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REVIEW ON ADVANCEMENTS IN LIPOSOMAL DOSAGE FORMS WITH NEWER METHODOLOGIES AND CLINICAL PERSPECTIVES

Debatri Roy[1, 2], Beduin Mahanti[1]*, Sudipta Das[2], Arnab Samanta[2], Setu Majumder[2]

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ABSTRACT
Background: Liposomes are widely used as drug delivery systems because of their reduced systemic toxicity. Over the past few decades, numerous drug-loaded liposomes have been approved for clinical use in the treatment of cancer, viral, and fungal infections. Various liposomal formulations have progressed to later phases of clinical trials. Liposomes are spherical vesicles composed of a single or multiple phospholipid bilayers surrounding an aqueous core. Drug-loaded liposomes can exhibit controlled or targeted drug delivery, low immunogenicity, high biocompatibility, biodegradability, prolonged drug half-life, increased efficiency, reduced systemic toxicity, and enhanced pharmacokinetic properties. **Methodology:** This review article addresses the characteristics and types of liposomes; novel methods for their preparation, such as the Supercritical Anti-solvent Method and the Dual Asymmetric Centrifugation Method; lipid preferences; future directions for liposomes; marketed liposomal formulations; and associated patents. **Results and Discussion:** It has the potential to protect the drug against degradation. The aforementioned drug delivery system increases in vivo drug distribution toward target sites. PEGylated liposomes can prolong circulation time. It requires expertise in techniques, such as thin-film hydration and reverse-phase evaporation, for preparation. It has been utilized in nanomedicine. This particular delivery system requires characterizations like size, drug loading, drug release, etc. **Conclusion:** Liposome-embedded delivery systems advance nanotechnology and biopharmaceutics. The role of modern medicine has continued to expand, particularly in the management of chronic diseases.

INTRODUCTION

Conventional therapeutic agents show bioavailability constraints, toxicity, and pharmacokinetic issues. Liposomes are compatible with drugs that have narrow therapeutic windows. Liposomes can enhance drug distribution, prolong circulation time, target drug molecules to desired tissues, increase intracellular drug concentration, increase drug solubility, and improve stability in the circulation [1,2]. Liposomes enhance specificity, sensitivity, and durability in the body, offering substantial advantages; thus, they are used in nanomedicines [3].

[1]*Department School of Pharmacy, Techno India University, EM-4, EM Block, Sector-V, Kolkata, WB 700091, India*
[2]*Department of Pharmaceutics, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, Roypara, Tatla, West Bengal 741222, India*

***For Correspondence:** **director.pharmacy@technoindiaeducation.com**

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Liposomes are comprised of lamellae enclosing an aqueous core. Lipid layers often include phospholipids, which have a polar head group and a nonpolar tail. Polar head moieties are positioned around the aqueous core [4]. Given these structural characteristics, liposomes can encapsulate and disperse drug molecules with varying solubilities. Polar substances will be encapsulated or captured within the aqueous core. At the same time, the lipid bilayer will surround non-polar substances, and amphiphilic molecules should be infused in the water-lipid bilayer interface [5]. Small Unilamellar Vesicles consist of a single bilayer. Large unilamellar vesicles consist of a single, larger bilayer. Multi-vesicular vesicles incorporate multiple lipid bilayers, while multi-vesicular vesicles bear multiple small vesicles within a large vesicle (detailed structure is available in Figure 1).

Lipids exhibit a phase transition temperature and exist in different physical states across the phase transition. Below the transition temperature, lipids are rigid, whereas above it they remain in a liquid-crystalline state [6]. Liposomes with a size constraint of 1-100 nm are optimal for drug delivery. A large surface area enhances cellular interactions with liposomes [7,8].

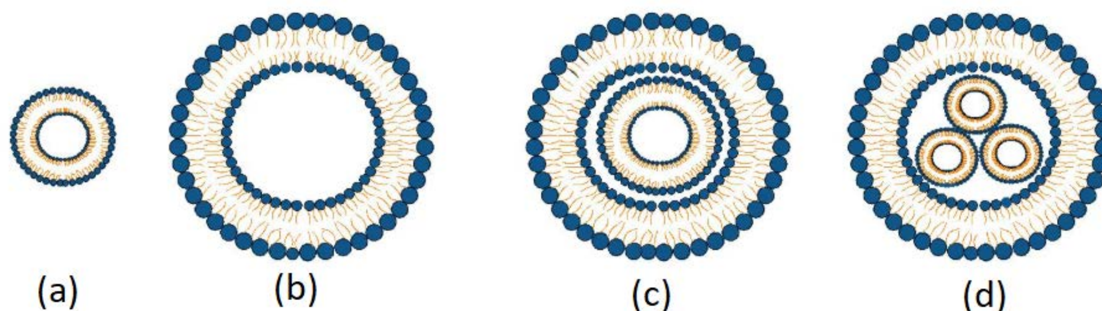

Figure 1: Liposomes with different structures a) SUV, b) LUV, c) MLV, d) MVV [9]

Liposome-encapsulated drug molecules can enhance clinical efficacy by targeting specific cells, improving pharmacokinetic properties, and crossing target membranes to achieve high concentrations at the cell surface [10,11,12]. Encapsulation of the drug reduces renal clearance and prolongs its residence time in the bloodstream [13]. Liposomes are recognized for their thermosensitive properties, such as an elevation in temperature of approximately 40-41 °C, which can alter bilayer characteristics and promote drug release. This attribute enables the release of cytotoxic agents in large amounts after external heat treatment of tumour sites, without damaging normal tissues [14]. Phospholipids' amphiphilic nature in solution can facilitate interactions between mammalian cell membranes and liposomes by mimicking natural cell membranes, thereby stimulating cellular uptake [15].

Liposomes are primarily investigated as carriers for nucleic acids, proteins, small-molecule drugs, and imaging agents [16,17,18]. Liposomes can be administered via various routes, including oral, transdermal, ophthalmic, nasal, parenteral, and pulmonary, with remarkable effectiveness and patient compliance [19,20,21]. This dosage form is preferably used in the food [22] & cosmetics [23] industries. Drug loading in liposomes minimizes exposure to healthy tissue, thus reducing the adverse effects as compared to the free drug form [24]. Formulation processes such as sonication, membrane extrusion, freeze-thawing, and homogenization are used to control liposome size. Liposomes can be processed in various sizes, charges, lamellarity, and compositions.

Liposomes are used in modern biotechnology to minimize obstacles encountered during *in vivo* delivery. Improved siRNA transfection efficiency can be achieved with liposomes, which are the main component of non-viral siRNA delivery vectors [25]. Chemotherapeutic drugs are unable to distinguish tumour cells from healthy cells; thus, their biodistribution is unfavourable for the desired site of action. Therefore, liposomes are the most practical approach for chemotherapeutic drugs [26]. The lymphatic drainage system facilitates liposome retention at the site of tumour and releases encapsulated drugs [27].

Liposomal drug delivery systems are prone to stability issues, including flocculation, self-aggregation, fusion, and sedimentation of liposomal vesicles, which may affect their in vivo and in vitro properties and, consequently, their therapeutic efficacy. Membrane fluidity analysis of liposomes, by evaluating physicochemical properties, can provide insights into stability during manufacturing, storage, and in vivo administration [28]. The transition of liposome production from laboratory scale to industrial-scale manufacturing poses

challenges, including maintaining uniform vesicle size, encapsulation efficiency & stability. High-pressure homogenization, extrusion, and 3D-printed microfluidic flow are preferred techniques for scale-up [29].

According to 2021 reports, the market for liposomal formulations is projected to reach approximately $7 billion by 2027, nearly double the 2019 value of $3.595 billion. It exhibits an annual growth rate of roughly 8.8% (2020–2027). The extension of liposomal applications from pharmaceutical products to cosmetics, agriculture, and food should drive international market growth by up to 13.2% from 2021 to 2028 [30].

STRUCTURE OF LIPOSOMES

Liposomes are categorized depending upon the number of lipid bilayers or lamellae and the size of the vesicle. According to the number of lamellae, liposomes are subdivided as ULV (various sizes), MLV (< 500 nm), and MVV (>1000 nm) vesicles [31, 32]. ULVs are again subdivided into three categories, like SUVs (20-100 nm), LUVs (>100 nm), and GUVs (>1000 nm). Multilamellar vesicles consist of several smaller non-concentric type vesicles enclosed inside the lipid bilayer (single), suitable for hydrophilic material [33]. The number of lamellae can be responsible for the amount of compound encapsulation in liposomes [34]. Classification of liposome categories is shown in Figure 2 [35].

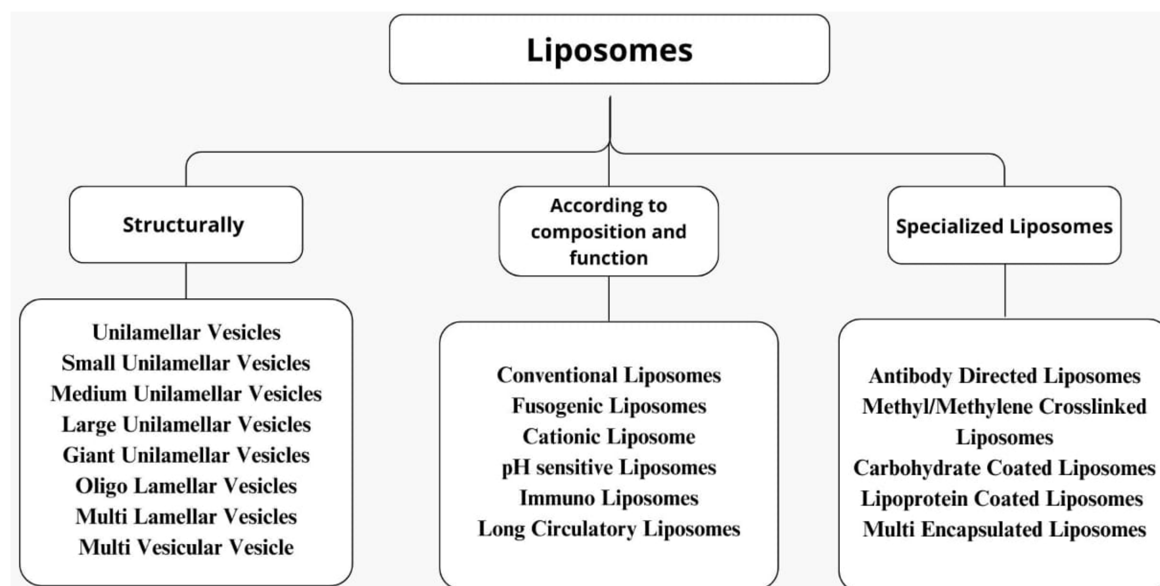

Figure 2: Classification of Liposomes according to their structure, composition, and specialization

MAIN COMPONENTS OF LIPOSOMES

Phospholipids

Liposomes are synthesized from lipids, especially phospholipids. Phospholipids are a hydrophilic group (head) and a hydrophobic group (tail). Phosphatidylcholine, phosphatidyl serine, phosphatidylinositol, phosphatidic acid, phosphatidyl ethanolamine, cardiolipin, and phosphatidyl glycerol are examples of lipids for liposome manufacturing. Charged vesicles can be generated by incorporating diethyl phosphate and stearyl amine into phospholipids. The use of sphingomyelin reduces water permeability and enhances proton permeability in several types of liposomes [36]. Lipids can control surface charge, permeability, bio-distribution, clearance, and release from liposomes. Phospholipids strongly influence the encapsulation efficiency, toxicity, and stability of liposomes [37].

Bilayer Excipients

Phospholipids cannot form a bilayer properly, leading to drug leakage during storage due to unsaturated fatty alkyl chains or low phase-transition temperatures. To control the outflow, bilayer-forming excipients, such as cholesterol and α-tocopherol, are incorporated into the liposomal formulation [38].

Differences in phospholipid bilayer composition can alter liposomal encapsulation efficiency. The rigidity of phospholipid bilayers can be improved by the addition of cholesterol in specific quantities based on the area of the liposome. Another excipient, α-tocopherol, has greater therapeutic potential for scavenging reactive oxygen species generated within injured tissues. Thus, liposomes can readily enter intracellular spaces and provide prolonged drug retention [39]. Several studies, after

characterization by mean particle size, size distribution, and zeta potential, have shown that particle size increased with cholesterol concentration; thus, to some extent, it stabilizes the liposomal suspension, but only up to a particular point (after which stability decreases) [40].

Surfactants

Surfactants can facilitate encapsulation and reduce surface tension between immiscible phases. Generally used surfactants include Span 60 and 80, sodium cholate, and Tween 60 and 80 [41, 42]. Liposomes with surfactants function as carrier systems to enhance drug penetration into the skin. Transfersomes (deformable liposomes) should be nanovesicles depending on the surfactant used in transdermal delivery systems [43, 44].

The cause of deformation is the edge activator or surfactant, which can alter the lipid bilayer properties of vesicles [45]. Nanovesicles have a variation from other conventional liposomes, where liposomes respond to osmotic pressure by shape transformations [46]. To mitigate problems associated with the in vivo target and severe side effects, stealth liposomes are combined with surfactants and an active drug-loading strategy. This can prolong blood circulation time, reduce cardiotoxicity, and improve tolerance [47].

Additional Excipients

Excipients such as PEG exhibit extended circulation characteristics and can protect encapsulated drug molecules from inactivation and metabolic degradation. PEG improves stability and intracellular intake [48]. It has the potential to reduce particle aggregation and improve stability during storage [49]. Ligands like antibodies, nucleic acids, vitamins, and proteins exist within the target cell's receptor surface, enhancing the functionality for PEGylated liposomes. PEG generates stealth or PEGylated liposomes imperceptible to the reticuloendothelial system of the body [50, 51].

Sialic acid, glycolipids [52], and dextran (unmodified or modified) are used for modification of the liposome surface [53]. PEG-coated liposomes enhance circulation time after intravenous administration and increase stability within the intestine via an adhesion mechanism mediated by intestinal mucus. This mechanism is due to mucoadhesive polymers (positively charged) and ionic interactions of negative compounds present within the mucus layer [54].

METHODS OF PREPARATION OF LIPOSOMES

Thin Film Hydration or Bangham Method

Thin-film hydration is a widely favoured and widely used technique for liposome generation. Organic solvents such as ethanol, chloroform, a mixture of chloroform and methanol, and dichloromethane can be applied to make solutions of lipids. After dissolving lipids, the solvent (organic) is vaporized under vacuum to build up a thin lipid film (45 to 60 ºC) [55]. Finally, hydration of the lipid film should be performed in an aqueous medium under agitation for 2 hours (60–70 °C). In this process, round closed liposomes are formed, as shown in Figure 3 [56].

Ethanol/ Ether Injection Method

Generating liposomes using the ether and ethanol injection method relies on dissolving lipids in solvents such as an ether-ethanol mixture, diethyl ether, or ethanol. A mixed solution is injected within an aqueous medium for the encapsulation process (55 to 65 ºC) under reduced pressure. An immiscible organic solvent in the aqueous phase is heated to ensure complete removal from the formulation. The inkjet method is a modern ethanol injection technique that incorporates the drug solution (hydrophilic/lipophilic) into ethanol. This prepared solution is transferred to an inkjet printer for the synthesis process, with greater control over liposome proportions; it is also applicable to large-scale production [57].

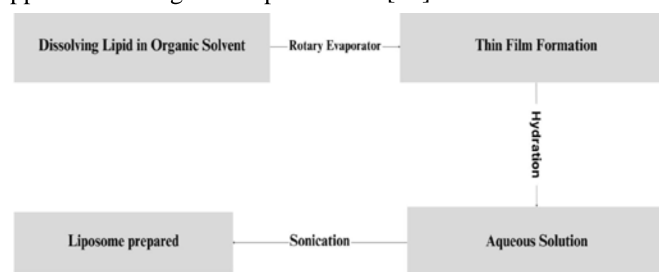

Figure 3: Steps involved in the thin film hydration method

Reverse-Phase Evaporation Method

This method involves dissolving drugs and lipids in aqueous and organic solvents, respectively. Both solutions should be sonicated together to generate inverted micelles. The organic solvent is removed using a rotary evaporator, thereby converting the micelles into a gel or viscous material. At a critical point, the gel state collapses, converting into inverted micelles. Reversed phospholipids finally form a bilayer surrounded by residual micelles and prepare liposomes. Modified reverse-phase evaporation techniques have achieved the highest encapsulation efficacy (80 %) [58].

Detergent Depletion Method

This method can synthesize homogeneous liposomes. This method alters lipid solubility by incorporation of specific detergent within an organic type of solvent (during critical micelle concentration) & formulates detergent-lipid micelles. When the detergent is removed, micelles form liposomes. Detergent removal is done by dialysis. A LipoPrep device can be applied for detergent elimination. Dialysis can also be performed by placing dialysis bags in detergent-free buffers, a method known as equilibrium dialysis [59]. The phospholipid-to-detergent ratio, the uniformity of liposomes, and the size vary with the rate of detergent elimination.

Heating Method

In the heating method, hydration of phospholipids takes place by using glycerol or PEG/ethylene glycol for 1 hr (60°-120 °C) with ceaseless stirring. After this process, the mixture is cooled and centrifuged to prepare liposomes (4000rpm, 15 min). Liposomes synthesized by the heat method do not suffer from the phospholipid’s deterioration. In this process, sterilization is unnecessary because it operates at high temperatures (120 °C) [60]. In the refurbished heating method in an aqueous medium, lipids are hydrated, and components are heated, rather than using organic solvents, thereby increasing stability [61].

Membrane Extrusion Process

This ideal technique should be applied to transform MLVs into SUVs and LUVs. Sizes can be decreased by using polycarbonate membrane filters at low pressure (less than 100 psi). LMVs are passed through freeze–thaw cycles or a large-pore-size filter (0.2–1 μm) before expulsion. During the extrusion process, vesicles in a dispersion medium are passed through a polycarbonate membrane multiple times, thereby breaking the vesicles and resealing the phospholipid layers. Vesicle size decreases as transmembrane pressure increases, driven by various extrusion processes. Finally, the generated liposomes should be characterized as large unilamellar vesicles (120–140 nm) [62].

Microfluidic Channel Method

The microfluidic channel method was developed to control liposome size [63]. During the process, solutions are made with lipids and 2-propanol. This solution is passed through a dual-channel centre containing an aqueous phase. A stream of lipids and an isopropyl alcohol mixture forms liposomes. The size of liposomes will be controlled by laminar flow and lipid concentrations within microfluidic channels. Finally, self-assembled liposomes can be generated via direct drug encapsulation [64].

Homogenization

The homogenization method is most commonly used for the size reduction of liposomes. In this process, a suspension containing liposomes is continuously pumped through the gap at a constant high pressure. Liposomes collide with stainless-steel walls, reducing their size.

Sonication method

It is applied to the generation of SUVs. Probe and bath sonication techniques are preferred for MLVs sonication under a significant atmosphere [65]. In probe sonication, the sonicator tip is immersed in the liposomal formulation. Energy input is very high, resulting in heating. To avoid heating, the vessel is placed in a water or ice bath during sonication (1 hour). In bath-type sonication, the formulation is added to a beaker and placed inside the sonicator. The method will be performed with automatic temperature control, unlike probe sonication. After sonication, liposomes are kept within sterile vessels.

Drug Loading Methods of Liposomes

The most challenging aspect of liposomal drug delivery is drug loading, which is governed by hydrophobicity and lipophilicity. For multi-step procedures, it is necessary to determine at which stage a drug should be encapsulated. Mainly, two types of drug loading are known: active loading and passive loading. In the case of the active loading technique, entrapment of drugs initiates after the completion of the generation of the liposome. This method is based on gradient loading with buffered ammonium sulphate (gradient). Efficient weak base loading can take place with less than 11 of pKa and − 2.5 to 2 of log P values [66]. The passive loading technique involves generating lipid films and hydrating them to form liposomes. Remote or gradient loading is another advanced technique for liposomal formulations that utilizes a transmembrane gradient, typically based on pH or ion concentration, to promote the movement of specific drugs into vesicles, thereby achieving high encapsulation efficiency and improved retention.

This technique is predominantly effective for amphipathic weak bases like doxorubicin and vincristine. Compared with passive

loading, gradient loading yields higher drug-to-lipid ratios, reduces waste, and improves stability. It is the most designated method for chemotherapeutics [67]. Improved loading techniques, particularly remote or gradient-based methods, have enabled the development of numerous marketed liposomal drugs for clinical use, with enhanced patient safety profiles.

Table 1: Quantitative data for Remote or Gradient loading techniques for several drugs

Drug	Entrapment Efficiency	Drug Release
Doxorubicin	Up to 98%	High internal concentration
Sirolimus	Up to 98%	Sustained release
Curcumin–cyclodextrin	16–51% (according to complex)	Improvement over passive
Vincristine sulfate	Highest entrapment efficiency at 1:15 w/w drug/lipid	Release sensitivity with pH buffering
Resiquimod	33–44 % for remote, but 0.2–8 % for passive loading	Initial 6 hours linear release

NEWER METHODOLOGIES FOR LIPOSOME PREPARATION

Freeze Drying Method

This technique can be employed by dissolving the drug and lipid in tert-butyl alcohol (45°C) and by dissolving the lyo-protectant in water (45°C). The two generated solutions are finally mixed, yielding a monophasic solution. The final solution is filtered and freeze-dried to produce proliposomes. Freeze drying takes place in two steps: firstly, a frozen sample should be formed (− 40 ºC), and secondly, the samples are dried (room temperature) and hydrated to manufacture liposomes (100–300 nm) [68]. The method is illustrated in Figure 4.

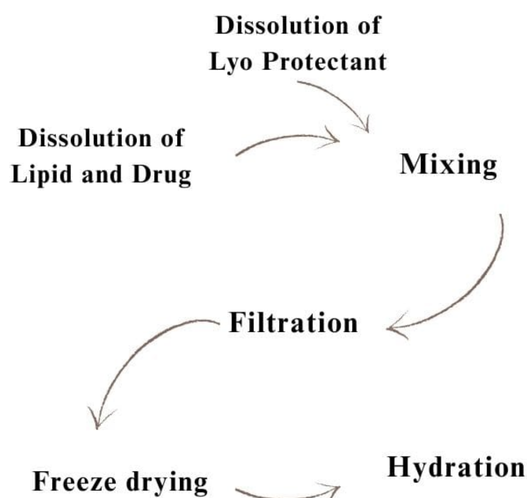

Figure 4: Schematic representation of the Freeze-Drying Method

Dual Asymmetric Centrifugation Method

The Dual Asymmetric Method of Centrifugation should be performed in sealed vials that are rotated at a determined speed (rotational axis) and also rotate on their own axis. On the other hand, in the general method of centrifugation, vials rotate only about their own axes. The sample moves outward during the main rotation, whereas the own-axis rotation pushes the sample in the opposite direction due to adhesion between the sample and the rotating vial. Through mechanical turbulence, energy can be transferred to the sample preparation, reducing liposome size to 60 nm [69]. The schematic representation is shown in Figure 5.

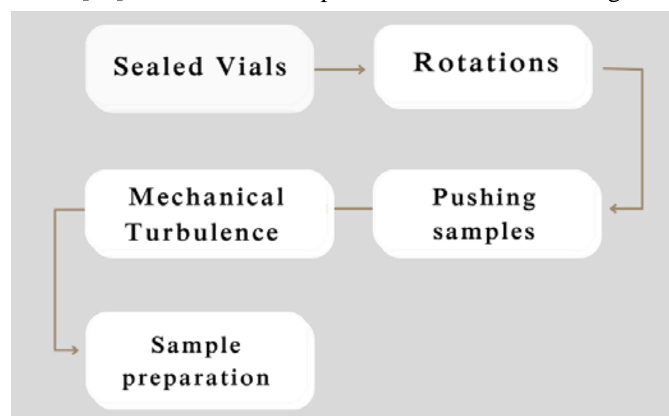

Figure 5: Schematic representation of the Dual Asymmetric Centrifugation Method

Supercritical Fluid Methods

To reduce restrictions on toxic limits and degradation, this method has emerged as a promising technique, including depressurization of an expanded liquid organic solution-suspension method, supercritical-assisted atomization, supercritical anti-solvent method, supercritical-assisted liposome formation, and supercritical reverse-phase evaporation method [70]. These methods are better alternatives for conventional methods of liposome formation [71, 72].

Supercritical Anti-solvent Method

This technique is used to generate proliposomes. This method applies to low-solubility drugs and can be used with low-boiling-point solvents [73]. In this technique, a supercritical fluid is passed from the top portion of a high-pressure chamber. Subsequently, drug-containing solutions in the form of small

droplets are sprayed into the Supercritical Fluid using an atomized nozzle. Finally, liposomes are formed after hydration with an aqueous solution. Supercritical CO_2 can behave like an anti-solvent for solute, whereas it can be mixed with an organic solvent. Liposomes produced are organic solvent-free in comparison to liposomes generated by conventional methods.

The CAS method is an upgraded method for SAS. CAS has two different procedures. The first method is the single-exit process, in which a liposomal solution is introduced into the autoclave; CO_2 is then loaded, and the organic solution is sprayed into the liposomal solution under continuous stirring. Liposome suspension and CO_2 are removed from the same valve. The second method has two exit processes: liposomes suspended in the liquid phase are recovered from the bottom of the autoclave, and CO_2 is recovered from the top [74]. This is shown in Figure 6.

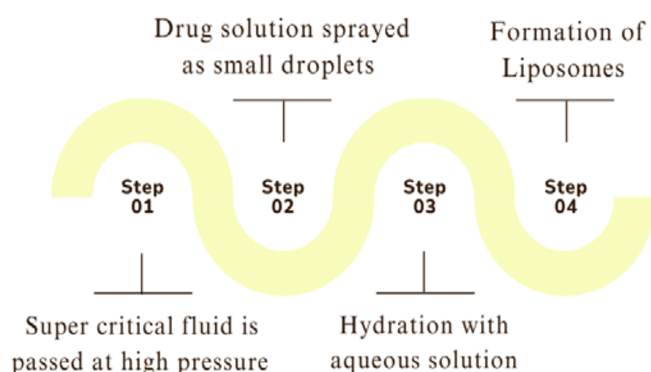

Figure 6: Schematic Representation of Supercritical Anti-Solvent

Supercritical CO_2 Reverse Phase Evaporation Process

Lipid, organic co-solvent, and compressed gas are mixed and stirred at a temperature higher than the transition temperature of the lipid phase within variable volume cells (10 to 30 bar). To volume cells, an aqueous solution has been introduced slowly under reduced pressure, and the release of compressed gas occurs to form liposomes with a mean diameter of 200-1200 nm. With decreased lipid concentration, the mean size also decreases (100-250 nm). This method is almost similar to the decompression technique. Recently, a new technology has been developed as an improved SCRPE method, where a phospholipid with a drug is enclosed within a vesicle-type cell at 60°C. Subsequently, for 40 min, CO_2 is pressurized to generate a liposome dispersion [75]. This method is shown in Figure 7.

Rapid Expansion of the Supercritical Solution Method

This method is beneficial for micronizing a poorly water-soluble drug. The most important factor in this method is the stability of the drug in the Supercritical Fluid, which alters particle size distribution and supersaturation. This technique has two steps: the first is the dissolution of solids in a supercritical fluid, and the second is the synthesis of supersaturated particles. The process is initiated by passing a supercritical fluid through the extractor at a constant pressure and temperature. Supercritical Fluid enters the extractor to dissolve the solids at low pressure. After that, the heated nozzle solution is depressurized. Cooling with this technique may cause nozzle block and particle de-aggregation [76].

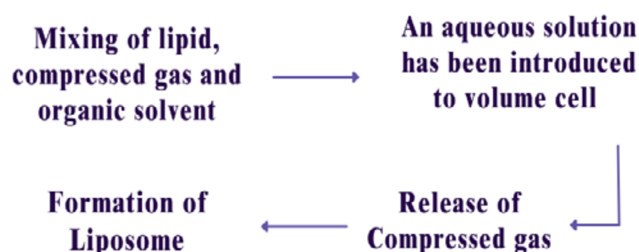

Figure 7: Supercritical CO_2 Reverse Phase Evaporation

Depressurization of an Expanded Liquid Organic Solution Suspension Method

During this methodology, phospholipids must be mixed with a solvent (organic) at the required temperature. Finally, in a vessel, the solution is mixed with supercritical CO_2 to generate a cosolvent. Depressurization of this solution is used to create liposomes via a nozzle. Thermosensitive substances can be readily used in this method to develop liposomes at low temperatures and pressures [77].

Supercritical Assisted Liposome Formation

For this technique, the lipid is dissolved in ethanol, and in a saturator, mixed with CO_2 (pure) to get an expanded fluid. Saturator has been loaded with baffles (high pressure requires) and bands (thin) are warmed up by heat to generate Supercritical Fluid. The mixture is passed through high-pressure generation vessels, and the drug in aqueous solution is finally atomized. The temperature of the saturator and the vessel is maintained at 40 °C, while the pressure is held constant at 100 bar. Suspension with liposome is collected from the ground section of the vessel. Separation of CO_2 and ethanol is taking place with a stainless steel separator (at 30°C). For the aqueous mixture, encapsulation

efficiency depends on flow rate, with the highest flow rate corresponding to the lowest entrapment efficiency [78, 79].

REGULATORY CHALLENGES ACCORDING TO QUALITY CONTROL [80]

Current regulatory challenges related to liposomes have arisen from manufacturing sensitivity, complex structure, and the absence of globally harmonized guidelines. USFDA, EMA, PMDA, and Health Canada suggest recommendations with different terminology, testing requirements, and emphasis. Quality attributes such as microstructural equivalence, size distribution, lamellarity, lipid composition, morphology, zeta potential, and drug-release characteristics play pivotal roles. Gaps and challenges in analytical methods may complicate submissions. Sterility and aseptic techniques also bring further difficulties because liposomes can be applied for parenteral products that may not always be subjected to sterilization (terminal) while ensuring integrity, endotoxin control, and requiring stringent aseptic manufacturing and container-closure system assurance. Stability is the primary concern for liposomes, as it is affected by factors such as aggregation, drug leakage, and lipid degradation.

To justify shelf life, extended real-time stability data are required. Minor manufacturing changes, such as pump models, extrusion membrane suppliers, or nozzle dimensions, may alter critical quality attributes. Finally, safety concerns associated with PEGylated formulations include immunogenicity risks, such as accelerated blood clearance and pseudo-allergy. Overall, liposomes are evaluated using adapted conventional drug frameworks, resulting in case-by-case decisions that leave persistent uncertainty for developers.

Table 2: Comparative summary of newer Methodologies for liposome preparation

Method	Advantages	Drawbacks	Scalability Potential
Freeze Drying (Lyophilization)	It maintains liposomal stability during long-term storage and is particularly suitable for heat-sensitive drugs.	It is a time-consuming process that requires cryoprotectants and expensive equipment with operational costs.	Moderately scalable batch processing, widely used across industries.
Dual Asymmetric Centrifugation (DAC)	It is suitable for viscous formulations. This method is solvent-free and can be processed rapidly.	For a limited batch size, high-speed heating is used, and industrial availability is limited.	Low to Moderate scaling, depending on batch nature.
Supercritical Fluid Methods (General)	It requires little or no organic solvent. It employs a green-chemistry approach with CO_2 and can provide high-purity products.	High-pressure equipment is required, thereby increasing operational costs and limiting compatibility with certain drugs.	Moderate to High scalability is possible with large-scale reactors, but costly.
Supercritical Anti-solvent (SAS) Method	It ensures precise control over particle size and enables processing of thermolabile substances with efficient solvent removal.	Requires complete solubility of the solute in the organic solvent, and process optimization will be complex.	Highly scalable with continuous SAS systems.
Supercritical CO_2 Reverse Phase Evaporation Process (SCRPE)	This technique generates large unilamellar vesicles with high encapsulation efficiency and can minimize residual solvents. It is appropriate for sensitive molecules.	Process control can be complex; specialized equipment is required.	Moderate scaling is feasible but requires expensive equipment.
Rapid Expansion of Supercritical Solution (RESS) Method	It generates uniform, small particles via a single-step solvent removal process and is environmentally friendly.	Limited to solutes soluble in supercritical CO_2 and shows low drug loading for some compounds.	Solubility constraints limit low-to-moderate scalability.
Depressurization of an Expanded Liquid Organic Solution Suspension (DELOS) Method	It has good control over surface morphology with reduced solvent residues.	Depressurization control is complex and needs precise temperature or pressure management.	Moderate scaling is possible with specialized setups.
Supercritical Assisted Liposome Formation (SuperLip)	It shows a continuous process with high encapsulation efficiency and uses less organic solvent.	Requires high-pressure pumps or reactors. Equipment cost and maintenance cost are high.	High and consistent processing makes it promising for industrial-scale production.

LIPOSOME CHARACTERIZATION

- **SEM and TEM for Size Distribution**

Liposome sizes are based on the type of preparation and composition of phospholipid. Preferred methods for size detection include microscopy, hydrodynamic techniques, and diffraction-based light scattering. Microscopic techniques include SEM, TEM, and optical microscopy. Images of liposomes can be obtained by TEM and SEM, which provide measurements of interbilayer and bilayer distances (thickness) [81]. Atomic force microscopy has recently been developed for high-resolution scanning. It produces micrographs at nanometer-scale 3D resolution. Hydrodynamic methods, such as field-flow fractionation, ultracentrifugation, and gel-exclusion chromatography, are used to estimate compound molecular mass [82]. This methodology determines the uniformity of liposomes, elution characteristics, and size distribution. Diffraction-based light-scattering methods, such as laser or quasi-elastic light scattering and spectroscopy (photon correlation), provide details for size determination.

- **Lamellarity Estimation**

Lamellarity is determined by the number of lipid bilayers in liposomes. It is measured using cryo-electron microscopy, small-angle X-ray scattering, and nuclear magnetic resonance, which reveal lamellarity, size, and homogeneity of liposomes [83].

- **Zeta Potential**

Zeta potential affects cellular uptake and drug delivery to the target. Zetasizer and electrophoresis (laser Doppler) can measure the liposome formulation's zeta potential. This method uses an electric field and laser scattering to detect the moving particles. Factors such as ionic strength, pH, and particle concentration can alter zeta potential. The positive and negative charges of phospholipids may influence the timing of blood circulation and the surface charge of liposomes [84].

- **Encapsulation Efficiency**

Encapsulation efficiency is the percentage of the drug entrapped within liposomes, depending on its lipophilic or hydrophilic nature. The bioavailability of drugs is directly proportional to entrapment efficiency [85]. It is estimated using solid-phase extraction, size-exclusion chromatography, hollow-fibre centrifugal ultrafiltration, protamine aggregation, and mini-column centrifugation. Liposome purification and separation should be performed using mini-column centrifugation. Negatively or neutrally charged liposomes are used in the protamine aggregation technique.

- ***In vitro* release study and *in vitro* cytotoxicity assay**

An *in vitro* release study of the drug should be initiated within a dialysis bag at 37 °C. Finally, the dialysis bag is immersed in the basic or acidic dissolution medium under sink conditions and continuous stirring. The required volume of sample is withdrawn after a specified time interval, and an equal volume of fresh medium is returned (minimum 4-hour study). Samples are measured using a UV spectrophotometer to determine absorbance. The cholesterol content of the formulations can modulate liposomal release. An increase in the concentration of cholesterol can improve the release (rate) of the drug [86]. *In vitro* cytotoxicity of selective formulations can be assessed using cell counting kits. For this method, cells should be incubated at 37 °C for 4 hours. After removing the culture medium, 100 µl of the cell counting kit should be diluted in phosphate buffer (v/v, 10:90) and added to each well. Absorbance will be finally measured at a specific wavelength under a microplate reader [87].

- ***In Vivo* study**

In vivo characteristics of liposomes (drug incorporated) are altered due to the nature of the vesicle. Liposomal formulations are administered intravenously or intramuscularly for *in vivo* study. The addition of cholesterol to liposomes increases stability and reduces drug leakage. *In vivo* study of liposomes can be examined by injecting liposomes (tail vein) in mice. Fluorescent images should be taken after injection [88]. After blood samples were collected at a specified time, they were centrifuged to separate plasma from blood and stored at −80 °C before further analysis.

- **Stability Studies**

A stability study determines the shelf life of the liposomal formulation and entails biological, chemical, and physical stability. Physical stability must be confirmed by visual inspection or by transmission electron microscopy. The oxidation, hydrolysis, and degradation of drugs can be determined using TLC, HPLC, and HPTLC [89].

MARKETED FORMULATIONS OF LIPOSOMES

The marketed liposomal formulation is shown in Table 3.

Table 3: Liposome formulation related to the market

Drug Name/ Vaccine type	Approval Year	Approved by	Market Status	Method of preparation	Route of Adm	Disease
Amphotericin B (AmBisome)	1997	FDA	Approved and marketed globally	Conventional technique	I.V Infusion	Fungal Infection
Doxorubicin (Doxil)	1995	FDA	Approved and marketed globally	Stealth liposome technology	I.V injection	Breast neoplasms
Daunorubicin (DaunoXome)	1996	FDA	Discontinued	Conventional method	I.V injection	AIDS-related Kaposi's sarcoma
Morphine sulphate (DepoDur)	2004	USFDA	Discontinued	Depofoam technology	Epidural	Analgesic
Cytarabin and daunorubicin(Vyxeos)	2017	FDA	Approved and marketed (US, EU, Japan)	Depofoam technology	I.V injection	Cancer therapy
Verteporfin (Visudyne)	2000	FDA	Approved and marketed globally	Conventional technique	I.V injection	Photodynamic therapy
Irinotecan (Onivyde)	2015	FDA	Approved and marketed globally	PEGylated Liposome	I.V injection	First-line metastatic pancreatic adenocarcinoma

Table 4: List of several Patents of Liposomal formulations with year

Title of Patent	Year	U.S. Patent No.	Key Innovations
Remote loading of sparingly water-soluble drugs into liposomes	2017	**US 9737485 B2**	Demonstrates successful remote loading of sparingly water-soluble drugs, overcoming a significant formulation challenge.
Liposome composition for use in peritoneal dialysis	2019	**US 20190105270 A1**	Novel therapeutic approach using liposome-supplemented dialysate.
Tailored liposomes for the treatment of bacterial infections	2020	**US 10744089 B2**	Invention provides empty (drug-free) liposomes designed to sequester bacterial toxins
Methods and devices for liposome preparation by centrifugation	2021	**US 11191725 B2**	Centrifugal Extrusion for Uniform Liposome Sizing
Liposome Containing Compositions & Their Use in Personal Care & Food Products	2020	**US 10702475 B2**	Non-solid liposomal particles for personal care and food
Controlled drug release liposome compositions and methods thereof	2021	**US 11147881 B2**	Liposomal formulation containing both free (soluble) and precipitated forms of an active agent inside the same liposome.
A composition comprising an onion extract and liposomes	2017	**EP 2723319 B1 / WO 2012/175626 A1**	Using liposomes to deliver onion extract more effectively for scar prevention and treatment,

NOVEL LIPOSOME FORMULATIONS COMPARED WITH TRADITIONAL

A novel liposomal formulation exhibits advantages over traditional methods in targeting, *in vivo stability,* and release. Conventional liposomes are generally composed of phospholipid–cholesterol bilayers, which are prone to rapid clearance by the reticuloendothelial system, increased drug leakage, and passive tumour accumulation via the enhanced permeability and retention effect.

In contrast, next-generation systems employ design features such as PEGylation and optimized lipid composition to maximize circulation and prevent premature leakage, whereas the remote loading method stabilizes drug encapsulation. For release control, stimuli-responsive liposomes can exploit internal triggers (e.g., pH) and external stimuli (e.g., heat, light, ultrasound) to achieve site-specific release.

Overall, novel formulations can provide predictable pharmacokinetics, controlled drug release, and improved targeting potential to enhance universal clinical success. It should be concluded that the development of liposomes is necessary for their optimal use in the pharmaceutical industry and clinical practice. An example of a successful liposomal formulation is Doxil®, approved by the FDA for cancer therapy, which was applied for treating patients with Kaposi's sarcoma and ovarian cancer [90].

CONCLUSION

This review has outlined various methodologies for liposome formulation and evaluation. Liposomes are evolving to improve performance and address prior limitations. A limited number of liposomal formulations have been developed and approved for market use, whereas clinical investigation is underway for more than 500 liposomal formulations. Liposomes are challenging dosage forms due to their stability issues. An emerging focus on personalized liposomal therapies is underway, in which formulations are tailored to individual patient profiles by applying proteomic, genomic, and metabolomic data to maximize therapeutic outcomes. Similarly, theranostic liposomes could allow real-time assessment of drug delivery and therapeutic efficacy.

Green and sustainable manufacturing approaches should be emphasized to reduce the environmental footprint during large-scale liposome preparation. In addition, the integration of artificial intelligence (AI) and advances in data analytics for the design, formulation, and optimization of shows significant potential. AI-generated predictive modelling and machine learning algorithms can promote discovery, improve reproducibility, and lead to patient-focused liposomal systems. Directions pointing toward a future of liposomes: the generalization of nanocarriers into intelligent, sustainable, and personalized therapeutic platforms.

ABBREVIATIONS

siRNA: Small Interfering Ribonucleic Acid, SUV: Small Unilamellar Vesicles, LUV: Large Unilamellar Vesicles, MLV: Multilamellar Vesicles, MVV: Multivesicular Vesicles, GUV: Giant Unilamellar Vesicles, PEG: Polyethylene glycol, SAS: Supercritical Anti-Solvent, CAS: Continuous Anti-Solvent, SCRPE: Supercritical CO_2 Reverse Phase Evaporation, IV: Intravenous, SEM: Scanning Electron Microscopy, TEM: Transmission Electron Microscopy, TLC: Thin-Layer Chromatography, HPLC: High Performance Liquid Chromatography, and HPTLC: High Performance Thin Layer Chromatography.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Sudipta Das and Beduin Mahanti planned and conceptualized the manuscript. Debatri Roy designed the figures, conducted the literature review, and drafted the manuscript. Based on an analysis of various data sources, Arnab Samanta drafted a literature review. Setu Majumder evaluated the data available across multiple research papers

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