Micro Nano Bio Aspects

https://www.mnba-journal.com

Online ISSN: 2980-8081

Conventional and novel methods for the preparation of micro and nanoliposomes

Mehran Alavi[1,2*], Mahendra Rai[3,4], Rajender S. Varma[5], Mehrdad Hamidi[6], M. R. Mozafari[7,8]

[1]*Department of Biological Science, Faculty of Science, Kurdistan University, Sanandaj, Kurdistan, Iran*

[2]*Nanobiotechnology Department, Faculty of Innovative Science and Technology, Razi University, Kermanshah, Iran*

[3]*Head of the Department of Biotechnology, Department of Biotechnology, UGC-Basic Science Research Faculty Fellow, SGB Amravati University, Amravati- 444 602, Maharashtra, India*

[4]*Department of Microbiology, Nicolaus Copernicus University, 87-100 Toruń, Poland*

[5]*Regional Centre of Advanced Technologies and Materials, Czech Advanced Technology and Research Institute, Palacky University in Olomouc, Olomouc, 78371, Czech Republic*

[6]*Department of Pharmaceutics, School of Pharmacy, Pharmaceutical Nanotechnology Research Center, Zanjan University of Medical Sciences*

[7]*Australasian Nanoscience and Nanotechnology Initiative (ANNI), 8054 Monash University LPO, Clayton, Victoria 3168, Australia*

[8]*Supreme Pharmatech Co. LTD, 399/90-95 Moo 13 Kingkaew Rd. Soi 25/1, T. Rachateva, A. Bangplee, Samutprakan 10540, Thailand*

ARTICLE INFO

Review paper

Article history:
Received: 07 May 2022
Revised: 25 May 2022
Accepted: 27 May 2022
Published: 29 May 2022

Keywords:
Thin film hydration, Detergent removal, Solvent injection, Reverse phase evaporation, Emulsion, Microfluidic methods, Microliposome, Nanoliposome

ABSTRACT

Liposomes mainly comprise an aqueous core surrounded by a lipid bilayer, akin to cellular membranes. The encapsulation, loading, and release properties of therapeutic agents in both, the core and lipid bilayer can be prominently affected by the preparative processes for liposome which include hydration of lipid thin films, detergent removal, solvent injection, reverse phase evaporation, emulsion, microfluidic methods (micro hydrodynamic focusing, microfluidic droplets and pulsed jet flow microfluidics), among others. Each method has its advantages and disadvantages for loading various hydrophilic and hydrophobic drugs. Therefore, selecting an appropriate method for the synthesis of micro- and nano liposome is a critical issue, particularly on the large-scale commercial enterprise. Herein, the main aim of this review is to present and compare the relative advantages of conventional and novel preparative methods of liposome in view of the emerging trend in their utilization. As the main conclusion, a combination of methods, for instance, supercritical fluids (SCFs) and microfluidic approaches can be employed along with emulsion method to synthesize micro and nanoliposomes encapsulated lipophilic drugs.

DOI: https://doi.org/10.22034/mnba.2022.150564

Introduction

Lipids are amphipathic molecules, comprising one part as hydrophilic (head) and the other portion being hydrophobic (tail) [1]. When lipids come in contact with water, the interaction of the molecule's hydrophobic components with the solution causes the lipids to spontaneously accumulate and often form liposome vesicles [2, 3]. Liposomes are composed of aqueous core surrounded by two lipid layers, very similar to cell membranes, which separate the cytoplasm from the outer media [4]. In 1961 (article was published in 1964), Alec D Bangham, a British hematologist, and coworkers explained prominent phospholipid spherical systems of liposomes for the first time [5]. In the following years, a large number of two-layer phospholipid structures were introduced, called "Banghasomes". The title was later changed to liposome (derived from the Greek word liposuction meaning fat and soma meaning body or mass) [6]. Liposomes have been used to improve the therapeutic value of new drugs by altering drug uptake, reducing toxicity, and enhancing the drug half-life. Later, drug distribution was not only controlled by its physicochemical properties but also by the characteristics of the carriers [7, 8].

*Corresponding author. E-mail: mehranbio83@gmail.com

The lipids, those synthesize liposomes may be of natural or synthetic origin [9]. Also, the components of liposomes may not only be made of lipids, for example, new liposomes can be made from polymers (sometimes called polymerosomes) [10]. When liposomes are constructed deploying natural or synthetic lipids or polymers, they are biocompatible and biodegradable and often leading to favorable outcomes for possible usage of liposomes in the field of molecular medicine [11]. The unique feature of liposomes is the solubility and degradability of both hydrophilic and hydrophobic parts by nature. This is an important attribute, along with biocompatibility and biodegradability, which has led to the widespread use of these structures as carriers [12]. Hydrophobic drugs are placed inside the two-layer liposome, while hydrophilic drugs are placed inside the aqueous core or in two-layer contact [11, 13]. Due to changes in biological distribution, liposomal formulations have increased the therapeutic effect of drugs in clinical models compared to the traditional formulation [14]. Immunogenic lipids such as didehydroxymyocobactin and β-D-glucopyranosylceramide (β-GlcCer) have amphiphatic structures that interaction with T-cell receptors [15]. Because liposomes are generally made up of non-immunogenic and biological lipids such as phosphatidylserine, phosphatidylglycerol, and, phosphatidylcholine [16], liposomal-linked drugs are transported in or on the membrane without rapid decomposition and with minimal side effects to the appropriate tissue [17]. In addition, these structures have barely any allergic, antiseptic or antigenic effects with very low toxicity. All these characteristics, besides the ease of changing the surface to tolerate targeted properties, render liposomes superior to other formulations comprising nanoparticles (NPs), microspheres, and microemulsions [18-21]. In 1979, liposomes were introduced as drug carriers, but the initial clinical results were unsatisfactory due to the biological and colloidal instability of these structures, and the ineffective encapsulation of the drug [22].

Liposomes are today recognized drug delivery nanosystems for a wide range of biologically active compounds [23]. The final values of the encapsulated drug are influenced by the choice of a suitable preparation method for liposomes of different sizes and physicochemical properties. By encapsulating anti-cancer drugs in the hydrophilic and hydrophobic parts, the toxicity of these drugs on healthy tissues can be prevented [24]. Changes in liposomes allow the active targeting of tumor sites, thereby increasing the efficacy of these drugs on malignant tumors, while non-malignant cells are relatively less affected [25]. Today, various liposomal products (in a semi-solid form or suspended aerosols such as gels, creams, and powders) are available on the market as anti-cancer, antifungal, antibacterial and cosmetic drugs. In addition, due to the enhanced efficiency of gene transfer through liposomal gene carriers, liposomes have recently been used in gene therapy [26].

The benefits and limitations of liposomal drug carriers are largely dependent on physicochemical and colloidal characteristics such as size, composition, loading efficiency, stability, as well as their biological interaction with cell membranes [27]. Overall, there are three major interactions between liposomes and cells, the most common effects being simple absorption or endocytosis. When attractive forces exceed repulsive ones, surface adsorption occurs, and this being governed by the surface electrostatic characteristics of the liposomes. In endocytosis delivery, the liposome and its contents are indirectly inserted into the cytoplasm. The destabilization of endosomal membrane of lung endothelial cells can be realized by modification of liposomes by the GALA peptide (WEAALAEALAEALAEHLAEALAEALEALAA) [28, 29].

After cell entering by clathrin-mediated pathway, the conformational alteration of a random coli of peptide to an alpha-helix under pH of endosome has resulted in the destabilization of endosomal membrane [30]. The combination of liposomes with cell membranes, that is, the delivery of liposomal content directly into the cell via the liposomal lipid’s entry into the membrane, is very rare. The last possible interaction is lipid exchange, which is the exchange of lipid bilayers, cholesterol, and the molecules with cell membrane components. Upon entering the cell, the liposomes delivered through one of these interactions activate the immune system's response, and as a result, the encapsulated material may become inactive. Therefore, basic research need to

be conducted on the development of liposomal levels to increase the ability of biocompatibility and their proper detection by target cells [31] besides additional strategies to enhance cellular uptake. Improved cell penetration in human prostate cancer cell lines and suitable *in vitro* efficiency has been instigated by mild hyperthermia therapy in a temperature range of 40–42 °C [32].

It should be taken into account that the physicochemical and biological properties of liposomes can be affected by liposome preparation methods [33]. In this regard, assorted liposomes can be prepared by different preparation methods, depending on their applications [34]. Herein, these preparative methods are briefly presented to provide an overview of the relationship between the structure and function of liposomes to encapsulate both, the hydrophilic and hydrophobic drugs as one of the advantages of the liposomal formulation. In contrast to previous reviews [1, 16, 19], we have tried to compare conventional and novel methods for the liposomal preparation in the micro and nano scales.

The main components of liposomes

There are several available bipolar lipids that serve as raw materials and additives for the production of liposomes. These substances include phospholipids; natural phospholipids such as phosphatidylcholine, also a major component of biological membranes, (extracted from soybeans, egg yolk, sunflower, and mustard sources) [35], phosphatidylserine originating from soybean, vegetarian and, bovine cortex [36], phosphatidylethanolamine (the second most abundant phospholipid in mammalian cells) [37], phosphatidylinositol (crucial roles in modulation of membrane protein functions) [38], glycosphingolipids such as gangliosides (combination a glycosphingolipid with one or more sialic acids) [39], steroids such as cholesterol and other substances, for example, stearyl amines [40]. There are many reports about conventional liposomal preparation techniques including thin-film hydration, solvent injection, detergent dialysis, and ultra-sonication [5]. Several disadvantages are associated with such preparative techniques such as heterogeneous size distribution, high temperature deployment in liposomal preparation and the phospholipid instability resulting from incomplete removal of the organic phases [41].

Liposomal preparation methods

Hydration of lipid thin films

This method includes three main steps: firstly, the lipid material is solubilized in an organic solvent, and then a thin lipid film can be formed by removing solvents deploying rotary evaporation and finally self-assembly of phospholipids can hydrate lipid film to produce liposomes [42, 43]. Moreover, the diameter and the size distribution of the liposomes necessitate the controlled usage by extrusion technique. The inherent disadvantages commonly entail, column chromatographic separation method, dialysis, ion-exchange resin, ultrafiltration, and centrifugation to remove organic solvents and purify liposomes [44]. Hydrating via evaporation (freezing-drying) of milky lipid mixture with a suitable buffer (prepared by manual or mechanical technique) is employed at elevated temperatures for the phase transfer temperature (Tm) of phospholipids [45]. In this context, the liquid sucrose solution can be added to the bottom of the flask to obtain the large unilamellar vesicles (LUV) from the multilamellar vesicles (MLV) followed by swelling and centrifugation, wherein the suspended MLV layer can be removed from the surface to procure the LUV in the solution [11]. Moreover, thin-film hydration in micro-tubes is another type of hydration procedure with advantages of mild conditions and simplicity, which can be applied without any specific tools. Generally, there are two main ways to fabricate giant liposomes involving gentle hydration and electroformation. The giant unilamellar liposomes of dioleoyl phosphatidylcholine (DOPC) in the diameter range of 10–100 μm have been prepared by the gentle hydration (various aqueous solutions including distilled water, Tris–10 mM NaCl, and Tris–100 mM NaCl) of a thin-film of fructose-containing lipid in a disposable glass microtube [46]. Liposomes encompassing egg phosphatidylcholine as well as liposomes composed of this phospholipid and cholesterol (weight ratio: 9+1) together exhibited negative zeta potential with a diameter of 118±3 and 121±4 nm, respectively [47].

Detergent removal

A micellar mixture can be prepared by mixing phospholipid and a detergent in aqueous medium followed by removing the detergent deploying techniques of column chromatography, dialysis, and the adsorption of detergent onto biobeads (such as adsorption of Triton X-100 onto hydrophobic Bio-Beads SM_2) to induce liposome formation. Time consumption and impurity of encapsulated compounds are the main disadvantages of this method [44, 48].

The solvent injection

The lipid ingredients are solubilized in a water-miscible organic solvent and then ensued solutions are injected into an aqueous media to induce liposome formation. The removal of organic solvent is the main drawback for the large-scale production via this approach [49]. Drug type, dimensions of the reaction vessel, the volume of solvent specifically ethanol, viscosity/pH/osmolality of the aqueous phase, concentration of lipid, diameter of injection hole, velocity of injection, and rate of stirring are effective factors in this process [50].

Double solvent displacement (DSD)

This method is suitable for the generation of a large amount of small diameter lipid vesicles with homogeneous property. The main steps include an ethanol phase comprising phospholipids dispersed in polyethylene glycol (PEG) in non-aqueous media, and then the mixtures are dispersed in an aqueous phase encompassing glycerin. By this procedure, a higher concentration of more than 12.5 mg/mL resulted in liposomes with a low size change (123 up to 141 nm; size difference of 18 nm) and a high homogeneity compared to ethanol injection from 129 to 318 nm (size difference of 189 nm), respectively [51].

Reverse phase evaporation

In this technique, the lipid material is dissolved in a water-immiscible solvent, and emulsions of water/oil (W/O) are formed by the addition of an aqueous solution followed by removal of the solvent via rotary evaporation to acquire a liposomal suspension. By this method, PEGylated nanoliposome comprising etoposide (hydrophobic chemotherapy medication applied to therapy lung cancer, ovarian cancer, testicular cancer, leukemia, lymphoma, and neuroblastoma) has been prepared against the lung cancer (Calu6 and A-549 cell lines) [52].

Emulsion

A solvent with water-immiscible property is deployed to solubilize the lipid material and then amount of an aqueous solution is added to prepare a W/O emulsion which can be added to a large volume of aqueous solution to attain a water/oil/water (W/O/W) emulsion. For example, the emulsion (W/O/W composed of soybean lecithin) method with single-step microfluidic was exploited to load β-carotene in giant liposomes (a diameter range of 100-180 μm with ~7 days stability) [53].

Microfluidic methods

Fluid flow in channels with cross-sectional dimensions particularly in the range of 5–500 μm are classified under the system, termed microfluidics [54]. According to the type of fluid phase, there are three main microfluidic systems comprising single-phase flow, gas-liquid multiphase flow, and liquid-liquid multiphase flow. Recently, several novel microfluidics-based techniques such as transient membrane ejection, micro hydrodynamic focusing, electroformation and hydration, ice droplet hydration, double emulsion templating, pulsed jetting, microfluidic mixing and droplet emulsion transfer have been developed to generate liposomes. There are several advantages and disadvantages related to microfluidic methods for the preparation of liposomes, which are presented in Table 1. For example, the double emulsion (W/O/W) method has been used as a microfluidizer to encapsulate curcumin in liposomes with diameter of 98.68 nm and polydispersity index (PDI) of 0.177 (Figure 1c). Using nonhalogenated and food-compatible solvents encompassing ethyl acetate, acetone, *n*-hexane, and isopropyl alcohol instead of chloroform, having side effects on the central nervous system, was a major advantage of this study [55].

Micro hydrodynamic focusing (MHF)

Small unilamellar vesicles (SUVs) and LUVs can be prepared by MHF method at a monodisperse level wherein two channels containing aqueous buffer

flow oppositely through a rectangular channel, while a phospholipid organic solution (such as isopropyl alcohol) flows between aqueous phases (Figure 1a). Self-assembly of phospholipid units ensues from the counter diffusion of organic solution and aqueous buffer along the channel [56]. The smaller size of liposomes is obtained by increasing of flow rate ratio (FRR) of organic phase. Production approach based on chip, unilamellar structure, and low encapsulation efficiency have been the typical characteristics of this system [57]. Several sections such as liposome stabilization, buffer exchange, amphiphatic weak base loading, and drug mixing may be exploited to modify the liposomal formulation [58].

Table 1. Advantages and disadvantages of microfluidic methods for preparation of liposomes [59].

Advantages	**Disadvantages**
High degree of synthesis reproducibility with control of physicochemical properties of products such as low level of PDI	Probability of channel clogging
Consumption of low precursors	Diffusion of particles through polydimethylsiloxane (PDMS)
experimental parameters can be easily controlled such as FRR, volumetric flow rate (VFR), and mixing index (MI)	Not completely automated
Passive and active mixing of reactants	Labor intensive
Combination of online optimization with control of feedback	Complexity of microfluidic design
potential of scale up with less time for liposome synthesis	Special experimental facilities are needed

Microfluidic droplets and Pulsed jet flow microfluidics (PJM)

The encapsulation of macromolecules involving DNA, RNA, and proteins, in giant liposomes with a diameter 10 µm can be accomplished via microfluidic droplets method. As illustrated in Figure 2, W/O/W emulsion was prepared in two steps: formation of W/O droplet followed by encapsulation of W/O in water solution by flowing two immiscible phases in a micro-channel [60, 61]. In PJM method, liposomal vesicles are formed from a planar lipid membrane by a pulsed flow of liquid executed from a fine jet nozzle [62].

The encapsulation of therapeutic agents, cells, chromosomes and cellular organelles in a wide range of sizes and shapes can be directly performed by PJM as a major advantage. Interestingly, the encapsulation of materials by this method is not affected by their chemical, properties concentration and size [63].

Additionally, the appropriate particle size control has been realized for PJM. However, this method is not desirable for large-scale generation as it involves purifying final products from organic solvent [64]. However, the pulsed jet flow method under higher pressure and longer duration conditions can be employed to prepare lipid unilamellar vesicles with asymmetric morphology, diameter of 50–300 nm and thicknesses of 5–6 nm from an asymmetric planar lipid bilayer [65].

Microfluidic mixing

A rapid and complete mixing of multiple precursors in microscale devices can be accomplished via passive (the augmented contact time and contact area of the samples) and active (using an external force to perturb the samples) microfluidic mixing in various chip designs of coaxial tubes, Y-channel, herringbone structure, and Y-channel by increasing the diffusion effect between the different samples [66].

Lamination, zigzag channels, 3-D serpentine structure, embedded barriers, twisted channels, and surface-chemistry (T-/Y- mixer) may be applied as passive mixers, wherein there are numerous options namely acoustic/ultrasonic, dielectrophoretic, electrokinetic time-pulsed, electrohydrodynamic force, thermal actuation, magneto-hydrodynamic flow, and electrokinetic instability for active mixing [67]. As an example, in a Y-channel mixer, acridine orange hydrochloride and doxorubicin were loaded in liposomes formed in-line with preparation in a single integrated chip, which showed encapsulation

efficiency values of 69.8% and 71.8%, respectively [58].

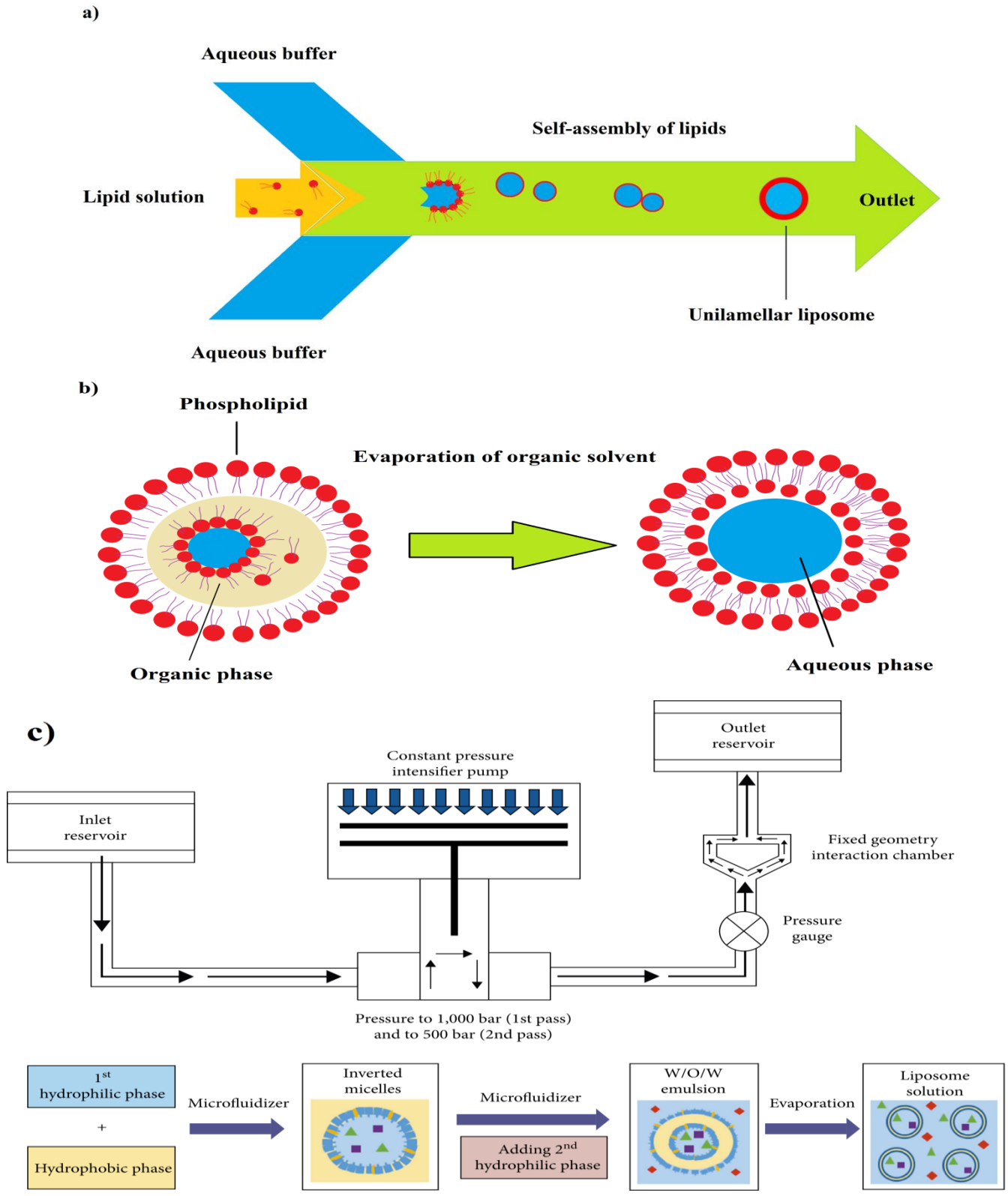

Fig. 1. Schematic images presenting a) MHF method of SUVs, LUVs generation, b) formation of W/O/W emulsion based on microfluidic droplets, and c) the double emulsion (water/oil/water) method was used as microfluidizer to encapsulate curcumin by liposomes [55].

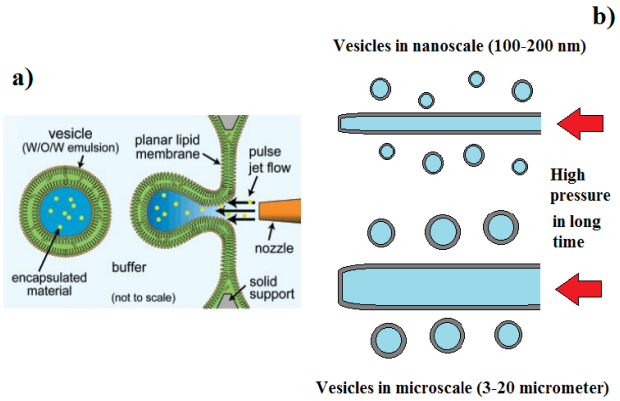

Fig. 2. a) Diagram presenting formation of vesicle (W/O/W) from a planar lipid membrane via a pulsed jet flow method. Organic solvent is illustrated in green color (reprinted with permission from [63]). b) Preparation of lipid vesicles in micro and nano-size under high pressure and time (reprinted with permission from [65]).

Supercritical fluids (SCFs) method

Liposome preparation via this method does not need large amounts of organic solvents which makes processes unsuitable for large scale production in industrial context. In addition, gas-like viscosity, liquid-like density, enhanced solvent power, high miscibility in organic solvents, and high diffusively are the main advantages of supercritical conditions of CO_2 [42]. Supercritical fluids can function as a cosolvent and a solvent (without an organic cosolvent) for the lipid molecules, an antisolvent to obtain liposomes after a hydration, and a dispersing agent of the lipids into pure water [68, 69]; combination of SCFs method with other processes has been used to prepare micro and nanoliposomes. For instance, supercritical assisted emulsion method was used to encapsulate lipophilic antioxidants encompassing linalool, limonene, and farnesol in nanoliposomes with a diameter range of 116 -230 nm and by encapsulation efficiency up to 54%, 87%, and 74%, respectively [70].

Electroformation

As a natural swelling, lipids such as phospholipids need to be dissolved in chloroform followed by deposition on a solid substrate. After evaporation of the solvent, the clustering of several stacks of lipid bilayers and finally, vesicles ensue slowly by adding a buffer solution to lipids. To increase the swelling process during liposome formation, an alternating current (AC) field is applied in electroformation method. In this method, the lipids components are deposited on two electrodes (indium tin oxide (ITO) coated glasses and platinum (Pt) wires), and liposomes can be produced as consequences of electro-osmotic effects, tension changes of membrane surface and line, redistribution of bilayer counter ions, and electrostatic interaction [71]. As illustrated in Figure 3, electroformation may be carried out for the production of giant unilamellar vesicles (with a diameter of >1 µm, visible by basic light microscope) on the electrodes of ITO-coated glasses and Pt wires [72].

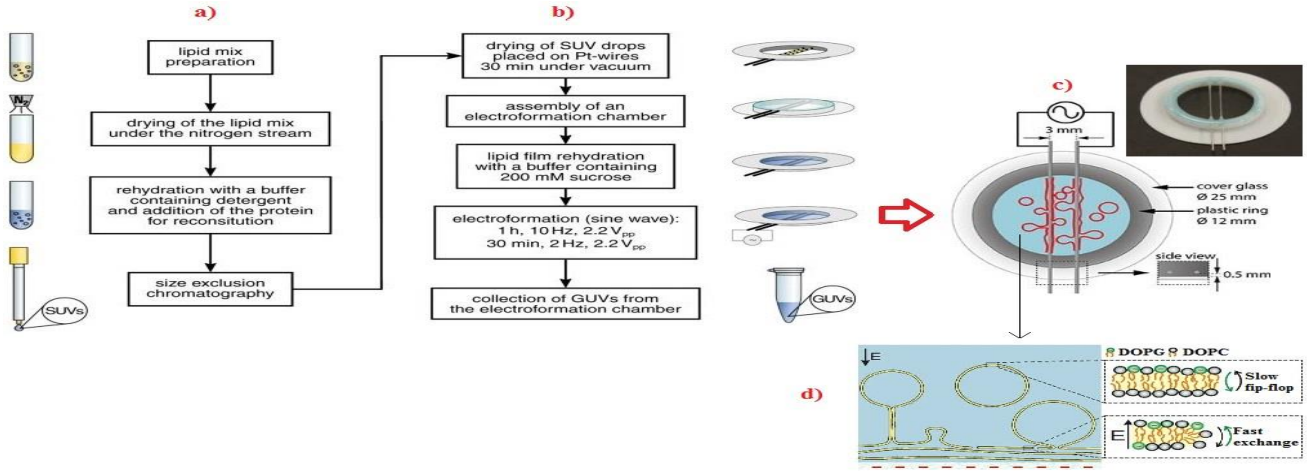

Fig. 3. Preparation processes of protein-containing SUVs (a) and GUVs (b) [72]. Top view of a Pt chamber (c) [71] and probable mechanisms for the formation of bilayer asymmetry by vesicle electroformation (1,2-dioleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DOPG)) (d) [73].

Conclusions

This review explained some conventional and novel preparative methods for micro and nanoliposomes involving hydration of lipid thin films, detergent removal, solvent injection, reverse phase evaporation, emulsion, and microfluidic methods (micro hydrodynamic focusing and microfluidic droplets and pulsed jet flow microfluidics). The advantages and limitations of each method have been delineated which should be considered in future investigations to obtain suitable attributes of encapsulation, loading, and release properties for micro and nanoformulations of therapeutic agents. In this manner, a combination of methods, for example, SCFs and microfluidic approaches can be deployed along with emulsion methodology to prepare micro and nanoliposomes encapsulated lipophilic agents.

Conflict of interest

Authors declare no conflict of interest.

Study Highlights

- The efficiency of the liposomal preparation method is dependent on their therapeutic applications.
- A combination of methods can be applied to prepare micro and nanoliposomes encapsulated hydrophilic or lipophilic drugs.
- Future studies should be focus on achieving appricpiate attributes of encapsulation, loading, and release properties for micro and nanoformulations of therapeutic agents.

Abbreviations

AC: Alternating current
DOPC: Dioleoyl phosphatidylcholine
DOPG:1,2-dioleoyl-sn-glycero-3-phospho-(1′-rac-glycerol)
DSD: Double solvent displacement
FRR: Flow rate ratio
LUV: Large unilamellar vesicles
MHF: Micro hydrodynamic focusing
MI: Mixing index
MLV: Multilamellar vesicles
NPs: Nanoparticles
PDI: Polydispersity index
PDMS: Polydimethylsiloxane
PEG: Polyethylene glycol
PJM: Pulsed jet flow microfluidics
SCFs: Supercritical fluids
SUVs: Small unilamellar vesicles
Tm: Transfer temperature
VFR: Volumetric flow rate
W/O/W: Water/oil/water
W/O: Water/oil
β-GlcCer: β-D-glucopyranosylceramide

Funding

This work was not supported by any institutes.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with animals or human participants.

Authors' contribution

MA: conceptualization, preparing the first draft, and revising; MR, RSV, MH, and MRM: revising of the manuscript.

Acknowledgment

None.

References

1. Alavi M, Webster TJ. Nano liposomal and cubosomal formulations with platinum-based anticancer agents: therapeutic advances and challenges. Nanomedicine. 2020;15(24):2399-410. doi:https://doi.org/10.2217/nnm-2020-0199
2. Akimov SA, Volynsky PE, Galimzyanov TR, Kuzmin PI, Pavlov KV, Batishchev OV. Pore formation in lipid membrane I: Continuous reversible trajectory from intact bilayer through hydrophobic defect to transversal pore. Scientific Reports. 2017;7(1):1-20. doi:https://doi.org/10.1038/s41598-017-12127-7
3. Nakhaei P, Margiana R, Bokov DO, Abdelbasset WK, Jadidi Kouhbanani MA, Varma RS, et al. Liposomes: Structure, Biomedical Applications, and Stability Parameters With Emphasis on Cholesterol. Frontiers in Bioengineering and Biotechnology. 2021;9. doi:https://doi.org/10.3389/fbioe.2021.705886
4. Zhang X, Zong W, Bi H, Zhao K, Fuhs T, Hu Y, et

al. Hierarchical drug release of pH-sensitive liposomes encapsulating aqueous two phase system. European Journal of Pharmaceutics and Biopharmaceutics. 2018;127:177-82. doi:https://doi.org/10.1016/j.ejpb.2018.02.021
5. Alavi M, Karimi N, Safaei M. Application of Various Types of Liposomes in Drug Delivery Systems. Advanced pharmaceutical bulletin. 2017;7(1):3-9. doi:https://doi.org/10.15171/apb.2017.002
6. Watts G. Alec Douglas Bangham. The lancet. 2010;375(9731):2070. doi:https://doi.org/10.1016/S0140-6736(10)60950-6
7. Huang G, Huang H. Hyaluronic acid-based biopharmaceutical delivery and tumor-targeted drug delivery system. Journal of Controlled Release. 2018;278:122-6. doi:https://doi.org/10.1016/j.jconrel.2018.04.015
8. Alavi M, Varma RS. Overview of novel strategies for the delivery of anthracyclines to cancer cells by liposomal and polymeric nanoformulations. International Journal of Biological Macromolecules. 2020;164:2197-203. doi:https://doi.org/10.1016/j.ijbiomac.2020.07.274
9. Alavi M, Karimi N, Safaei M. Application of Various Types of Liposomes in Drug Delivery Systems. Adv Pharm Bull. 2017;7(1):3-9. doi:https://doi.org/10.15171/apb.2017.002
10. Kauscher U, Holme MN, Björnmalm M, Stevens MM. Physical stimuli-responsive vesicles in drug delivery: Beyond liposomes and polymersomes. Advanced Drug Delivery Reviews. 2019;138:259-75. doi:https://doi.org/10.1016/j.addr.2018.10.012
11. Çağdaş M, Sezer AD, Bucak S. Liposomes as potential drug carrier systems for drug delivery. Application of nanotechnology in drug delivery. 2014:1-100. doi:https://doi.org/10.5772/58459
12. Alavi M, Asare-Addo K, Nokhodchi A. Lectin Protein as a Promising Component to Functionalize Micelles, Liposomes and Lipid NPs against Coronavirus. Biomedicines. 2020;8(12):580. doi:https://doi.org/10.3390/biomedicines8120580
13. Lombardo D, Kiselev MA, Caccamo MT. Smart Nanoparticles for Drug Delivery Application: Development of Versatile Nanocarrier Platforms in Biotechnology and Nanomedicine. Journal of Nanomaterials. 2019;2019:3702518. doi:https://doi.org/10.1155/2019/3702518
14. Alavi M, Hamidi M. Passive and active targeting in cancer therapy by liposomes and lipid nanoparticles. Drug Metabolism and Personalized Therapy. 2019;34(1). doi:https://doi.org/10.1515/dmpt-2018-0032
15. Brennan PJ, Tatituri RVV, Brigl M, Kim EY, Tuli A, Sanderson JP, et al. Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals. Nature Immunology. 2011;12(12):1202-11. doi:https://doi.org/10.1038/ni.2143
16. Liu W, Hou Y, Jin Y, Wang Y, Xu X, Han J. Research progress on liposomes: Application in food, digestion behavior and absorption mechanism. Trends in Food Science & Technology. 2020;104:177-89. doi:https://doi.org/10.1016/j.tifs.2020.08.012
17. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017;9(2):12. doi:https://doi.org/10.3390/pharmaceutics9020012
18. Broecker F, Götze S, Hudon J, Rathwell DCK, Pereira CL, Stallforth P, et al. Synthesis, liposomal formulation, and immunological evaluation of a minimalistic carbohydrate-α-GalCer vaccine candidate. Journal of medicinal chemistry. 2018;61(11):4918-27. doi:https://doi.org/10.1021/acs.jmedchem.8b00312
19. Alavi M, Webster TJ. Recent progress and challenges for polymeric microsphere compared to nanosphere drug release systems: Is there a real difference? Bioorganic & Medicinal Chemistry. 2021;33:116028. doi:https://doi.org/10.1016/j.bmc.2021.116028
20. Alavi M. Bacteria and fungi as major bio-sources to fabricate silver nanoparticles with antibacterial activities. Expert Review of Anti-infective Therapy. 2022:1-10. doi:https://doi.org/10.1080/14787210.2022.2045194
21. Alavi M, Adulrahman NA, Haleem AA, Al-Râwanduzi ADH, Khusro A, Abdelgawad MA, et al. Nanoformulations of curcumin and quercetin with silver nanoparticles for inactivation of bacteria. Cellular and Molecular Biology. 2022;67(5):151-6. doi:https://doi.org/10.14715/cmb/2021.67.5.21
22. Wagner A, Vorauer-Uhl K. Liposome Technology for Industrial Purposes. Journal of Drug Delivery. 2011;2011:591325. doi:https://doi.org/10.1155/2011/591325
23. Kontogiannopoulos KN, Dasargyri A, Ottaviani MF, Cangiotti M, Fessas D, Papageorgiou VP, et al. Advanced drug delivery nanosystems for shikonin: a calorimetric and Electron Paramagnetic resonance study. Langmuir. 2018;34(32):9424-34. doi:https://doi.org/10.1021/acs.langmuir.8b00751
24. Alavi M, Nokhodchi A. Micro- and nanoformulations of paclitaxel based on micelles, liposomes, cubosomes, and lipid nanoparticles: Recent advances and challenges. Drug Discovery Today. 2022;27(2):576-84. doi:https://doi.org/10.1016/j.drudis.2021.10.007
25. Lee Y, Thompson DH. Stimuli-responsive liposomes for drug delivery. Wiley Interdisciplinary

Reviews: Nanomedicine and Nanobiotechnology. 2017;9(5):e1450. doi:https://doi.org/10.1002/wnan.1450
26. Souto EB, Fernandes AR, Martins-Gomes C, Coutinho TE, Durazzo A, Lucarini M, et al. Nanomaterials for Skin Delivery of Cosmeceuticals and Pharmaceuticals. Applied Sciences. 2020;10(5):1594. doi:https://doi.org/10.3390/app10051594
27. Zoghi A, Khosravi-Darani K, Omri A. Process variables and design of experiments in liposome and nanoliposome research. Mini reviews in medicinal chemistry. 2018;18(4):324-44. doi:https://doi.org/10.2174/1389557516666161031120752
28. Yokoo H, Oba M, Uchida S. Cell-Penetrating Peptides: Emerging Tools for mRNA Delivery. Pharmaceutics. 2022;14(1):78. doi:https://doi.org/10.3390/pharmaceutics14010078
29. Tanaka H, Sakurai Y, Anindita J, Akita H. Development of lipid-like materials for RNA delivery based on intracellular environment-responsive membrane destabilization and spontaneous collapse. Advanced Drug Delivery Reviews. 2020;154-155:210-26. doi:https://doi.org/10.1016/j.addr.2020.07.001
30. Santiwarangkool S, Akita H, Khalil IA, Abd Elwakil MM, Sato Y, Kusumoto K, et al. A study of the endocytosis mechanism and transendothelial activity of lung-targeted GALA-modified liposomes. Journal of Controlled Release. 2019;307:55-63. doi:https://doi.org/10.1016/j.jconrel.2019.06.009
31. Corbo C, Molinaro R, Taraballi F, Furman NET, Sherman MB, Parodi A, et al. Effects of the protein corona on liposome–liposome and liposome–cell interactions. International journal of nanomedicine. 2016;11:3049. doi:https://doi.org/10.2147/IJN.S109059
32. Eleftheriou K, Kaminari A, Panagiotaki KN, Sideratou Z, Zachariadis M, Anastassopoulou J, et al. A combination drug delivery system employing thermosensitive liposomes for enhanced cell penetration and improved in vitro efficacy. International Journal of Pharmaceutics. 2020;574:118912. doi:https://doi.org/10.1016/j.ijpharm.2019.118912
33. Yang K, Mesquita B, Horvatovich P, Salvati A. Tuning liposome composition to modulate corona formation in human serum and cellular uptake. Acta Biomaterialia. 2020;106:314-27. doi:https://doi.org/10.1016/j.actbio.2020.02.018
34. Lujan H, Griffin WC, Taube JH, Sayes CM. Synthesis and characterization of nanometer-sized liposomes for encapsulation and microRNA transfer to breast cancer cells. International journal of nanomedicine. 2019;14:5159. doi:https://doi.org/10.2147/IJN.S203330
35. Alemán A, Marín D, Taladrid D, Montero P, Gómez-Guillén MC. Encapsulation of antioxidant sea fennel (Crithmum maritimum) aqueous and ethanolic extracts in freeze-dried soy phosphatidylcholine liposomes. Food Research International. 2019;119:665-74. doi:https://doi.org/10.1016/j.foodres.2018.10.044
36. Reljic R, Hart P, Copland A, Kim M, Tran AC, Poerio N, et al. Immunization with Mycobacterium tuberculosis antigens encapsulated in phosphatidylserine liposomes improves protection afforded by BCG. Frontiers in Immunology. 2019;10:1349. doi:https://doi.org/10.3389/fimmu.2019.01349
37. Jovanović AA, Balanč BD, Djordjević VB, Ota A, Skrt M, Šavikin KP, et al. Effect of gentisic acid on the structural-functional properties of liposomes incorporating β-sitosterol. Colloids and Surfaces B: Biointerfaces. 2019;183:110422. doi:https://doi.org/10.1016/j.colsurfb.2019.110422
38. Inoh Y, Tsuchiya Y, Nakanishi Y, Yokawa S, Furuno T. Involvement of intracellular caveolin-1 distribution in the suppression of antigen-induced mast cell activation by cationic liposomes. Cell Biology International. 2019. doi: https://doi.org/10.1002/cbin.11297
39. Jordan LR, Blauch ME, Baxter AM, Cawley JL, Wittenberg NJ. Influence of brain gangliosides on the formation and properties of supported lipid bilayers. Colloids and Surfaces B: Biointerfaces. 2019;183:110442. doi:https://doi.org/10.1016/j.colsurfb.2019.110442
40. Venkatraman S, Natarajan JV, Wong T, Boey YCF. Liposomal formulation for ocular drug delivery. Google Patents; 2019. doi:US10272040B2
41. Zacheo A, Quarta A, Zizzari A, Monteduro AG, Maruccio G, Arima V, et al. One step preparation of quantum dot-embedded lipid nanovesicles by a microfluidic device. RSC advances. 2015;5(119):98576-82. doi:https://doi.org/10.1039/C5RA18862H
42. William B, Noémie P, Brigitte E, Géraldine P. Supercritical fluid methods: An alternative to conventional methods to prepare liposomes. Chemical Engineering Journal. 2020;383:123106. doi:https://doi.org/10.1016/j.cej.2019.123106
43. Thabet Y, Elsabahy M, Eissa NG. Methods for preparation of niosomes: A focus on thin-film hydration method. Methods. 2022;199:9-15. doi:https://doi.org/10.1016/j.ymeth.2021.05.004
44. Lin M, Qi X-R. Purification Method of Drug-Loaded Liposome. In: Lu W-L, Qi X-R, editors. Liposome-Based Drug Delivery Systems. Berlin,

Heidelberg: Springer Berlin Heidelberg; 2021. p. 111-21. doi:https://doi.org/10.1007/978-3-662-49320-5_24
45. Bhattacharjee A, Das PJ, Dey S, Nayak AK, Roy PK, Chakrabarti S, et al. Development and optimization of besifloxacin hydrochloride loaded liposomal gel prepared by thin film hydration method using 32 full factorial design. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2020;585:124071. doi:https://doi.org/10.1016/j.colsurfa.2019.124071
46. Tsumoto K, Matsuo H, Tomita M, Yoshimura T. Efficient formation of giant liposomes through the gentle hydration of phosphatidylcholine films doped with sugar. Colloids and Surfaces B: Biointerfaces. 2009;68(1):98-105. doi:https://doi.org/10.1016/j.colsurfb.2008.09.023
47. Płaczek M, Wątróbska-Świetlikowska D, Stefanowicz-Hajduk J, Drechsler M, Ochocka JR, Sznitowska M. Comparison of the in vitro cytotoxicity among phospholipid-based parenteral drug delivery systems: Emulsions, liposomes and aqueous lecithin dispersions (WLDs). European Journal of Pharmaceutical Sciences. 2019;127:92-101. doi:https://doi.org/10.1016/j.ejps.2018.10.018
48. Palanirajan SK, Govindasamy P, Gummadi SN. Polystyrene adsorbents: rapid and efficient surrogate for dialysis in membrane protein purification. Scientific Reports. 2020;10(1):16334. doi:https://doi.org/10.1038/s41598-020-73522-1
49. Shah S, Dhawan V, Holm R, Nagarsenker MS, Perrie Y. Liposomes: Advancements and innovation in the manufacturing process. Advanced Drug Delivery Reviews. 2020;154-155:102-22. doi:https://doi.org/10.1016/j.addr.2020.07.002
50. Gouda A, Sakr OS, Nasr M, Sammour O. Ethanol injection technique for liposomes formulation: An insight into development, influencing factors, challenges and applications. Journal of Drug Delivery Science and Technology. 2021;61:102174. doi:https://doi.org/10.1016/j.jddst.2020.102174
51. Sala M, Miladi K, Agusti G, Elaissari A, Fessi H. Preparation of liposomes: A comparative study between the double solvent displacement and the conventional ethanol injection—From laboratory scale to large scale. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2017;524:71-8. doi:https://doi.org/10.1016/j.colsurfa.2017.02.084
52. Zare Kazemabadi F, Heydarinasab A, Akbarzadeh A, Ardjmand M. Preparation, characterization and in vitro evaluation of PEGylated nanoliposomal containing etoposide on lung cancer. Artificial Cells, Nanomedicine, and Biotechnology. 2019;47(1):3222-30. doi:https://doi.org/10.1080/21691401.2019.1646265
53. Michelon M, Huang Y, de la Torre LG, Weitz DA, Cunha RL. Single-step microfluidic production of W/O/W double emulsions as templates for β-carotene-loaded giant liposomes formation. Chemical Engineering Journal. 2019;366:27-32. doi:https://doi.org/10.1016/j.cej.2019.02.021
54. Antognoli M, Stoecklein D, Galletti C, Brunazzi E, Di Carlo D. Optimized design of obstacle sequences for microfluidic mixing in an inertial regime. Lab on a Chip. 2021;21(20):3910-23. doi:https://doi.org/10.1039/D1LC00483B
55. Yang E, Yu H, Park J-Y, Park K-M, Chang P-S. Microfluidic Preparation of Liposomes Using Ethyl Acetate/n-Hexane Solvents as an Alternative to Chloroform. Journal of Chemistry. 2018;2018:7575201. doi:https://doi.org/10.1155/2018/7575201
56. Patil YP, Jadhav S. Novel methods for liposome preparation. Chemistry and Physics of Lipids. 2014;177:8-18. doi:https://doi.org/10.1016/j.chemphyslip.2013.10.011
57. Deshpande S, Dekker C. On-chip microfluidic production of cell-sized liposomes. Nature Protocols. 2018;13(5):856-74. doi:https://doi.org/10.1038/nprot.2017.160
58. Hood RR, Vreeland WN, DeVoe DL. Microfluidic remote loading for rapid single-step liposomal drug preparation. Lab on a Chip. 2014;14(17):3359-67. doi:10.1039/C4LC00390J
59. Jahn A, Vreeland WN, DeVoe DL, Locascio LE, Gaitan M. Microfluidic Directed Formation of Liposomes of Controlled Size. Langmuir. 2007;23(11):6289-93. doi:https://doi.org/10.1021/la070051a
60. Yu B, Lee RJ, Lee LJ. Microfluidic methods for production of liposomes. Methods in enzymology. 2009;465:129-41. doi:https://doi.org/10.1016/S0076-6879(09)65007-2
61. Romanov V, McCullough J, Gale BK, Frost A. A Tunable Microfluidic Device Enables Cargo Encapsulation by Cell-or Organelle-Sized Lipid Vesicles Comprising Asymmetric Lipid Bilayers. Advanced biosystems. 2019;3(7):1900010. doi: https://doi.org/10.1002/adbi.201900010
62. Supramaniam P, Ces O, Salehi-Reyhani A. Microfluidics for Artificial Life: Techniques for Bottom-Up Synthetic Biology. Micromachines. 2019;10(5). doi:https://doi.org/10.3390/mi10050299
63. Funakoshi K, Suzuki H, Takeuchi S. Formation of giant lipid vesiclelike compartments from a planar lipid membrane by a pulsed jet flow. J Am Chem Soc. 2007;129(42):12608-9. doi:https://doi.org/10.1021/ja074029f
64. Maja L, Željko K, Mateja P. Sustainable technologies for liposome preparation. The Journal of

Supercritical Fluids. 2020;165:104984. doi:https://doi.org/10.1016/j.supflu.2020.104984
65. Kamiya K, Osaki T, Takeuchi S. Formation of nano-sized lipid vesicles with asymmetric lipid components using a pulsed-jet flow method. Sensors and Actuators B: Chemical. 2021;327:128917. doi:https://doi.org/10.1016/j.snb.2020.128917
66. Oevreeide IH, Zoellner A, Mielnik MM, Stokke BT. Curved passive mixing structures: a robust design to obtain efficient mixing and mass transfer in microfluidic channels. Journal of Micromechanics and Microengineering. 2020;31(1):015006. doi:https://doi.org/10.1088/1361-6439/abc820
67. Lee C-Y, Chang C-L, Wang Y-N, Fu L-M. Microfluidic mixing: a review. International Journal of Molecular Sciences. 2011;12(5):3263-87. doi:https://doi.org/10.3390/ijms12053263
68. Penoy N, Grignard B, Evrard B, Piel G. A supercritical fluid technology for liposome production and comparison with the film hydration method. International Journal of Pharmaceutics. 2021;592:120093. doi:https://doi.org/10.1016/j.ijpharm.2020.120093
69. Trucillo P, Campardelli R, Reverchon E. Liposomes: From Bangham to Supercritical Fluids. Processes. 2020;8(9). doi:https://doi.org/10.3390/pr8091022
70. Trucillo P, Campardelli R, Reverchon E. Antioxidant loaded emulsions entrapped in liposomes produced using a supercritical assisted technique. The Journal of Supercritical Fluids. 2019;154:104626. doi:https://doi.org/10.1016/j.supflu.2019.104626
71. Stein H, Spindler S, Bonakdar N, Wang C, Sandoghdar V. Production of Isolated Giant Unilamellar Vesicles under High Salt Concentrations. Frontiers in Physiology. 2017;8. doi:https://doi.org/10.3389/fphys.2017.00063
72. Witkowska A, Jablonski L, Jahn R. A convenient protocol for generating giant unilamellar vesicles containing SNARE proteins using electroformation. Scientific Reports. 2018;8(1):9422. doi:https://doi.org/10.1038/s41598-018-27456-4
73. Steinkühler J, De Tillieux P, Knorr RL, Lipowsky R, Dimova R. Charged giant unilamellar vesicles prepared by electroformation exhibit nanotubes and transbilayer lipid asymmetry. Scientific Reports. 2018;8(1):11838. doi:https://doi.org/10.1038/s41598-018-30286-z

HOW TO CITE THIS ARTICLE:
Alavi M, Rai M, Varma RS, Hamidi M, Mozafari MR. Conventional and novel methods for the preparation of micro and nanoliposomes. Micro Nano Bio Aspects. 2022; 1(1):18-29.
doi: https://doi.org/10.22034/mnba.2022.150564