Review On Comparative Study of Methods Preferred in Formulation of Liposomes for Anticancer Drug Delivery

[1]Gitanjali Bhatjire, [2]Vandana Shirsath, [3]Bhavesh Akbari, [4]Anil Jadhav
[1]Research Scholar, [2]Assistant Professor, [3,4]Principal
[1,3]School of Pharmacy
[1,3]PP Savani University, Kosamba, Surat, Gujrat-394125
[2,4]Mahavir Institute of Pharmacy, Mhasrul-Varvandi Road, Nashik, Maharashtra 422202.
[1]8459492078, [1]gitsbhatjire88@gmail.com

Abstract-Liposomes are versatile nanocarrier vesicles, composed of Phospholipids, featuring both a hydrophilic core and hydrophobic membrane. This dual nature makes them ideal for delivering molecules with varying polarities. Their biocompatibility, biodegradability, and ability to target specific tissues have established them as a cornerstone in anticancer drug delivery system. Today, liposomes are actively used in tumor targeting, gene therapy, genetic vaccines, immunomodulation, photodynamic therapy, transdermal delivery. In the oncology sector, these nanovesicle formulations depict a significant advancement by offering innovative solutions for the delivery of chemotherapeutic agents. This article confronts latest developments in widely used liposome formulation methods including thin film hydration method, Solvent evaporation, microfluidic techniques. Each method presents distinct advantages, challenges and limitations, which are discussed in detail. Additionally, the review highlights advanced approaches and future directions in the field of liposomal drug delivery.

Index Terms—Oncology, Nano-vesicles, Microfluidic Method, Rotary evaporator, Film Hydration, Drug Delivery.

I. INTRODUCTION

Liposomes, as nanoscale vesicular structures, possess one or more concentric phospholipid bilayers encapsulating aqueous cores. It is one of the most promising drug delivery systems in modern pharmaceutical science. Structurally, Liposomes are lipid bilayer vesicles, known for their biodegradability and biocompatibility, making them effective carriers for targeted drug delivery.

They can encapsulate both hydrophilic and hydrophobic drugs, offering versatility in therapeutic applications. Due to their diverse composition and structural adaptability, liposome have become widely utilized in biomedical fields. In recent developments, malfunctional liposome have emerged as promising tool s for targeted tumor. One of the most promising and versatile drug delivery systems in modern pharmaceutical science is liposomes. Structurally, liposomes are nanosized vesicles formed by one or more concentric phospholipid bilayers encapsulating aqueous cores [1,6,10]. This unique amphiphilic architecture allows them to simultaneously carry both hydrophilic agents and lipophilic compounds, making them exceptionally adaptable for a wide range of therapeutic applications [32,34].

In the context of cancer therapy, liposomes are particularly advantageous. They offer improved bioavailability, prolonged circulation time, and reduced systemic toxicity compared to conventional chemotherapeutic formulations [36]. A key feature contributing to their clinical relevance is their ability to be modified for targeted delivery. Surface modifications such as the incorporation of polyethylene glycol (PEG) or specific ligands can enhance passive or active targeting, facilitating the accumulation of liposomes in tumor tissues through the enhanced permeability and retention (EPR) effect (Fig. 1). Furthermore, their structural similarity to biological membranes enhances their biocompatibility, enabling them to mimic natural cellular processes and evade immune recognition [40,41].

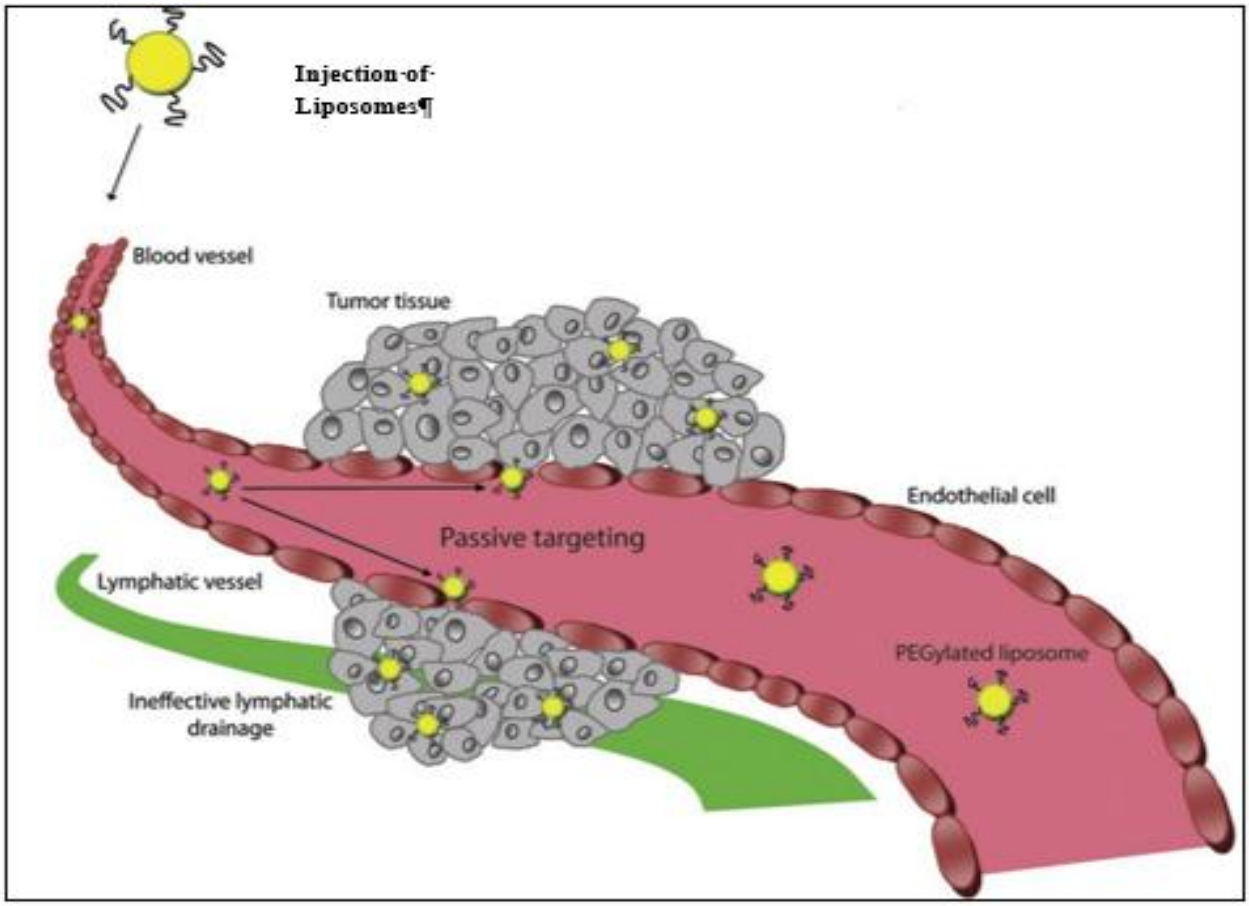

Figure 1 Enhancement in Permeability and retention effect by Liposomes [53]

The growing interest in liposomal systems is driven not only by their therapeutic versatility but also by ongoing advances in manufacturing technologies. These include reverse-phase evaporation, ethanol injection, freeze-drying, and more recently, microfluidic technologies. Such innovations have significantly improved the scalability, reproducibility, and drug loading capacity of liposome formulations, thereby strengthening their potential for clinical translation [36,42]. Among the significant innovations in liposome development has been the introduction of "stealth technology." By incorporating PEGylated lipids, researchers have developed long-circulating liposomes that are less readily identified and cleared by the mononuclear phagocyte system. This improves drug delivery to the targeted site of action and extends systemic circulation. However, despite their potential, liposome-based drug products are inherently complex. Even subtle changes in their composition or preparation method can markedly affect pharmacokinetics, biodistribution, and therapeutic outcomes. As such, meticulous attention to formulation parameters and manufacturing processes is essential to ensure consistency and efficacy [41,42]. Beyond oncology, liposomes have also found application in other therapeutic areas. In cardiovascular diseases, they have been used to deliver nitric oxide and antioxidants to improve vascular function and mitigate oxidative stress. Liposomes facilitate targeted gene expression with less immunogenicity in gene therapy by acting as non-viral vectors for the delivery of nucleic acids like DNA and RNA [44].

Importantly, the technique employed for liposome preparation plays a pivotal role in determining their physicochemical characteristics such as particle size, lamellarity, surface charge, and encapsulation efficiency, which directly influence their stability, biodistribution, and therapeutic performance (Fig. 2). Several techniques have been established for liposome production, each with unique advantages and challenges. This mini-review explores four widely adopted methods: thin-film hydration, solvent evaporation, ethanol injection, and microfluidic technology (Fig. 3). These methods differ significantly in terms of complexity, scalability, reproducibility, and the physical properties of the resulting liposomes. For instance, thin-film hydration remains a popular laboratory-scale technique known for producing multilamellar vesicles, while microfluidic methods represent a newer, more precise approach capable of generating uniform unilamellar liposomes with reduced batch-to-batch variability [34].

Figure 2 Features of Liposomal Formulation

Given the complexity and therapeutic importance of liposomal systems, this review aims to provide a comparative overview of these liposome preparation techniques, with a focus on their relevance in anticancer drug delivery. Emphasis is placed on how these methods influence key formulation parameters and how they can be optimized for clinical application [42].

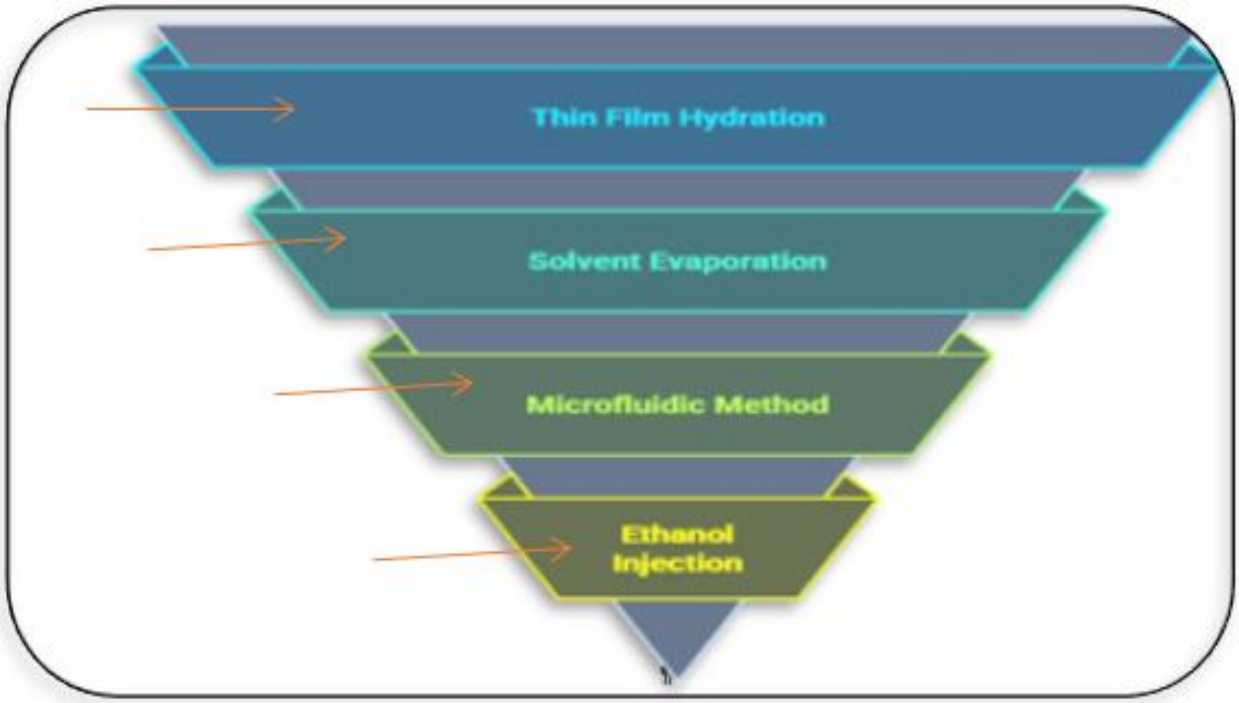

Figure 3 Liposomes Formulation Techniques

II. METHOD OF THIN FILM HYDRATION

Advanced Techniques in Liposome Preparation: A Narrative Overview

Because liposomes can encapsulate a wide range of therapeutic agents, they have become essential in contemporary pharmaceutical and biomedical research. Among the many fabrication techniques developed, certain methods stand out due to their efficiency, reproducibility, and suitability for specific drug types. Below is a detailed, narrative exploration of some of the key methods currently employed in liposomal formulation. [10] The thin film hydration method is a basic technique for creating liposomes and niosomes, and it is regarded as one of the most well-established and extensively used approaches [1–6,10–19]. Lipid components are first dissolved in an organic solvent, usually methanol or chloroform [1,2]. A thin lipid film is then formed on the inner surface of a round-bottom flask as the solvent is evaporated under reduced pressure, typically using a rotary evaporator [3,4]. Upon hydration with an aqueous solution potentially containing the therapeutic compound this dry lipid film spontaneously swells and peels off, forming multilamellar vesicles (MLVs) [5,6]. These MLVs can then be processed via extrusion, sonication, or freeze-thaw cycles to produce unilamellar vesicles with controlled size and improved homogeneity [10,11]. Technique involves lipid dissolution in organic solvents, followed by solvent evaporation to form a thin lipid film, which is then hydrated to generate liposomes [12]. This technique offers

advantages, including high encapsulation efficiency for hydrophilic drugs, as the aqueous core formed during hydration effectively traps these agents [13,14]. Particle size control is achievable through downstream processing such as extrusion, which yields vesicles with uniform diameters [15]. Additionally, the rigid structure of lipid bilayers contributes to enhanced stability, which in turn supports better drug retention and shelf life [16]. However, the method has limitations as it is time-consuming, involving multiple steps from film formation to size reduction [17]. It is also less effective for lipophilic drug loading, as these compounds must be incorporated into the lipid bilayer during the initial lipid dissolution stage, which may restrict their loading capacity [18,19].

Microfluidic Techniques

Microfluidic-based liposome production represents a cutting-edge innovation in nanotechnology. Unlike traditional bulk processes, microfluidic systems manipulate fluids within micron-sized channels, enabling precise control over liposome formation conditions. In this process, lipid materials dissolved in organic solvents are introduced into microfluidic chips alongside aqueous buffer solutions. Rapid and controlled mixing within these microchannels initiates the self-assembly of liposomes. [20,22] What distinguishes this method is its ability to produce highly uniform, unilamellar vesicles with consistent particle size and superior encapsulation efficiency. This precision is critical for reproducible performance in drug delivery applications, particularly in personalized medicine.[21] Its benefits include fine-tuning of liposome size and polydispersity by adjusting flow rates, temperature, and lipid-to-buffer ratios. Higher batch-to-batch reproducibility compared to thin film hydration, reducing variability in drug delivery performance. Enables co-encapsulation of multiple agents (e.g., therapeutic and diagnostic molecules) for theragnostic applications.[21] Some limitations observed, such as initial setup of microfluidic systems may be cost-intensive and require specialized fabrication. Solvent removal post-formation can be challenging and must be optimized for large-scale applications. [20,22]

Supporting Techniques in Liposome Preparation

To improve liposome production and refinement, a number of auxiliary techniques are employed in addition to the main ones mentioned below, either alone or in combination.

- Sonication Technique: This approach employs ultrasonic energy to reduce the size of multilamellar vesicles into small unilamellar vesicles (SUVs). It's commonly used after hydration in thin film methods to fine-tune liposome diameter.
- Extrusion Method: Liposomal suspensions are passed through polycarbonate membranes with defined pore sizes, yielding vesicles of uniform size. This is particularly effective for producing nano-liposomes for intravenous applications.
- Emulsification-Evaporation Method: involves dispersing a lipid-drug solution in an organic phase into an aqueous phase to create an emulsion. As the solvent evaporates, lipid vesicles form, ideal for encapsulating lipophilic drugs.[72]

- Lipid Layer Hydration Method: Similar to thin film hydration, this technique focuses on hydrating a pre-formed lipid film to initiate liposome formation. It may be used for both small-scale research and large-scale manufacturing.[73]

Microfluidic Techniques Overview

Microfluidic systems have emerged as a cutting-edge method in liposome production, offering precision and scalability that surpass traditional techniques [20]. In microfluidic processes, lipid materials are initially dissolved in organic solvents and then mixed with aqueous buffer solutions within microfluidic chips. This controlled micro-mixing results in consistent and reproducible liposome formation [21]. The design of the microfluidic devices significantly influences their efficiency. Configurations like Y-shaped channels and 3D integrated chip structures enhance the mixing process and support continuous production [22]. This capability is particularly important in overcoming limitations related to solvent removal and scale-up. A major advantage of microfluidic methods is their precise control over particle size. By adjusting flow rates and solution composition, manufacturers can produce liposomes with narrowly distributed particle sizes, offering better uniformity than traditional techniques [23]. Devices such as static mixers and staggered herringbone micromixers have shown varied performance, with static mixers notably improving particle size distribution [24]. Advancements in 3D printing and device fabrication have improved throughput, making microfluidic liposome production viable for industrial applications. The ability to effectively remove residual solvents enhances both shelf-life and safety of the resulting formulations, a critical factor for clinical usage [71].

Solvent Evaporation Method

For the preparation of liposomes, the solvent evaporation method is still one of the most widely used and scalable techniques [2]. Phospholipids and medication ingredients are dissolved in a volatile organic solvent, which is subsequently emulsified into an aqueous phase [6]. After that, the solvent is evaporated, usually at a lower pressure, which promotes the formation of vesicles and gas bubbles that aid in the formation of liposomes [7]. It is a versatile method, accommodating a wide range of lipid mixtures and drug types and is often combined with techniques such as homogenization or membrane extrusion to yield liposomes with controlled particle sizes and uniformity [11,12]. One of the method's key strengths is its adaptability to industrial-scale production as it has simplicity and high throughput [13]. The removal of organic solvents, although a critical step, must be managed carefully to ensure product safety [6]. The choice of solvent significantly influences the final product. Polar solvents tend to enhance the encapsulation of hydrophilic drugs, thereby increasing efficacy [11]. However, the possibility of residual solvent presence is a major concern, potentially impacting liposome stability and safety [7]. This can be a benefit as it is a quick and scalable process suitable for industrial use, and effective for encapsulating both hydrophilic and lipophilic drugs [12,13]. Also, drawbacks include the possibility that residual solvent may compromise safety and stability [6], and the method often shows lower encapsulation efficiency for hydrophilic drugs compared to other methods [2]

Ethanol Injection Method

The ethanol injection method is another widely employed strategy in liposome formulation [4]. This technique involves dissolving lipids in ethanol and injecting the solution into an aqueous buffer under controlled conditions [6]. The temperature is typically maintained between 60–70 °C to facilitate efficient liposome formation [8]. One of the key advantages is its scalability, with successful production volumes ranging from laboratory-scale (60 mL) to industrial-scale (3 L) [9]. The resulting liposomes usually fall within the 150–200 nm size range and display narrow polydispersity indices [14]. Encapsulation efficiency is typically high (above 90%), making this method particularly appropriate for drug delivery [15]. It is also compatible with a wide variety of active agents, from pharmaceutical drugs to plant-derived bioactive compounds [16]. The method has drawbacks despite being effective and simple to use. Notably, because of the quick mixing, it might result in reduced encapsulation efficiencies for hydrophilic medications [17]. Furthermore, ethanol residues in the final product may require additional purification steps, adding time and complexity to the process [6,18]. Some of the advantages include that it is a simple, cost-effective method with minimal equipment requirements and produces small, uniform vesicles suitable for targeted delivery [14,15]. However, its limitations include potentially poor encapsulation for hydrophilic compounds [17] and the requirement for purification to remove residual ethanol [18].

Ultrasonication

Ultrasonication is a simple and frequently used method for the preparation of liposomes [53]. This process is considered a green technology that utilizes ultrasonic waves to prepare emulsions, and sonication enhances dispersivity [54]. Although there are two different sonication techniques bath sonication and probe sonication the liposomes made using each have comparable properties [53]. However, when it comes to managing operational parameters, bath sonication is considered better due to its more uniform energy distribution [54]. The understanding of the exact mechanism behind liposome formation through sonication is still evasive; however, it involves the application of ultrasonic pulsation to a hydrated lipid solution to create a more uniform population of liposomes [53]. The high-frequency vibration likely a combination of physical agitation and water cavitation induced by sonication results in the shattering of large lipid structures, which then reform as small, unilamellar liposomes [54].

Additional Context: Production Techniques for Lipid Nanoparticles

In addition to the liposome-specific approaches mentioned above, a number of methods are used for the more general class of lipid nanoparticles (LNPs). These include nanoprecipitation, emulsification, thin film hydration, microfluidic mixing, and impingement jet mixing. Each approach offers unique benefits and challenges that influence the physicochemical properties, Entrapment efficiency, and therapeutic performance of the prepared nanoliposomes. To optimise drug delivery systems and achieve the intended clinical outcomes, it is crucial to comprehend the advantages and disadvantages of each technique.[52]

TABLE 1 COMPARATIVE ANALYSIS OF METHODS

Method	Process Description	Advantages	Limitations
Thin Film Hydration	Lipid film formation followed by hydration and extrusion [18]	High encapsulation efficiency, uniform size, and stability, simple process [18]	Time-consuming, complex process, low drug loading for lipophilic drugs [31]
Solvent Evaporation	Emulsification of lipid-drug solution in aqueous phase, followed by solvent evaporation [18]	High throughput, flexibility in drug encapsulation [18]	Residual solvent, poor encapsulation efficiency for hydrophilic drugs [31]
Ethanol Injection	Injection of ethanol-lipid solution into an aqueous phase under continuous stirring [18]	Simple method, cost-effective, produces small vesicles [18,31]	Low encapsulation efficiency, requires purification to remove ethanol residues [31]
Microfluidic technique	Allows for precise control over the liposome formation process by manipulating fluids at the microscale through tiny channels.[55]	Requires minimal sample volumes, High precision and reproducibility Reduced reagent consumption.[55]	Complex fabrication process, Risk of clogging in small channels, Limited scalability for large-scale production.[55]
Ultrasonication Method	Utilizes ultrasonic waves to form emulsion and followed by bath or probe sonication produces Liposome vesicles [53]	Simple, Fast method, High Efficiency [53]	Limited process capacity [53]

III. APPLICATIONS IN ANTICANCER DRUG DELIVERY

Conventional cancer treatments such as surgery, radiotherapy, and chemotherapy are often limited in their ability to address aggressive and metastatic forms of cancer [25]. These traditional modalities frequently result in high recurrence rates and suboptimal outcomes [26]. The development of advanced drug delivery systems (DDS) has therefore become a crucial strategy to maximize therapeutic effectiveness while minimizing adverse side effects [27,28]. Among these innovations, liposomal formulations have gained prominence for offering safe and effective ways to deliver chemotherapeutic agents [29,33].

This study highlights several liposome-based drug delivery techniques that have successfully transitioned from laboratory research to clinical applications [35,37,39]. These approaches are essential in modern oncology due to their ability to encapsulate a wide range of drugs, enhance bioavailability, and improve targeted delivery.

One of the most established techniques, Thin Film Hydration, is widely regarded as the benchmark for liposomal drug delivery, particularly for hydrophilic anticancer agents such as doxorubicin [25]. It facilitates the formation of multilamellar or unilamellar vesicles with high encapsulation efficiency. The rigid lipid bilayer structure contributes to enhanced liposome stability, making them suitable for systemic circulation and targeted tumor delivery [26,27]. A well-known example is Doxil, a commercially available liposomal formulation of doxorubicin that demonstrates improved pharmacokinetics, extended circulation time, and significantly reduced cardiotoxicity compared to conventional forms [28,29].

The Solvent Evaporation Method is particularly effective for encapsulating lipophilic compounds like paclitaxel, curcumin, and camptothecin [33]. It involves creating a lipid-drug emulsion followed by solvent removal, which results in liposome formation [35]. Its scalability and high throughput make it attractive for industrial applications [37]. Additionally, it allows for the integration of functionalized lipids or ligands to facilitate active targeting to cancer cells [39]. Researchers have also explored this method for combination therapies by co-encapsulating multiple hydrophobic drugs to enhance therapeutic efficacy and combat resistance [33,35].

The Ethanol Injection Method, known for its simplicity and rapid formulation, is especially suited for hydrophilic anticancer agents, including plant-derived bioactive compounds and cisplatin analogs [56]. It produces small unilamellar vesicles (SUVs) with a uniform size distribution, typically between 100–200 nm, enhancing cellular uptake and allowing better penetration into solid tumors with dense stromal barriers [57]. Due to its ease of use and minimal equipment requirements, this method is popular in academic research and early clinical development [58,59]. Its adaptability for quick formulation with customizable lipid compositions also makes it highly suitable for experimental nanomedicine [60].

The Microfluidic Technique represents a cutting-edge advancement in liposome formulation, offering precise control over vesicle size, shape, and drug loading [61–63]. Utilizing continuous-flow systems, this method achieves monodispersity and high encapsulation efficiency, which is particularly beneficial for cancer therapy [64,65]. In the realm of personalized medicine, microfluidic liposomes are employed for the co-encapsulation of therapeutic and diagnostic agents, enabling theranostic applications simultaneous diagnosis and treatment [66,67]. Furthermore, this technique is being used to deliver genetic materials such as siRNA, mRNA, and immune-modulatory therapeutics aimed at modulating the tumor microenvironment and overcoming drug resistance [68,69]. These technological advancements underscore the enormous potential of liposomal DDS in creating more effective, targeted cancer treatments while reducing side effects and improving patient outcomes [70].

IV. FUTURE PERSPECTIVES OF LIPOSOMES

Because of their versatility, biocompatibility, and capacity to be engineered for specific medical applications, liposomes are expected to play an increasingly important role in this endeavor. Some of these applications include the development of long-circulating liposomes that can bind and neutralize blood-borne toxins; localized drug depots that allow for the controlled and programmable release of agents such as morphine or cytosine arabinoside; ligand-targeted liposomes that target receptor saturation and macrophages in lymphatic and dermal tissues; inhalable liposomal aerosols for respiratory treatments; and the creation of liposomal blood substitutes and allergen-encapsulated formulations for desensitization therapies.

V. CONCLUSION

Several liposome formulation methods provide distinct benefits according to the characteristics of the drug and the intended therapeutic outcome. While solvent evaporation and ethanol injection facilitate high-throughput production and versatility, thin film hydration is still a dependable technique for encapsulating hydrophilic drugs. The next generation of nanocarrier systems is being shaped by microfluidic technology because of its accuracy and scalability. Liposomes enhanced solubility, stability, and targeted action have greatly improved the delivery of cancer drugs. Even though problems like leakage and poor stability persist, they are being quickly resolved by ongoing advancements in nanotechnology. Liposomes are therefore well-positioned to be a key component of more potent, precise, and patient-friendly treatments.

REFERENCES

[1] Enaru B, Fărcaș AC, Stănilă A, Socaci S, Diaconeasa Z. Novel Methods for Liposome Formulation: Advancements and Innovations. Bull Univ Agric Sci Vet Med Cluj-Napoca. 2023; 80:1–13. https://doi.org/10.15835/buasvmcn-hort:2023.0015

[2] Kumar V, Kewlani P, Singh A, Gautam A, Rajamanickam V. Multifunctional Liposomes to Attain Targeting, Stimuli Sensitive Drug Release and Imaging Cancer. In: Multifunctional Liposomes for Enhanced Tumor Targeting. Springer; 2023. p. 49–87. https://doi.org/10.1007/978-981-19-8760-2_3

[3] J. Carroll, N. Daly, J. R. Mondala, Alancasey, B. Tiwari, J. F. Curtin *et al.*, "Liposome synthesis protocol: Preparation of anionic and cationic unilamellar liposomal nanoparticles via thin-film hydration followed by extrusion," *Protocols.io*, 2023. [Online]. Available: https://doi.org/10.17504/protocols.io.dm6gpjyx1gzp/v1. [Accessed: Apr. 17, 2025].

[4] Tagrida M, Prodpran T, Zhang B, Aluko RE, Benjakul S. Liposomes loaded with betel leaf (Piper betle L.) ethanolic extract prepared by thin film hydration and ethanol injection methods: Characteristics and antioxidant activities. J Food Biochem. 2021;45(9):e14012. https://doi.org/10.1111/jfbc.14012

[5] Moudgil A, Chaudhari BP. Understanding Critical Aspects of Liposomal Synthesis for Designing the Next Generation Targeted Drug Delivery Vehicle. ChemistrySelect. 2023;8(12): e202302435. https://doi.org/10.1002/slct.202302435

[6] Chuprin EN, Gavrilov DI, Blynskaya EV. Methods for obtaining phospholipid vesicles to improve the bioavailability of sparingly soluble active pharmaceutical ingredients. Meditsinskaya Tekhnika. 2024; https://doi.org/10.33920/med-13-2402-03

[7] Chang H-I, Cheng M-Y. Clinically-Proven Liposome-Based Drug Delivery: Formulation, Characterization and Therapeutic Efficacy. Sci Rep. 2012. https://doi.org/10.4172/scientificreports.195

[8] Kaur L, Kaur P. Liposome as a drug carrier - a review. Int Res J Pharm. 2013;4(5):30–6.

[9] Liposomes as Targeted Drug-Delivery Systems. In: Torchilin VP, editor. Methods and Principles in Medicinal Chemistry. 2022; p. 69–125. https://doi.org/10.1002/9783527829991.ch3

[10] Al-Amin F, Bellato F, Mastrotto M, Garofalo A, Malfanti S, Salmaso P. Dexamethasone Loaded Liposomes by Thin-Film Hydration and Microfluidic Procedures: Formulation Challenges. Int J Mol Sci. 2020;21(5):1611. https://doi.org/10.3390/ijms21051611

[11] Deamer DW. Preparation of Solvent Vaporization Liposomes. In: Liposomes, Part E. CRC Press; 2019. p. 29–35. https://doi.org/10.1201/9780429505137

[12] Ahmed SA, Hassanzadeh H. Optimization of solvent evaporation method in liposomal nanocarriers loaded-garlic essential oil (Allium sativum): Based on the encapsulation efficiency, antioxidant capacity, and instability. IET Nanobiotechnol. 2023;17(1):e1214. https://doi.org/10.1049/nbt2.1214

[13] Cortesi R, Esposito E, Gambarin S, Telloli P, Menegatti E, Nastruzzi C. Preparation of liposomes by reverse-phase evaporation using alternative organic solvents. J Microencapsul. 1999;16(2):251–6. https://doi.org/10.3109/02652049909007238

[14] Charcosset C, Juban A, Valour JP, Urbaniak S, Fessi H. Preparation of liposomes at large scale using the ethanol injection method: Effect of scale-up and injection devices. Chem Eng Res Des. 2015; 94:508–15. https://doi.org/10.1016/j.cherd.2014.09.013

[15] Gentine P, Bourel-Bonnet L, Frisch B. Modified and derived ethanol injection toward liposomes: development of the process. J Liposome Res. 2013;23(1):11–9. https://doi.org/10.3109/08982104.2012.745629

[16] Huang G, Tian L, Hao Y. Artemether liposome for injection and preparation method and application thereof. CN108579666A. 2018.

[17] Can P, Yu J, Lingtao J, Junyu T, Xiushu L. Method for preparing paeonol-ozagrel conjugate lipidosome through ethanol injection method. CN106982312A. 2017.

[18] Harrison RM. Formulation and characterization of liposomes. In: Lipid-Based Nanocarriers for Drug Delivery and Diagnosis. Elsevier; 2023. p. 39–63. https://doi.org/10.1016/B978-0-12-823935-3.00010-2

[19] Popovska O, Simonovska J, Kavrakovski Z, Rafajlovska V. An Overview: Methods for Preparation and Characterization of Liposomes as Drug Delivery Systems. Int J Pharm, Pharm Sci. 2013;5(3):9–15.

[20] Xiu X, Ma SL, Li Z, Li Z, Li X, Ruan Y, et al. Novel 3D integrated microfluidic device: Design, construction, and application to the preparation of liposomes for vaccine delivery. J Drug Deliv Sci Technol. 2023; 81:105191. https://doi.org/10.1016/j.jddst.2023.105191

[21] Gong X, Wang X, Liu J. Controllable preparation method of liposome vesicle based on microfluidic device. *2019.* [No DOI available]

[22] Chen J, Jia Y, Zhang L. Microfluidic preparation method for regulating and controlling particle size of lipidosome. *2020.* [No DOI available]

[23] Ota A, Mochizuki A, Sou K, Takeoka S. Evaluation of a static mixer as a new microfluidic method for liposome formulation. *Front Bioeng Biotechnol*. 2023; 11:1229829. https://doi.org/10.3389/fbioe.2023.1229829

[24] Shan H, Lin Q, Wang D, Sun X, Quan B, Chen X, et al. 3D Printed Integrated Multi-Layer Microfluidic Chips for Ultra-High Volumetric Throughput Nanoliposome Preparation. *Front Bioeng Biotechnol*. 2021; 9:773705. https://doi.org/10.3389/fbioe.2021.773705

[25] Olusanya TOB, Haj Ahmad RR, Ibegbu DM, Smith JR, Elkordy AA. Liposomal Drug Delivery Systems and Anticancer Drugs. *Molecules*. 2018;23(4):907. https://doi.org/10.3390/molecules23040907

[26] James ND, Coker RJ, Tomlinson D, Harris JR, Gompels M, Pinching AJ, et al. Liposomal doxorubicin (Doxil): an effective new treatment for Kaposi's sarcoma in AIDS. *Clin Oncol (R Coll Radiol)*. 1994; 6:294–6. [No DOI available]

[27] Rommasi F, Esfandiari N. Liposomal Nanomedicine: Applications for Drug Delivery in Cancer Therapy. *Nanoscale Res Lett*. 2021;16(1):95. https://doi.org/10.1186/S11671-021-03553-8

[28] Hamad I, Harb AA, Bustanji Y. Liposome-Based Drug Delivery Systems in Cancer Research: An Analysis of Global Landscape Efforts and Achievements. *Pharmaceutics*. 2024;16(3):400. https://doi.org/10.3390/pharmaceutics16030400

[29] Rahman M, Beg S, Verma A, Kazmi I, Ahmed FJ, Kumar V, et al. Liposomal Drug Delivery Systems. *Pharm Nanotechnol*. 2020;10(2):178–85. https://doi.org/10.2174/2468187309666190618171332

[30] Rodriques P, Thacker K, G PB. Exploring lipid-based drug delivery in cancer therapy via liposomal formulations. *Asian J Pharm Clin Res*. 2022;15–22. https://doi.org/10.22159/ajpcr.2022.v15i5.43668

[31] Naghib SM, Mohammad-Jafari K. Microfluidics-mediated Liposomal Nanoparticles for Cancer Therapy: Recent Developments on Advanced Devices and Technologies. *Curr Top Med Chem*. 2024. https://doi.org/10.2174/0115680266286460240220073334

[32] Drug Delivery through Liposomes. *IntechOpen*. 2022. https://doi.org/10.5772/intechopen.97727

[33] Alhabib NA, Alehaidib SM, Almansour M, Aldosary MS, Almegbel MT, Alzahrani AM. Comprehensive overview of drug delivery systems for tumor treatment. *Int J Health Sci*. 2021. https://doi.org/10.53730/ijhs.v5ns1.15039

[34] Liposomes as Targeted Drug-Delivery Systems. *Methods Principles Med Chem*. 2022;69–125. https://doi.org/10.1002/9783527827855.ch4

[35] Wang S, Chen Y, Guo J, Huang Q. Liposomes for Tumor Targeted Therapy: A Review. *Int J Mol Sci*. 2023;24(3):2643. https://doi.org/10.3390/ijms24032643

[36] Kamalan KS, Balashanmugam D, Kalidoss A, Mohankumar N, Ilangovan SS. Preparation methods and applications of Lipid-based nanosomes for advancement in the field of Pharmaceutical Biotechnology: A review and outlook on future. *Res J Biotechnol*. 2024;20(2):202–21. https://doi.org/10.25303/202rjbt2020221

[37] Khatoon R, Huda S, Alam MA. WITHDRAWN: Smart Trends: Liposome-Based Nanocarriers for Cancer Treatment and its Applications. *Pharm Nanotechnol*. 2022;10: e100222201004. https://doi.org/10.2174/2211738510666220210115953

[38] Delcassian D, Patel AK. Nanotechnology and drug delivery. In: *Academic Press*; 2020. p.197–219. https://doi.org/10.1016/B978-0-12-813886-1.00009-7

[39] Cazzolla A, Mondala JRM, Wanigasekara J, Carroll J, Daly N, Tiwari B, et al. Synthesis of cationic liposome nanoparticles using a thin film dispersed hydration and extrusion method. *PLoS ONE*. 2024;19. https://doi.org/10.1371/journal.pone.0300467

[40] Drummond DC, Kirpotin DB, Benz CC, Park JW, Hong K. Liposomal Drug Delivery Systems for Cancer Therapy. In: *Liposome Technology*. 2004. p.191–213. https://doi.org/10.1007/978-1-59259-427-6_9

[41] Almajed Z, Al Sawaftah NM, Sulieman H, Husseini GA. Liposomal Encapsulation of Chemotherapeutics Agents Combined with the Use of Ultrasound in Cancer Treatment. *J Biomed Nanotechnol*. 2023;19(7):1128–40. https://doi.org/10.1166/jbn.2023.3617

[42] Lee MK. Clinical usefulness of liposomal formulations in cancer therapy: lessons from the experiences of doxorubicin. *J Pharm Investig*. 2019;49(2):203–14. https://doi.org/10.1007/s40005-018-0398-0

[43] Liu Y, Bravo KMC, Liu J. Targeted liposomal drug delivery: a nanoscience and biophysical perspective. *Nanoscale Horiz*. 2021;6(2):78–94. https://doi.org/10.1039/D0NH00605J

[44] Bhattacharya S, Saindane D, Prajapati BG. Liposomal drug delivery and its potential impact on cancer research. *Anti-Cancer Agents Med Chem*. 2022;22(15):2671–83. https://doi.org/10.2174/1871520622666220418141640

[45] Cheung CCL, Al-Jamal WT. Liposomes-based nanoparticles for cancer therapy and bioimaging. In: *Biomedical Applications of Nanoparticles*. Cham: Springer; 2018. p.51–87. https://doi.org/10.1007/978-3-319-89878-0_2

[46] Kumar P, Gulbake A, Jain S. Liposomes a vesicular nanocarrier: potential advancements in cancer chemotherapy. *Crit Rev Ther Drug Carrier Syst*. 2012;29(5):355–419. https://doi.org/10.1615/critrevtherdrugcarriersyst.v29.i5.10

[47] Alavi M, Hamidi M. Passive and active targeting in cancer therapy by liposomes and lipid nanoparticles. *Drug Metab Pers Ther*. 2019;34(1). https://doi.org/10.1515/dmpt-2018-0032

[48] Rohilla S, Dureja H. Recent patents, formulation and characterization of nanoliposomes. *Recent Pat Drug Deliv Formul*. 2015;9(3):213–24. https://doi.org/10.2174/1872211309666150629105900

[49] Dejeu IL, Vicaș LG, Marian E, Ganea M, Frenț OD, Maghiar PB, et al. Innovative approaches to enhancing the biomedical properties of liposomes. *Pharmaceutics*. 2024;16(12):1525. https://doi.org/10.3390/pharmaceutics16121525

[50] Ru F, Shaozong Y, Yanbin W, Qian H. Nanoscale drug delivery systems: a current review on the promising paclitaxel formulations for future cancer therapy. *Nano*. 2015;10(5):1530004. https://doi.org/10.1142/S1793292015300042

[51] Mills H, Acquah R, Tang N, Cheung L, Klenk S, Glassen R, et al. A critical scrutiny on liposomal nanoparticles drug carriers as modelled by topotecan encapsulation and release in treating cancer. *Evid Based Complement Alternat Med*. 2022; 2022:7702512. https://doi.org/10.1155/2022/7702512

[52] Mehta M, Bui TA, Yang X, Aksoy YA, Goldys EM, Deng W. Lipid-based nanoparticles for drug/gene delivery: an overview of the production techniques and difficulties encountered in their industrial development. *ACS Mater Sci Au*. 2023;3(6):600–19. https://doi.org/10.1021/acsmaterialsau.3c00032

[53] Abatchev G. Liposomes: production and applications for controlled drug delivery [dissertation]. Boise (ID): Boise State University; 2021. https://doi.org/10.18122/td/1888/boisestate

[54] Meure LA, Foster NR, Dehghani F. Conventional and dense gas techniques for the production of liposomes: a review. *AAPS PharmSciTech*. 2008;9(3):798–809. https://doi.org/10.1208/s12249-008-9097-x

[55] Has C, Sunthar P. A comprehensive review on recent preparation techniques of liposomes. *J* Liposome Res. 2020;30(4):336–65. https://doi.org/10.1080/08982104.2019.1668010

[56] Taher, Muhammad et al. "PEGylated liposomes enhance the effect of cytotoxic drug: A review." Heliyon vol. 9,3 e13823. 17 Feb. 2023, doi: 10.1016/j.heliyon. 2023.e13823

[57] Jiali Wu, Xinyu Wang, Bingchen Chen, Jiang Li, Youfa Xu, Zhizhe Lin, Chunfang Xu, Wei Fan, and Xin Wu, ACS Applied Nano Materials, DOI: 10.1021/acsanm.5c00803

[58] Izadiyan Z, Misran M, Kalantari K, Webster TJ, Kia P, Basrowi NA, Rasouli E, Shameli K. Advancements in Liposomal Nanomedicines: Innovative Formulations, Therapeutic Applications, and Future Directions in Precision Medicine. Int J Nanomedicine. 2025; 20:1213-1262 https://doi.org/10.2147/IJN.S488961

[59] Cheng, Z., Huang, H., Yin, M. et al. Applications of liposomes and lipid nanoparticles in cancer therapy: current advances and prospects. Exp Hematol Oncol 14, 11 (2025). https://doi.org/10.1186/s40164-025-00602-1

[60] Azimizonuzi, H., Ghayourvahdat, A., Ahmed, M.H. et al. A state-of-the-art review of the recent advances of theranostic liposome hybrid nanoparticles in cancer treatment and diagnosis. Cancer Cell Int 25, 26 (2025). https://doi.org/10.1186/s12935-024-03610-z

[61] Miatmoko, A., Christy, P.K., Isnaini, A. et al. Characterization and in vitro anticancer study of PEGylated liposome dually loaded with ferulic acid and doxorubicin. Sci Rep 15, 1236 (2025). https://doi.org/10.1038/s41598-024-82228-7

[62] Ernits, M., Reinsalu, O., Yandrapalli, N. et al. Microfluidic production, stability and loading of synthetic giant unilamellar vesicles. Sci Rep 14, 14071 (2024). https://doi.org/10.1038/s41598-024-64613-4

[63] Waheed Ibtesam, Ali Anwar, Tabassum Huma, Khatoon Narjis, Lai Wing-Fu, Zhou Xin,Lipid-based nanoparticles as drug delivery carriers for cancer therapy, JOURNAL=Frontiers in Oncology, Volume 14 – 2024,DOI=10.3389/fonc.2024.1296091

[64] Eman Jaradat, Adam Meziane, Dimitrios A. Lamprou, Conventional vs PEGylated loaded liposomal formulations by microfluidics for delivering hydrophilic chemotherapy, International Journal of Pharmaceutics, Volume 655, 2024, 124077, ISSN 0378-5173, https://doi.org/10.1016/j.ijpharm.2024.124077.

[65] Jiayi Chen, Siyuan Hu, Man Sun, Jianan Shi, Huan Zhang, Hongmei Yu, Zhaogang Yang, Recent advances and clinical translation of liposomal delivery systems in cancer therapy, European Journal of Pharmaceutical Sciences, Volume 193, 2024, 106688, ISSN 0928-0987, https://doi.org/10.1016/j.ejps.2023.106688.

[66] Andra, V.V.S.N.., Pammi, S.V.N., Bhatraju, L.V.K.P. et al. A Comprehensive Review on Novel Liposomal Methodologies, Commercial Formulations, Clinical Trials and Patents. BioNanoSci. 12, 274–291 (2022). https://doi.org/10.1007/s12668-022-00941-x

[67] Karthikeyan Elumalai, Sivaneswari Srinivasan, Anandakumar Shanmugam, Review of the efficacy of nanoparticle-based drug delivery systems for cancer treatment, Biomedical Technology, Volume 5, 2024, Pages 109-122, ISSN 2949-723X, https://doi.org/10.1016/j.bmt.2023.09.001.

[68] Botter, E., Caligiuri, I., Rizzolio, F., Visentin, F., & Scattolin, T. (2024). Liposomal Formulations of Metallodrugs for Cancer Therapy. International Journal of Molecular Sciences, 25(17), 9337. https://doi.org/10.3390/ijms25179337

[69] Pande, S. (2023). Liposomes for drug delivery: review of vesicular composition, factors affecting drug release and drug loading in liposomes. Artificial Cells, Nanomedicine, and Biotechnology, 51(1), 428–440. https://doi.org/10.1080/21691401.2023.2247036

[70] Chavda, V. P., Vihol, D., Mehta, B., Shah, D., Patel, M., Vora, L. K., … Paiva-Santos, A. C. (2022). Phytochemical-Loaded Liposomes for Anticancer Therapy: An Updated Review. Nanomedicine, 17(8), 547–568. https://doi.org/10.2217/nnm-2021-0463

[71] Zhang, H., Yang, J., Sun, R., Han, S., Yang, Z., & Teng, L. (2023). Microfluidics for nano-drug delivery systems: From fundamentals to industrialization. Acta pharmaceutica Sinica. B, 13(8), 3277–3299. https://doi.org/10.1016/j.apsb.2023.01.018

[72] Ridgway F, Payne NI, Timmins P, Groom CV. Method of preparing liposomes and products produced thereby 1984. Available from: https://patents.google.com/patent/US4744989A/en.
[73] Domb AJ, Maniar M. Lipospheres for controlled delivery of substances [Internet]. Dekker; 1990. Available from: https://patents.google.com/patent/US5188837A/en