporation of CO_2. This, in turn, induces supersaturation and precipitation of solids, which are collected from the gaseous stream. The RESS method can be used for liposome preparation, and the rapid depressurization promotes desolvation of lipids, facilitating the formation of layers around droplets and ultimately leading to liposome formation. The resulting liposomes typically exhibit an oligolamellar vesicle structure with a broad size distribution ranging from 50 nm to nearly 1 µm. This method allows for the encapsulation of both hydrophobic and hydrophilic drugs.

Future Prospects:

The future of liposomal drug delivery systems is highly promising, driven by advancements in innovative techniques such as dual asymmetric centrifugation, microfluidics, freeze drying, and supercritical fluid methods. These evolving technologies offer precise control over liposome size, uniformity, and drug encapsulation efficiency, paving the way for scalable and reproducible manufacturing processes. For instance, microfluidics and supercritical techniques hold potential for enhancing drug loading and minimizing variability in traditional production methods, improving therapeutic outcomes. Emerging technologies like artificial intelligence (AI) and computational modelling are expected to revolutionize liposome design by predicting optimal preparation conditions and improving stability. Integrating AI with microfluidic devices could facilitate the creation of liposomes with superior drug-loading capacities. Innovations in freeze-drying formulations are also anticipated,

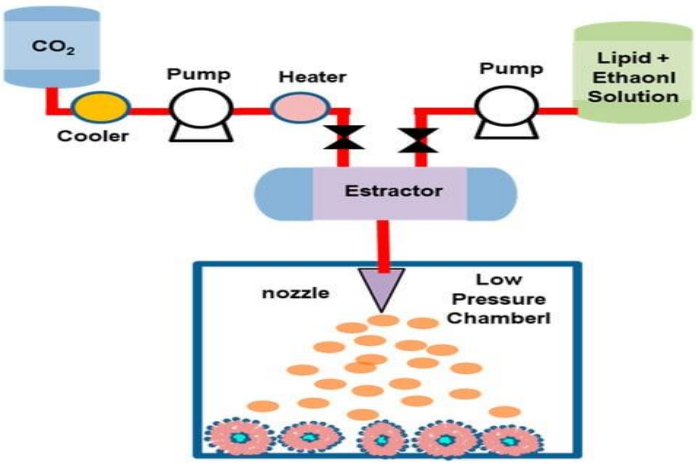

Fig. 7: RESS method for liposome formation.

aiming to improve rehydration efficiency while maintaining liposome integrity for long-term storage. Additionally, hybrid systems that combine liposomes with other nanocarriers are gaining attention, offering new possibilities for targeted drug delivery and personalized medicine. These multidisciplinary approaches are poised to overcome existing challenges and expand the clinical applications of liposomal drug delivery systems.

Conclusion

This review highlighted significant advancements in liposomal drug delivery systems, focusing on novel preparation and optimization techniques. Methods such as dual asymmetric centrifugation, freeze-drying, microfluidics, and supercritical fluid processing demonstrate potential in addressing challenges related to liposome formulation, drug loading, and stability. Dual asymmetric centrifugation ensures precise control over liposome size, freeze-drying enhances long-term storage, microfluidics enables high-throughput production with reproducibility, and supercritical fluids improve drug encapsulation and loading efficiency. Together, these innovations pave the way for more efficient and scalable production of liposomal systems. Looking ahead, advancements in technology and the integration of liposomes with other nanocarriers are expected to enable more targeted and personalized treatments. Streamlining production processes for scalability and consistent quality will be crucial for broader medical applications. Liposomal drug delivery systems hold great promise for improving outcomes, particularly in treating challenging conditions such as cancer, neurological disorders, and infections, marking a step forward in next-generation therapeutic strategies.

Ethical Statement

This is a review article hence no Ethical Approval needed.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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