A comprehensive review on: liposomes as drug delivery system

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Abstract:
Liposomes are round, bubble-like structures used to deliver drugs. They are made from one or more layers of phospholipids that form a barrier around a water-filled centre. These structures are popular in the pharmaceutical world because they are safe for the body, break down naturally, and can hold both water-soluble and fat-soluble drugs. Cholesterol is often added to make the structure more stable and prevent the drug from leaking out. Liposomes come in different forms depending on their structure and what they're made of, such as small single-layer vesicles, large single-layer vesicles, multi-layer vesicles, regular liposomes, PEG-coated liposomes, and targeted liposomes. To make them, scientists use methods like making a thin film and then adding water, using sound waves, pushing through tiny holes, adding alcohol, or evaporating a solvent in the reverse way. When they test these liposomes, they check for size, how uniform the size is, electrical charge, shape, how well the drug is trapped, how much drug is inside, how it releases in a lab setting, and how stable it is over time. Using liposomes helps drugs dissolve better, reach the body more effectively, release slowly, stay in the bloodstream longer, target specific areas, and be less harmful. PEG-coated liposomes can stay in the body longer, and liposomes with special molecules can help drugs gather at certain parts of the body. These systems are used for treating cancer, fungal infections, bacteria, vaccines, delivering genes, and giving proteins and peptides. However, there are some issues, like drugs leaking out, lipids breaking down, physical changes, high costs to make them, and problems scaling up production. Scientists are working on new types of liposomes that respond to certain signals and can actively target specific areas to overcome these problems. Overall, liposomes are a promising way to make medicines work better, be safer, and deliver drugs more effectively.

Keywords:
Liposomes, drug delivery, Phospholipids, cholesterol, Bloodstream, Fungal infections, PEG-coated liposomes.

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1. Introduction:
Drug delivery systems (DDSs) are designed to enhance the therapeutic effectiveness of medications by increasing drug accumulation and retention at target cells while minimizing unwanted side effects(Adepu S et al., 2021). These systems improve the pharmacological performance of conventional drugs by optimizing their pharmacokinetic behavior and distribution within the body. In addition, DDSs can function as drug reservoirs, enabling the controlled transport of therapeutic agents to specific sites of action through nanocarriers and thereby reducing drug-related adverse effects (Liu P et al., 2022).
Nanoparticles (NPs) generally range in size from a few nanometers to several hundred nanometers, depending on their intended purpose and application (Li Z et al., 2024). They can be developed using a wide variety of

natural, organic, and inorganic materials, including ceramics, polymers, metals, and lipids. These materials can be used to construct different nanostructures, such as micelles and liposomes (Adepu S et al., 2021).

Therapeutic agents can be incorporated into nanoparticles through several mechanisms, including physical entrapment, surface adsorption, and encapsulation (Coelho JF et al., 2010). The distinctive structural and physicochemical characteristics of different nanoparticles can be exploited to improve the performance of conventional therapeutic approaches. Nanomedicine has facilitated the development of innovative nanoscale strategies for delivering biologically active agents, thereby supporting the treatment, prevention, and diagnosis of various diseases (Allen TM et al., 2013).

Despite substantial progress in nanoparticle-based drug delivery, many existing systems continue to face challenges related to limited drug-loading capacity and insufficient specificity toward target tissues or cells. Consequently, the development of nanocarriers capable of achieving high and controllable drug loading, combined with recognition ligands that selectively bind to specific or overexpressed biomarkers, represents a promising approach to improving drug delivery efficiency.(Petros RA et al., 2010).

Among the various nanocarrier systems, liposomes have received considerable attention for their potential in targeted drug delivery. They are spherical vesicles consisting of one or more lipid bilayers and generally measure approximately 50–500 nm in diameter. Liposomes are produced by dispersing or emulsifying natural or synthetic lipids in an aqueous medium. Since their discovery by Bangham in the 1960s, liposomes have become one of the most extensively investigated and widely utilized platforms for drug delivery (Wen H et al., 2015).

The favorable characteristics of liposomal nanoformulations, including their stability, ease of preparation, high drug-loading capacity, biocompatibility, improved bioavailability, and use of safe excipients, have made them widely applicable in nanomedicine(Torchilin VP et al., 2005). Their versatility is further attributed to their nanoscale dimensions and amphiphilic structure, which enable them to encapsulate therapeutic agents within the aqueous core or lipid bilayer, depending on the physicochemical properties of the drug(Muzykantov VR et al., 2019). These features make liposomes promising carriers for efficient and targeted drug delivery.

Several liposome-based drug delivery formulations have received approval from the Food and Drug Administration (FDA) for the treatment of various diseases. In addition to their therapeutic applications, liposomes are also useful in diagnostic procedures and can be administered through multiple routes, including oral, parenteral, pulmonary, ophthalmic, and transdermal delivery (Yun YH et al., 2014, Kumari A et al., 2010, Mitchell MJ et al., 2021)

Phospholipids, such as soybean-derived phosphatidylcholine and synthetic dialkyl or trialkyl lipids, serve as the primary structural components of liposomes (Aminu N et al., 2020). Cholesterol is commonly incorporated into liposomal formulations because it regulates membrane fluidity, reduces membrane permeability, and enhances the stability of lipid bilayers in biological fluids, including blood and plasma (Tenchov R et al., 2021). In addition, polymers and membrane proteins may be incorporated into liposomes to improve drug encapsulation efficiency, modify biodistribution, and prolong circulation time within the body (Anselmo AC et al., 2016).

Furthermore, stealth liposomes containing polyethylene glycol (PEG) conjugated to phospholipids have emerged as an effective strategy for modifying the pharmacokinetic properties and biodistribution of liposomal formulations. The presence of PEG on the liposomal surface helps improve their circulation characteristics and supports prolonged drug delivery (Patel T et al., 2012, Ewii UE et al., 2024).

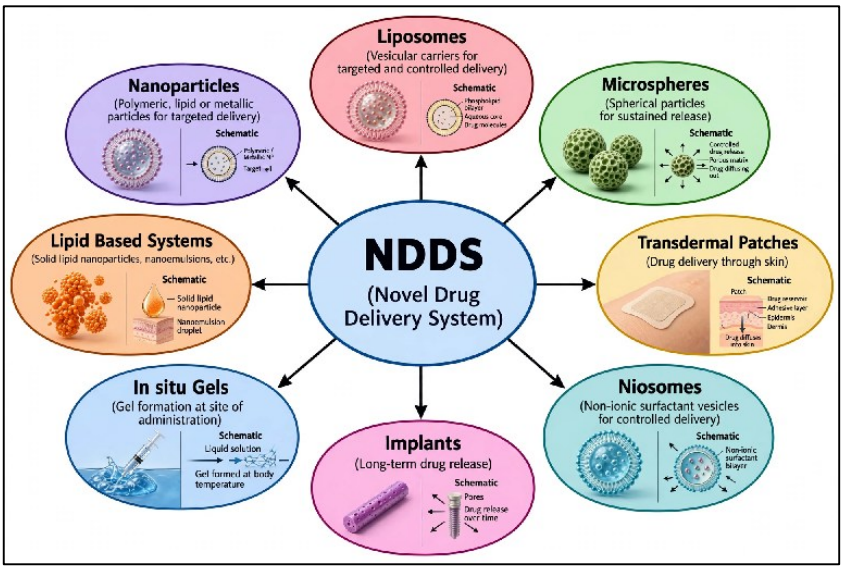


Figure. 1.1: Type of novel drug delivery system

2. Liposomes:

Liposomes are microscopic spherical vesicles composed of one or more phospholipid bilayers enclosing an aqueous core. They were first discovered by Alec D. Bangham and his colleagues in the 1960s and have since gained considerable importance in pharmaceutical and biomedical research. Due to their unique structure, biocompatibility, and ability to encapsulate both hydrophilic and hydrophobic drugs, liposomes are widely investigated as advanced drug delivery systems (Bangham et al., 1965).

Liposomes are primarily composed of phospholipids, which are amphiphilic molecules containing hydrophilic and hydrophobic regions. When dispersed in an aqueous medium, these molecules spontaneously arrange themselves into bilayer structures, forming vesicles capable of entrapping therapeutic substances. Hydrophilic drugs can be incorporated into the aqueous core, whereas hydrophobic drugs can be embedded within the lipid bilayer. This characteristic makes liposomes versatile carriers for a wide range of pharmaceutical agents (Bozzuto G et al., 2015).

Liposomes have attracted significant attention because of their potential to improve drug solubility, enhance therapeutic efficacy, protect drugs from degradation, and reduce adverse effects. They may also facilitate controlled drug release and improve the distribution of therapeutic agents within the body. Furthermore, their surface properties can be modified to enhance circulation time and promote targeted drug delivery, thereby increasing the possibility of delivering drugs to specific tissues or cells (Nsairat H et al., 2022).

2.1. Structure of liposomes:

Liposomes are spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core. Their size generally ranges from 20 nm to several micrometres, depending on their type and method of preparation. Each phospholipid molecule consists of a hydrophilic head and hydrophobic fatty acid tails. The hydrophilic heads face the aqueous environment, while the hydrophobic tails face inward toward each other, forming a bilayer membrane. Cholesterol may also be incorporated into the bilayer to improve stability and regulate membrane fluidity.

Depending on their compartment structure and lamellarity, liposomes are classified into unilamellar vesicles (ULVs), oligolamellar vesicles (OLVs), multilamellar vesicles (MLVs), and multivesicular liposomes (MVLs). OLVs contain two to five concentric bilayers, whereas MLVs contain more than five bilayers, giving them an onion-like structure. MVLs have a honeycomb-like structure with multiple non-concentric aqueous compartments. ULVs contain a single bilayer and are further classified into small unilamellar vesicles (SUVs, 30–100 nm), large unilamellar vesicles (LUVs, generally greater than 100 nm), and giant unilamellar vesicles (GUVs, greater than 1000 nm).

The aqueous core of liposomes can encapsulate water-soluble drugs, whereas lipid-soluble drugs can be incorporated into the phospholipid bilayer. Due to their unique structure and drug-carrying ability, liposomes are widely used in pharmaceutical formulations, targeted drug delivery, and vaccine development (Musumeci T et al., 2024, Nakhaei P et al., 2021).

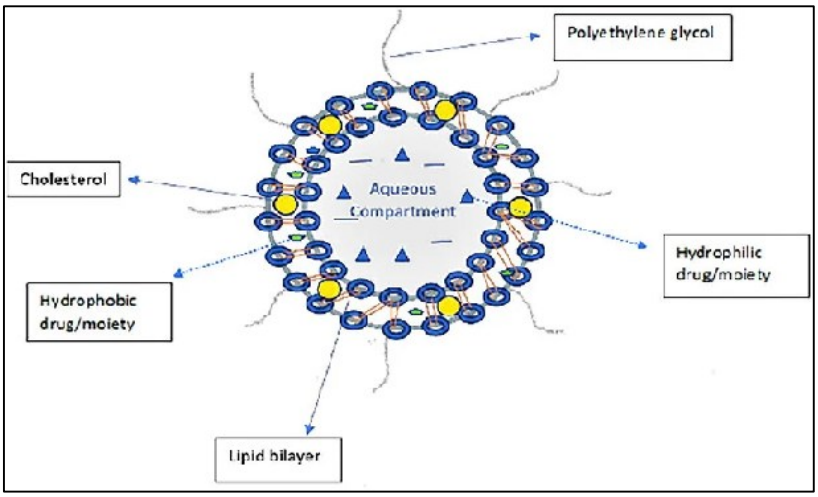


Figure. 1.2: Structure of liposomes

3. Composition of liposomes:

3.1. Phospholipid used for liposomes:

Phospholipids are the primary building blocks of liposomes due to their amphiphilic nature (having a hydrophilic polar head and hydrophobic non-polar tails), which allows them to self-assemble into lipid bilayers.

Here is a breakdown of the most commonly used phospholipids for liposome preparation, categorized by their source and function:

3.2. Natural Phospholipids:

Extracted from natural sources, these are cost-effective and biocompatible, though they often consist of a heterogeneous mixture of lipid species.

1. Egg Phosphatidylcholine (Egg PC): Widely used for neutral, relatively fluid liposomes at room temperature.
2. Soy Phosphatidylcholine (Soy PC): Contains a higher proportion of polyunsaturated fatty acids, yielding more fluid bilayers. Often used in nutritional and cosmetic formulations (Ahmed KS et al., 2019).

3.3. Synthetic Phosphatidylcholines (PCs):

These are chemically pure lipids with defined fatty acid chain lengths, allowing precise control over membrane fluidity, phase transition temperature (T_m), and stability.

1. DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine): Has saturated long chains (C18) and a high T_m (~55°C). Used for rigid, highly stable liposomes that retain contents effectively at body temperature.
2. DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine): Has C16 saturated chains with an intermediate T_m (~41°C). Commonly used in thermo-sensitive drug delivery.
3. DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine): Contains unsaturated chains with a very low T_m (~-17°C). Provides high membrane fluidity at room temperature.

3.4. Charged Lipids (To Prevent Aggregation):

Adding charged lipids creates electrostatic repulsion between liposomes, preventing them from fusing or aggregating.

1. DPPG / DSPG (Phosphatidylglycerols): Provide a negative charge to the liposome surface.
2. DOTAP / DDAB: Provide a positive charge, frequently used in gene delivery (cationic liposomes/lipoplexes) to complex with negatively charged DNA or RNA.

3.5. PEGylated Lipids (Stealth Liposomes):

1. DSPE-PEG(1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N[methoxy(polyethylene glycol)]): Conjugating polyethylene glycol (PEG) to the lipid head creates a steric barrier that prevents opsonization by plasma proteins, significantly extending the circulation time of liposomes in the bloodstream(Makino K et al., 2006)..

3.6. Hydrophilic layer in liposomes:

The hydrophilic layer of liposomes is formed by the polar phosphate heads of phospholipid molecules, which have an affinity for water. In a liposome, the hydrophilic heads of the outer layer face the surrounding aqueous medium, while the hydrophilic heads of the inner layer face the aqueous core. These layers are separated by a hydrophobic region formed by the fatty acid tails of phospholipids. The hydrophilic layer helps maintain the stability of liposomes in an aqueous environment and allows water-soluble drugs to be encapsulated within the inner aqueous core.

3.7. Hydrophobic layer in liposomes:

The hydrophobic layer of liposomes is formed by the non-polar fatty acid tails of phospholipid molecules. These tails repel water and are arranged inward, facing each other in the phospholipid bilayer, while the hydrophilic heads face the aqueous environment and the inner aqueous core. The hydrophobic region provides a barrier to water-soluble substances and helps maintain the structural integrity of the liposome. It also facilitates the incorporation and encapsulation of lipophilic drugs within the lipid bilayer, making liposomes useful carriers for drug delivery.

3.8. Surfactant in liposomes:

Surfactants are used in liposomes to improve their stability and drug delivery properties. They help reduce surface tension and influence the permeability of the lipid bilayer. Surfactants can also improve drug encapsulation and control drug release. They help maintain the uniformity of liposomal formulations. Common examples of surfactants used in liposomes include Tween 80, Span 60, and Poloxamer 188. The selection of surfactants depends on the type of drug and the desired formulation properties (Caracciolo G et al., 2014).

4. Method of preparation of liposomes:

Liposomes are spherical vesicles composed of one or more lipid bilayers surrounding an aqueous core. Because of their unique structure, they can encapsulate both hydrophilic (in the aqueous core) and hydrophobic (within the lipid bilayer) compounds, making them vital carriers for drug delivery, cosmetics, and gene therapy.

4.1. Thin film hydration (bangham method):

This is the classic and most widely used laboratory technique for preparing multi-lamellar vesicles (MLVs).

Step 1: Dissolution: The desired lipids (e.g., phosphatidylcholine, cholesterol) are dissolved in an organic solvent (such as chloroform or a chloroform-methanol mixture) in a round-bottom flask.

Step 2: Solvent Evaporation: The organic solvent is removed using a rotary evaporator under reduced pressure. This deposits a thin, uniform lipid film on the walls of the flask. (Traces of solvent are often removed by placing the flask under a vacuum overnight).

Step 3: Hydration: The dry lipid film is hydrated by adding an aqueous buffer (containing the drug or molecule to be encapsulated) while gently agitating/swirling the flask above the phase transition temperature of the lipids.

Step 4: Vesicle Formation: The resulting suspension yields heterogeneous Multi-Lamellar Vesicles (MLVs)(Thabet Y et al., 2022, Varona S et al., 2011).

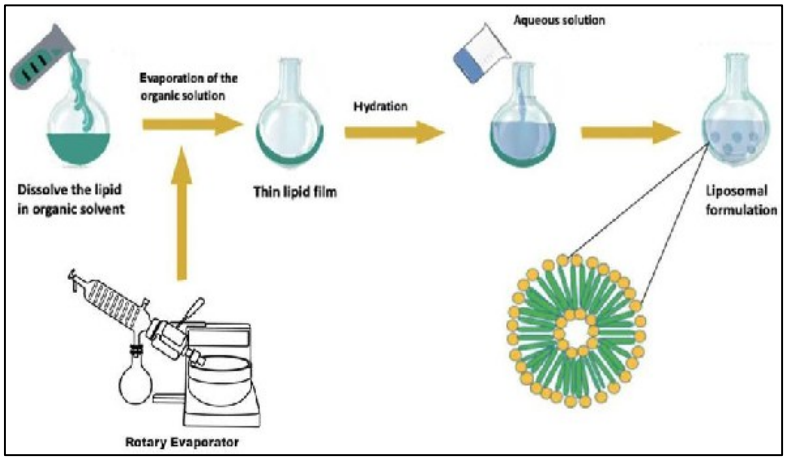


Figure. 1.3: Diagram of thin film hydration method

5. Sonication method:

Sonication is commonly used to reduce the size and lamellarity of MLVs produced by the thin-film hydration method, converting them into Small Uni-lamellar Vesicles (SUVs).

1. **Probe Sonication:** A titanium-alloy probe tip is immersed directly into the liposome suspension. This delivers high energy, but can cause titanium shedding into the solution and lipid degradation.
2. **Bath Sonication:** The liposome suspension in a glass vessel is placed into an ultrasonic cleaning bath. It is milder and keeps the sample sterile, though it takes longer (Alsaif MM et al., 2014, Ghaleb ZA et al., 2014).
3. Sonication is usually performed under an inert atmosphere (like nitrogen) to prevent oxidation.

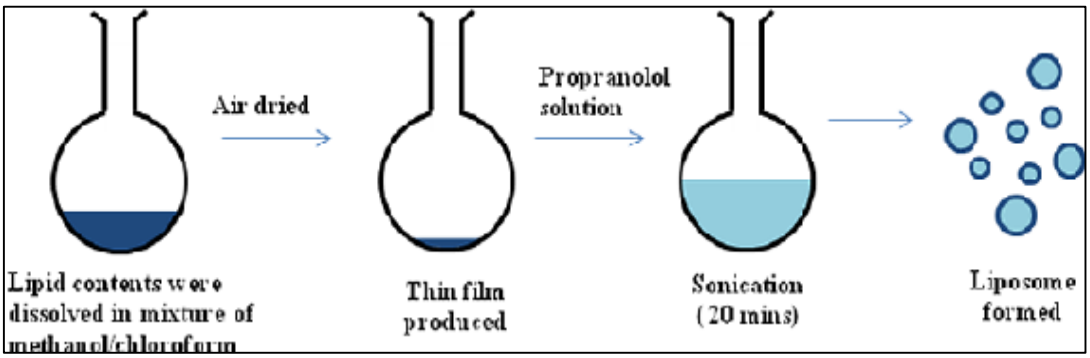


Figure. 1.4: Diagram of sonication method

6. Reverse-phase evaporation method:

This method is particularly useful for encapsulating large macromolecules (like DNA, RNA, or large proteins) with high encapsulation efficiency.

Step 1: Lipids are dissolved in an organic solvent (like diethyl ether or isopropyl ether).

Step 2: An aqueous buffer containing the water-soluble molecule to be encapsulated is added to the organic phase.

Step 3: The mixture is sonicated briefly to form a water-in-oil (w/o) emulsion.

Step 4: The organic solvent is removed under reduced pressure using a rotary evaporator, which causes the emulsion to pass through a viscous gel state and eventually collapse into Large Uni-lamellar Vesicles (LUVs) or oligo-lamellar vesicles (Guinedi AS et al., 2005, Taylor KM et al., 1990).

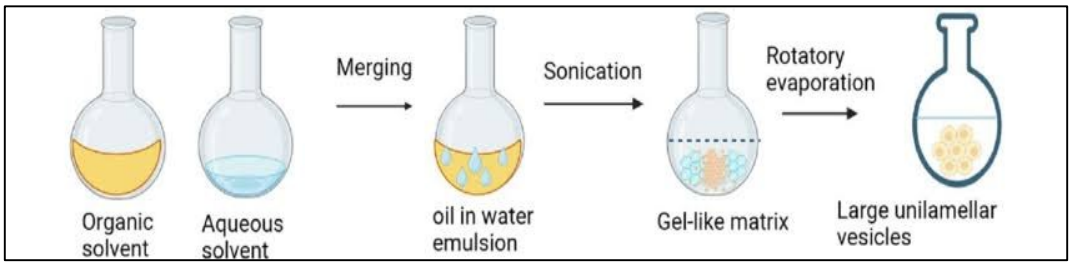


Figure. 1.5: Diagram of reverse phase evaporation method

7. Solvent injrction method:

These methods avoid the need to create a dry lipid film and are easier to scale up.

1. **Ethanol Injection:** Lipids dissolved in a small volume of ethanol are rapidly injected into a large volume of an aqueous buffer. The rapid mixing leads to spontaneous formation of liposomes (usually SUVs).
2. **Ether Injection:** Lipids dissolved in diethyl ether are slowly injected into an aqueous solution of the material to be encapsulated kept at around 55°C to 65°C. The ether evaporates simultaneously, leaving behind unilamellar liposomes (Meure LA., 2008, Charcosset C et al., 2015).

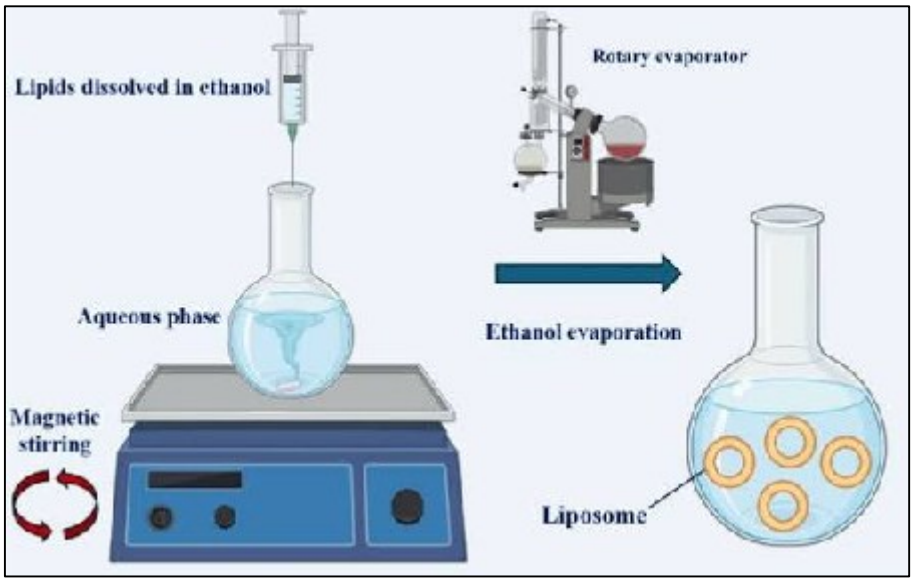


Figure. 1.6: Diagram of solvent injection method

8. Extrusion method (size reduction):

Regardless of the preparation method, raw liposomes often vary widely in size. To achieve a uniform (monodisperse) size distribution:

1. The liposome suspension is passed repeatedly (using gas pressure) through **polycarbonate membrane filters** with defined pore sizes (ranging from 100 nm to 400 nm).
2. This yields large unilamellar vesicles of a highly reproducible, controlled size (Zhang H, 2016, Zhang H, 2023).

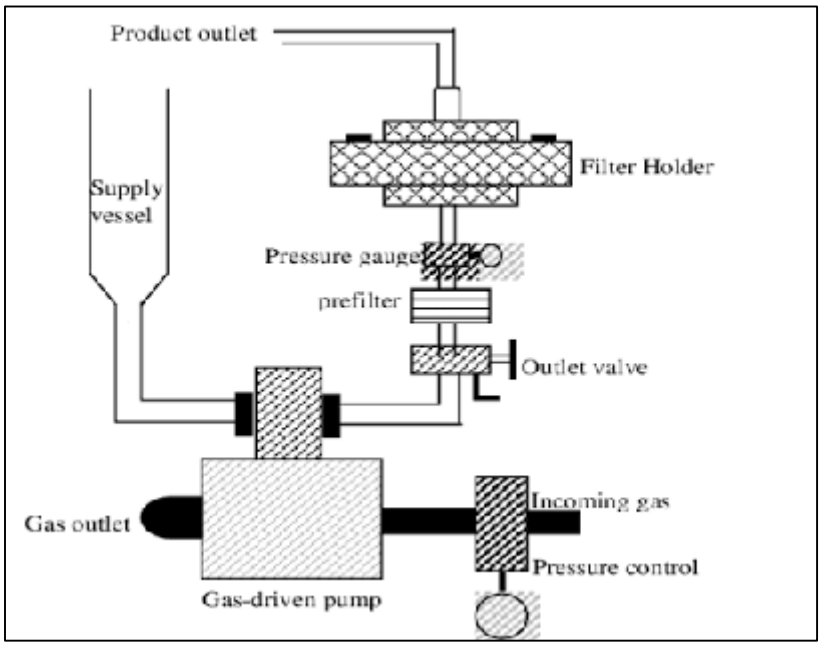


Figure. 1.7: Diagram of extrusion method

9. Advantage of liposomes:

Improved drug delivery: Help carry drugs to specific tissues or cells in the body.
Reduced toxicity: May lessen the harmful effects of certain drugs on healthy tissues.
Improved stability: Protect drugs from breaking down due to environmental changes.
Targeted drug delivery: Can be designed to go to specific organs, tissues, or cells.
Suitable for different drugs: Can hold both water-soluble and fat-soluble drugs.
Controlled drug release: Allow for a steady or controlled release of the drug over time.
Biocompatible: Usually well-tolerated when made with the right kinds of lipids.
Biodegradable: Many of the ingredients in liposomes break down naturally in the body.

Enhanced therapeutic effect: May make certain drugs more effective.
Reduced side effects: Can lower unwanted exposure of the drug in parts of the body not meant to receive it (Bozzuto G et al., 2015, Banerjee R et al., 2001).

10. Disadvantage of liposomes:

High production cost: Making and manufacturing liposomes can be expensive.
Physical instability: Liposomes may clump together, merge, or leak their contents when stored.
Chemical instability: The phospholipids used can break down through oxidation or hydrolysis.
Short shelf life: Some types need special storage conditions to last longer.
Low drug-loading capacity: Certain drugs may not be able to be fully enclosed within liposomes.
Difficult sterilization: It can be hard to sterilize them without harming the structure of the liposomes.
Scale-up difficulties: Producing large amounts with the same quality can be challenging.
Rapid clearance: Some liposomes are quickly taken out of the bloodstream by the body's immune system.
Drug leakage: The drug inside may escape before it reaches its target.
Special storage requirements: Some formulations need to be kept cold or protected from light and air (Hatakeyama H et al., 2013, Akbarzadeh A et al., 2013).

11. Application of liposomes:

1. Cancer treatments use special medicines like Doxil, Marqibo, and DaunoXome to reduce damage to the heart and deliver drugs directly to tumors using the EPR effect.
   2. Gene therapy and mRNA vaccines use special lipid nanoparticles to safely carry delicate genetic material into cells.
2. Antibiotics and antifungals like AmBisome help fight serious infections while being kinder to the kidneys.
3. Imaging tests use liposomes filled with radioactive materials or dyes to find tumors and inflamed areas in the body.
4. Cosmetic products use liposomes to help active ingredients like vitamins, antioxidants, and anti-aging peptides get deeper into the skin.
5. Cosmetics use controlled release systems to slowly release active ingredients like Vitamin C, retinol, and coenzyme Q10 over time.
6. Liposomes help keep skin moist and repair the skin's natural protective layer by using their natural moisturizing qualities.
7. Vitamins and minerals like Vitamin C, B-complex, D, and iron are protected from stomach acid by being wrapped in liposomes to improve how well they are absorbed.
   Liposomes help hide bitter or metallic tastes in foods that contain nutrients and plant extracts.
   Liposomes help keep strong smells from food ingredients from disappearing during processing and storage.
8. Agricultural pesticides and weed killers are delivered slowly to protect crops and reduce runoff into the environment.
9. Pest control uses slow-releasing insect pheromones to stop insects from mating in a targeted way.
10. These carriers are safe, break down in the body, and don't cause immune reactions because they are made from natural phospholipids.
11. Liposomes can carry both water-based and fat-based substances at the same time, with one inside and one in the outer layer.
12. They can be changed on the surface with PEG or antibodies to make them target specific cells or organs more precisely (Daraee H et al., 2016).

12. Conclusion:

Liposomes have emerged as one of the most promising and innovative drug delivery systems in modern pharmaceutical sciences due to their unique structure, biocompatibility, biodegradability, and ability to encapsulate both hydrophilic and lipophilic drugs. They offer several significant advantages, including improved drug solubility and bioavailability, enhanced therapeutic efficacy, controlled drug release, targeted drug delivery, and reduced toxicity to healthy tissues. Their applications in cancer therapy, antimicrobial treatment, vaccine development, gene delivery, and other therapeutic areas highlight their importance in advancing modern medicine. Furthermore, recent developments in PEGylated, ligand-targeted, and stimuli-responsive liposomes have expanded their potential for precise and efficient drug delivery. Despite certain limitations, such as formulation instability, drug leakage, high production costs, and manufacturing challenges,

continuous research and technological advancements are improving their safety, stability, and clinical performance. In conclusion, liposomes represent a versatile and valuable platform for pharmaceutical drug delivery, with considerable potential to enhance patient outcomes and support the development of safer, more effective, and targeted therapeutic formulations in the future.

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