Nano sizing liposomes by extrusion technique and its application.

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Abstract: Due to their outstanding biocompatibility and capacity to encapsulate both hydrophilic and hydrophobic pharmaceuticals, liposomes are attractive drug delivery vehicles. Extrusion has emerged as a potential technique among the several liposome preparation techniques for the effective and repeatable synthesis of liposomes with limited size dispersion. We give an overview of the extrusion process for making liposomes in this article, along with its guiding principles, benefits, and drawbacks. We go over the various kinds of extrusion devices and how they operate. We also point out the elements such lipid content, extrusion pressure, and temperature that affect the size and characteristics of extruded liposomes. Finally, we discuss the various ways extruded liposomes are used to deliver drugs for chemotherapy, gene therapy, and vaccinations. Overall, the extrusion technique is a flexible and effective method for producing liposomes that are uniform in size and characteristics, and it has a lot of potential for use in clinical settings in the future.

Index Term: Extrusion; Pro-liposomes; Liposomes; Particle size; Membrane filter.

INTRODUCTION.

LIPOSOMES:-

Liposomes are macroscopic, spherical vesicles comprised of phospholipids, the same kind of chemicals that make up cell membranes. Liposomes are made up of one or more layers of these phospholipids. Liposomes can be created to contain a variety of compounds, including medications, vaccinations, and genetic material. Its size can range from 50 nanometers to several microns.

Phospholipid bilayers, which have their hydrophilic ("water loving") heads facing outward and their hydrophobic ("water fearing") tails facing inward, make up the basic structure of a liposome. A closed sphere, or vesicle, made of this bilayer can contain aqueous liquids.

Because they can be utilised to encapsulate pharmaceuticals, keeping them from deterioration and enhancing their pharmacokinetic profile, liposomes are appealing for medication delivery. By adding ligands to the surface of liposomes that bind to receptors on the surface of target tissues or cells, they can also be made to target particular cells or tissues.

Many different types of medications, such as antibiotics, vaccinations, and anticancer medications, have been delivered by liposomes. They can be given by a number of different ways, including as intravenous injection, oral consumption, and topical use. In gene therapy, liposomes have also been investigated for their potential to carry nucleic acids like DNA or RNA into cells.

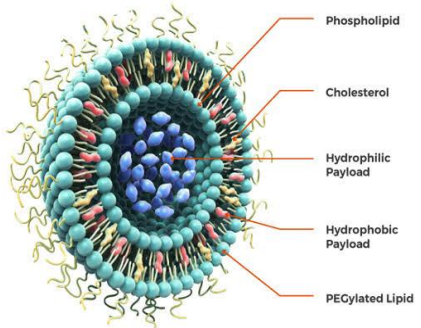

Figure 1:- Liposomes

Types of liposomes:-

1. ***Multilamellar liposomes (MLVS)*:** These are composed of multiple concentric lipid bilayers separated by aqueous compartments.
2. ***Large unilamellar vesicles (LUVs)*:** These are large liposomes composed of a single phospholipid bilayer enclosing an aqueous compartment.
3. ***Small unilamellar vesicles (SUVs):*** These are small liposomes composed of a single phospholipid bilayer enclosing an aqueous compartment.

4. ***Giant unilamellar vesicles (GUVs)*:** These are very large liposomes composed of a single phospholipid bilayer enclosing an aqueous compartment.
5**.** ***Cationic liposomes:*** These are liposomes with a positively charged surface, which allows them to interact with negatively charged molecules such as DNA or RNA.
6. ***PEGylated liposomes:*** These are liposomes coated with polyethylene glycol (PEG) chains, which prolong their circulation time in the body and decrease their uptake by the immune system.
7. ***pH-sensitive liposomes****:* These are liposomes that change their structure in response to changes in pH, allowing them to release their contents in acidic environments such as tumors .
8. ***Temperature-sensitive liposomes:*** These are liposomes that change their in response to changes in temperature, allowing them to release their comments at a specific temperature.

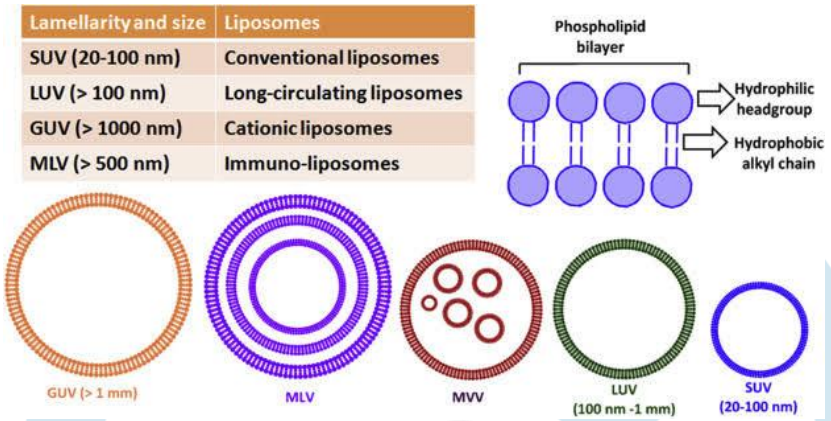

Figure 2:- Type and its Size of Liposomes

Nanosizing liposomes:-
A number of medications, nutrients, or other physiologically active chemicals can be included within liposomes, which are spherical, bilayered structures formed of phospholipid molecules. Because they may shield the encapsulated molecules from deterioration, increase their bioavailability, and target particular cells or tissues, they are frequently utilised as drug delivery systems.
Liposomes can be "nanosized" by shrinking them to the nanoscale, which is typically between 10 and 200 nanometers in size. Many techniques, such as sonication, extrusion, or homogenization, can be used to accomplish this.
Liposomes can become more effective drug delivery vehicles by becoming nanosized since it increases their stability, circulation time, and tissue penetration. This is owing to the fact that nanosized liposomes have a higher surface area to volume ratio, increasing their interactions with cells and tissues. Moreover, because they are smaller, nanosized liposomes can be absorbed by cells more readily.
Liposomes physicochemical characteristics can also be impacted by the nanosizing process. For instance, shrinking liposomes can alter their interactions with proteins and cells by raising their surface charge. The release profile of the molecules that are enclosed can also be impacted by nanosizing, as smaller liposomes may release their contents more quickly than bigger liposomes.
Overall, nanosizing liposomes is a powerful tool for improving the properties and functionality of liposomal drug delivery systems, and has the potential to enhance the efficacy and safety of a wide range of drugs and therapies.

Extrusion Techniques and its Characteristics:-
Extrusion is a frequently employed method for nanosizing liposomes. To create uniformly sized and shaped liposomes, the procedure involves filtering a solution of lipids and other ingredients via a small hole size filter. The liposome solution is subjected to shear strain during the extrusion process, which causes the liposomes to elongate before being pushed through the filter. Due to the filter's pore size, the liposomes become more consistent in size and shape as they pass through it.
For applications like medication delivery, where the size of the liposomes can alter their behaviour in the body, the extrusion technique can be utilised to manufacture liposomes in a predefined size range. The size of the manufactured liposomes can be accurately regulated by adjusting the pore size of the filter.
The extrusion process and the size of the generated liposomes can be influenced by a number of factors. They include the amount of extrusion passes, filter pore size, and lipid makeup of the liposome solution.
Extrusion Techniques:

1) ***Liposome Preparation***: There are numerous ways to make liposomes, including sonication, solvent evaporation, and reverse-phase evaporation. The technique used should result in liposomes that are the desired size and contain the intended medication.
2) ***Selection of Membrane Filters****:* To regulate the size of the liposomes, it is essential to manage the pore size of the membrane filters employed in extrusion. To make sure that liposomes flow through the filter while larger particles are kept, the pore size should be smaller than the liposome size.

3) ***Extrusion Process:*** The extrusion chamber is loaded with the liposome suspension, and pressure is then applied to push the suspension through the filter. To guarantee uniform liposome size, the suspension is passed through the filter numerous times.
4) ***Characterization:*** Techniques including dynamic light scattering, transmission electron microscopy, and atomic force microscopy are used to determine the size and form of the liposomes created by extrusion.

Advantages:-

1. Increased bioavailability: Increasing the surface area and decreasing the particle size of liposomes can increase their ability to absorb their contents by cells and tissues, hence enhancing their bioavailability.
2. Enhanced drug targeting: Smaller liposomes have a greater ability to cross biological barriers, target particular cells or tissues, and deliver drugs more effectively while posing a lower risk of harm to untargeted organs.
3. Improved stability: Comparatively speaking to larger liposomes, smaller ones can be more stable and have a longer shelf life.
4. Reduced side effects: By limiting drug exposure to tissues other than those intended for the drug's target, nanosized liposomes can lessen the possibility of side effects.

Disadvantages:-

1. Increased production cost: Due to the requirement for specific tools and procedures, nanosizing liposomes might raise production costs.
2. Potential toxicity: Due to their easier uptake by cells and potential for unexpected interactions with biological systems, smaller liposomes may be more hazardous.
3. Reduced drug loading capacity: Liposomes' ability to carry drugs may decline as they get smaller.
4. Decreased stability: Due to their smaller size and higher surface area, nanosized liposomes can have less stability, which increases the possibility of breakdown or aggregation.

EQUIPMENT USED IN EXTRACTION TECHNIQUES.

1) Materials:

The pro-liposome (Pro-lipo duo®) was purchased from France's Lucas Meyer. Pro-lipo duo comprised hydrophilic medium made of glycerol and ethanol that was suspended in 50% unsaturated soybean phosphatidylcholine.

2) Instruments:

The Waters Millipore® 510 HPLC pump, an Upchurch Scientific guard column, a stainless steel changeable frit (filter) with a pore size of 2 µm, and polyethersulfone membrane filters with various pore sizes (0.5, 0.2, and 0.1 µm) make up the extrusion apparatus (Sartorius AG, Goettingen, Germany). Figures 1 and 2 depict, respectively, how these devices are put together for the extrusion process and how the membrane filter is placed in the guard column.

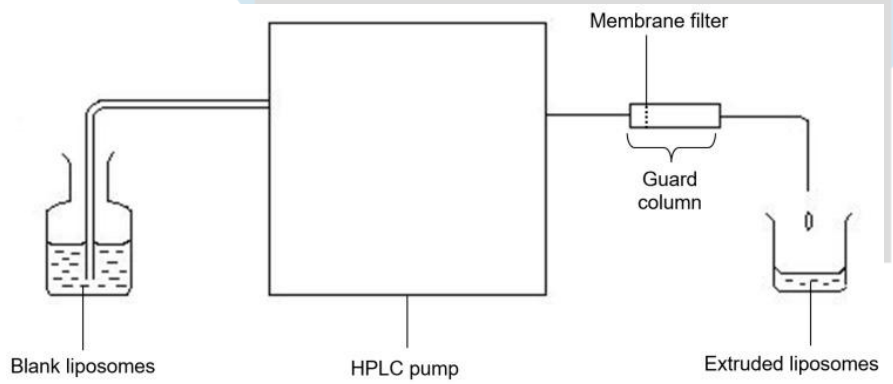

Figure 3. Assembly of instrument used for the extrusion process.

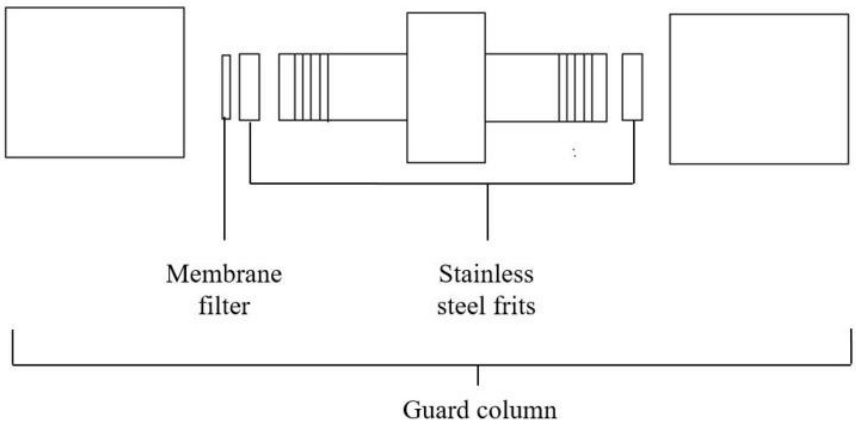

Figure 4. Placement of membrane filter in the guard column.

3) Preparation of Liposome Suspension

The unencapsulated blank liposome By hydrating the Pro-lipo duo® with clean water from a water purification system, the blank liposome (without an encapsulated medication) was created (Milli-Q Plus, Millipore, Bedford, MA, USA). The Pro-lipo duo® contained phosphatidylcholine from soybeans that was 50% unsaturated and suspended in hydrophilic medium made of ethanol and glycerol. To make sure that there was a very low concentration of pollutants in the suspension, filtered water was employed. The amount of purified water added\swas two parts to one part of the pro-liposome. Then, using a medium-speed stirrer, the mixture was 30 minutes at room temperature using a hot plate stirrer (model 502-P, PMC Industries, Inc., San Diego, CA, USA). Prior to usage, the resultant blank liposome solution was further diluted (relative to the pro-liposome) with five parts of purified water and swirled for an additional five minutes to create a homogeneous suspension. The final dispersion's lipid content was 6.25% w/v.

4) Extrusion Procedure and Influence of Process Parameters

Five times the membrane filter was used to push the liposomal suspension through. After each cycle, aliquots were collected to determine the size and dispersion of the liposomes, and samples that were eluted during the first 5 minutes of each cycle were discarded. Several process variables have been used to investigate their effects on the size and dispersion of the liposomes that were extruded. Below is a summary of them:

(a) Flow rate:

The liposome suspension was put through a 0.2 µm membrane filter while being pumped at 1, 5, and 9 mL/min of various flow rates.

(b) Membrane pore size :

The suspension of liposomes was run through a membrane filter with pores that ranged in size from 2, 0.5, 0.2, and 0.1 µm. The pump was running at 5 mL/m during the extrusion process.

(c) Temperature :

membrane filter was used to filter the liposome suspension at temperatures ranging from 25, 30, 35, 40, 45, and 50 °C. The liposomes were kept at the desired temperature using a water bath. A flow rate of 5 mL/min and membrane filters of 0.2 and 0.1 µm were used for the extrusion process.

The room temperature used for all extrusion procedures was approximately 25 ℃.

5) Comparison with Size Reducing Methods

Other methods for reducing the size of the liposome suspension included sonication for 30 minutes (Sonorex Super, Bandelin Electronic, Berlin, Germany), ultrasonication for 30 minutes (Digital PRO, Beijing, China), homogenization at 13,500 rpm for 30 minutes (Ultra-Turrax® T25, Janke & Kunkel GMBH & Co. KG, Staufen im Breisgau, Germany), and FTS for 10 cycles. The liposome suspensions were subjected to a FTS cycle in which they were first rapidly frozen for 3 min in an acetone-dry ice (solid carbon dioxide) bath, quickly thawed for 3 min in a water bath (40 °C), and then sonicated (Sonorex Super, Bandelin Electronic, Berlin, Germany) for 5 min at room temperature. During the course of 24 weeks, the sizes, size distributions, and physical stability of all the items produced by these various processes were compared. They were created using the extrusion technique explained in Part 2.4, which employed a 0.1 µm membrane filter. They were compared immediately upon preparation as well as after 1, 2, 3, 4, 8, and 12 weeks of storage at 4 °C.

6) Particle Size Analysis

Photon correlation spectroscopy was used to evaluate the size and distribution of the liposomes (Zetasizer 1000HS, Malvern Instruments Ltd., Malvern, Worcestershire, UK). In a low volume disposable size cuvette, 10 µL of liposome suspensions were mixed with 500 µL of purified water before to measurement. The polydispersity index (PDI) and ZAve measurements, respectively, were used to determine the particle size and size distribution. Three measurements were made on two different samples for each preparation. To gauge the repeatability of the measurements, the standard error mean (SEM) of the average was used.

7) Statiscal Analysis

If appropriate, the average particle size was compared using a student t-test or a one-way ANOVA. A p value of < 0.05 was considered to be significant.

DISCUSSION.

Due to its ease of use, reproducibility, and scalability, the extrusion approach for nanosizing liposomes has gained popularity. The method involves pushing the liposome solution through a series of filters with progressively smaller pore diameters, producing liposomes that are more consistent in size and shape.

In comparison to bigger liposomes, nanosized liposomes have a higher surface area to volume ratio, better cellular absorption, and increased bioavailability. As a result, they are very appealing for drug delivery applications, particularly for medications that are weakly soluble, where nanosizing can greatly increase drug solubility and bioavailability.

Furthermore, to achieve precise targeting and improve their durability in vivo, nanosized liposomes can be modified with various surface coatings, such as polyethylene glycol (PEG) or antibodies. This makes it possible to create highly targeted drug delivery systems that efficiently administer medications to particular tissues or cells.
Nanosized liposomes have potential outside of medication delivery applications in the cosmetics industry. They can be utilised to deliver cosmetic actives to particular skin layers and enhance their absorption and effectiveness.

CONCLUSIONS.

Extrusion-based liposome nanosizing has demonstrated tremendous promise in a number of fields, including medicine, gene therapy, and the cosmetics industry. In order to create smaller, more homogeneous liposomes, the liposome solution is forced through a succession of filters with progressively smaller pore diameters. This technique has shown to be very scalable and reproducible.
The higher surface area to volume ratio of nanosized liposomes, which enables improved drug loading and bioavailability, is one of its main advantages. Additionally, because of their small size, they are easier for cells to absorb and can be delivered precisely to the right regions, improving the effectiveness of treatments.
Nanosized liposomes have found usage in the cosmetic sector in addition to medicine delivery since they can be utilised to deliver active ingredients for cosmetics to particular layers of the skin and improve their absorption and effectiveness.
Overall, the nanosizing liposome extrusion technology presents a viable route for the creation of novel and creative medication delivery systems and cosmetics.

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