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Liposomes: A Promising Pulmonary Drug Delivery Carrier

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Abstract:

The liposomes,sphere-shaped aqueous vesicles consisting of one or more concentric lipid bilayers.They are mainly made up of phospholipids. Liposomes made with phospholipids identical to natural lung surfactant (Dipalmitoylphosphadylcholine)and lipids offers biocompatibility and versality in the field of pulmonary medicine, treating disorders like lung cancer,cystic fibrosis, lung infections etc.Improved pharmacokinetics with lower toxicity,higher therapeutic efficiency,increased distribution of poorly soluble medicines,and taste masking,sustained drug release, targeted drug delivery are all advantages of liposomal pulmonary drug delivery. Because of the lung's large absorptive surface area,high permeability (above 70-140m^2),low protective activity and enough blood supply.pulmonary route is today considered as one of the most successful drug delivery systems. Liposomal pulmonary carrier has also been used for systemic distribution of drugs like insulin,human growth hormone, and calcitonin. There are different types of inhalation devices like pressurized metered dose inhale, soft mist inhaler,dry power inhaler,Nebulizer etc. are used.This review provides an overview of liposomal pulmonary drug delivery system.

Keywords: Liposome, Phospholipids, Sustained Drug Release, Targeted Drug Delivery Pulmonary Drug Delivery Carrier.

Introduction:

According to the WHO, lower respiratory infection is the most lethal communicable disease in the world. It is the world's 4th leading cause of death. In 2019,2.6 million people died and 460000 fewer than in 2020 [1].The only currently recognized treatment for this condition is high-dose antibiotics that must be given often. Instead, pulmonary drug delivery has been regarded one of the most efficient ways of drug delivery to the targeted locations because it gives rapid beginning of action, direct deposition of pharmaceuticals into the

lungs and superior therapeutic benefit at low doses,as well as being self-administrable [2].Hence,the main aim of pulmonary drug delivery is to inhale drug formulations through the mouth and deposit the inhaled pharmacological agent in the lower airways [2].

Liposomes are enclosed potential pulmonary drug carriers that are nano-scale in size because of their aqueous vesicles have the ability to contain hydrophilic medications and their lipid bilayer localized or concentrate and lipophilic medications,liposome resemble the structure of animal cell semipermeable membrane [3].liposomal pulmonary delivery of medication has advantages over conventional systemic therapy in the treatment of lung disorders such as asthma, lung cancer and COPD since it is a non-invasive route of administration and reduces pulmonary toxicity. Despite they can act as a pulmonary sustained release reservoir facilitate intracellular delivery of drugs. Specifically, to alveolar macrophages, show good compatibility with lung surfactant components and effective for delivering very tiny amount of medications to target areas.liposomes are kept at the targeted receptor for a long time, reducing bioavailability all through circulatory system [4].A complex mixture of phospholipids (90% dipalmitophosphatidylcholine) andprotein (10%) makes up natural lung surfactant. This surfactant primary purpose is to lower surface tension at the lung's alveolar air- liquid contact, preventing alveolar collapse. Phospholipids have also been found to be improve the absorption of active agents in the lungs [5].

Systemic distribution of medications including insulin, human growth hormone and calcitonin has also been done by liposome vesicular pulmonary delivery. The pulmonary route is now regarded one of the most successful methods for drug delivery due to the lungs huge absorptive surface area,around 70-140m^2 high permeability, little protective activity and adequate blood supply [6].

- **Structural component of liposome**:

The main component of liposome: Phospholipid, Cholesterol

- **Phospholipids:**

Phospholipids have great incompatibility and are amphillicin nature,thus when they are introduced into an aqueous environment, they get self-assemble to form vesicle based on their quality and conditions.Phospholipids with hydrophilic head and a hydrophobic tail are used to make liposomes. The hydrophilic part is mostly phosphoric acid coupled to a water-soluble molecule, whereas the hydrophobic part is made up of two fatty acid chains each with 10-24 carbon atoms and 0-6 double bonds.They form a lamella is a flat plate like structure that appears during the formation of liposomes. Self-assembly, emulsifying and wetting property are due to amphipathic nature.liposome are made up from several types of phospholipids, including natural and synthetic phospholipids. When compared to unsaturated phospholipids, saturated phospholipids have better stability and less impermeability.phospholipids act by reducing the high surface tension fat the air -water interface within alveoli thereby reducing the pressure needed to expand the lungs.E.g., Phosphatidylcholine Phosphatidyl serine, Phosphatidyl inositol, Phosphatidyl ethanolamine [7,8].

- **Cholesterol**

Cholesterol strengthens the lipid membrane, although its concentration in the liposome structure cannot exceed 50%.Incorporated in the ratio of 1:1 or even 1:2. It reduces content leakage from liposomes and tightens the fluid bilayer. Enhances the bilayer stability and reduces the water permeability[9]. E.g., Cholesterol Hemi Succinate, Lysine based Cholesterol, PEGylatedCholesterol Neutral.

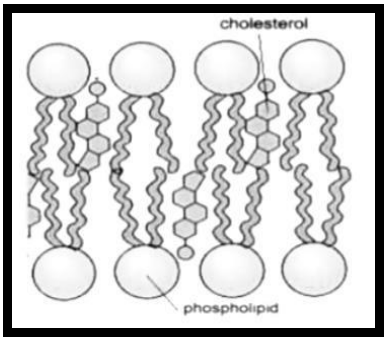

Fig.No1: Structure of Phospholipids and Cholesterol

- ## Classification of liposomes:

I) Based on structural parameters [10].

Sr.no.	Type	Diameter size	No.of bi-layers
I)	Unilamellar vesicle (UV)	All size range	One
a.	Small unilamellar vesicles (SUV)	20-100nm	One
b.	Medium unilamellar vesicles (MUV)	>100nm	One
c.	Large unilamellar vesicles (LUV)	>1000nm	One
d.	Giant unilamellar vesicles (GUV)	>1um	One
II)	Oligolamellar vesicles (OLV)	0.1-1 um	5
III)	Multilamellar vesicles (MLV)	>5 .0 um	5-25
IV)	Multivesicular vesicles (MV	>1.0 um	Multicompartmental structure

Table No 1: Liposomes, based on Structural Parameter

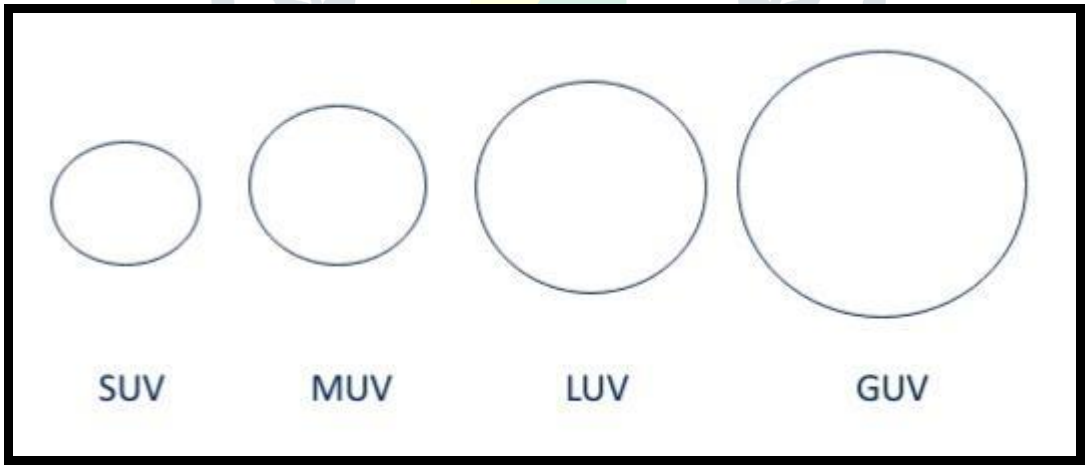

Fig. No. 2: Types of Liposomes

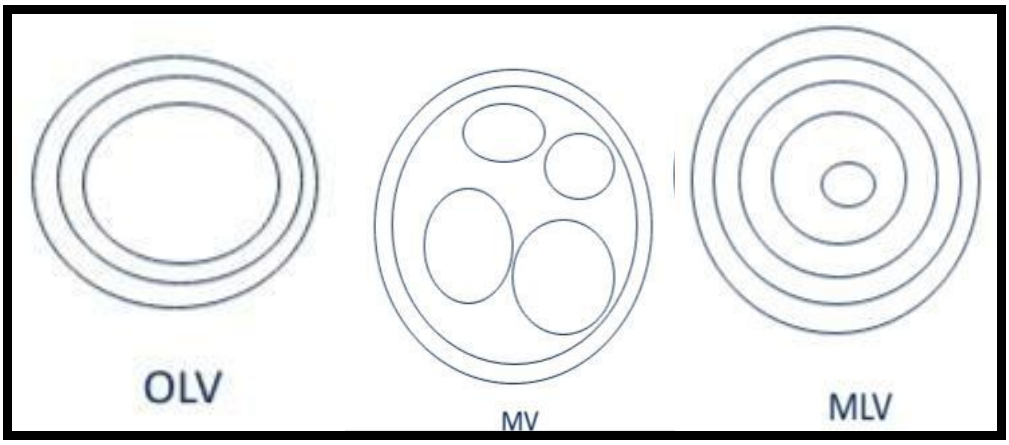

Fig. No. 3: Types of Liposomes

II) Based on method preparations [10]

Sr.no.	Types	Methods of preparation
1.	SUV-REV, OLV-REV	Single or Oligolamellar vesicles made by reverse phase evaporation method
2.	MLVREV	Multilamellar vesicles prepared by reverse phase evaporation method
3.	SPLV	Stable plurilaminar vesicles
4.	FATMLV	Multilamellar vesicles made by freeze -thawing method
5.	VET	Vesicles prepared by membrane extrusion method
6.	DRV	Dehydration- rehydration method
7.	BSV	Bubblosomes

Table No 2: Liposomes, based on method of Preparation

III) Based on composition [10]

Sr.no.	Types	Composition
1.	Conventional Liposomes (CL)	Neutral or negatively charged phospholipids and cholesterol
2.	Fusogenic Liposomes (RSVE)	Reconstituted sendai virus envelops
3.	pH Sensitive Liposomes	Phospholipids such as PER or DOPE with either CHEMS or OA
4.	Cationic Liposomes	Cationic lipid with DOPE
5.	Long Circulating Liposomes	Neutral high temp,Cholesterol and 5-10%PEG, DSP
6.	Immuno Liposomes	CL or LCL with attached monoclonal antibody or recognition sequences

Table No 3: Liposomes, based on composition

IV) Based on speciality liposome [10]

- Bipolar fatty acid
- Antibody directed
- Methyl /Methylene x-linked

- Lipoproteins coated
- Carbohydrate coated
- Multiple encapsulated

V) Based on conventional liposomes

- Natural lecithin mixture
- Synthetic identical,chain phospholipids
- Liposome with Glycolipids

Types of drug loading:

I. Active loading:

The addition of pharmaceutical into preformed empty liposomes using diverse approaches like as pH gradient,electric gradient and potential gradient differences across the lipid membrane are all examples of active active loading procedures. Encapsulation efficiency and capacity are good and encapsulated drug leakage is low, but it is not ideal for encapsulating hydrophobic drug [11].

II. Passive loading:

The addition of medications during the creation of vesicles is referred to as passive loading. liposome can be made in a variety of ways, each of which alters liposome feature including size, familiarity and encapsulation efficiency as a result, the medications are passively loaded into the liposomes, include the three options listed below [12].

- **Methods of preparation:**

I) Mechanical dispersion methods:

- Lipid film hydration method
- Sonication method
- French pressure cell extraction method
- Membrane extraction method
- Freeze -thawed liposomes

II) Solvent dispersion methods:

- Ethanol injection method
- Ether injection method
- Reverse phase evaporation method

III) Detergent removal methods:

- Dilution
- Gel chromatography
- Dialysis
- Bio bead

I)Mechanical dispersion methods:

- **Sonication method:**

Sound energy is converted into physical vibrations during the sonication process which can be utilized to break down MLVs liposomes in suspension into SUVs using either a bath sonicator or a probe sonicator. In the ultrasonication procedure, sound waves with a frequency of >20kHz are employed. Temperature rises during the process, causing liposome destruction, which is prevented by an ice bath and N_2 gas is fed through a probe sonicator into the liposomal suspension tube. liposome in naso-pharynx are a critical component in pulmonary transport, probe sonicator is not commonly used because Titanium metal particles discharged from probe tip contaminate the liposome dispersal. Huge molecules eradication in presence of MLVs along with SUVs [13].

- **French pressure cell extrusion method:**

To disseminate the lipids the french pressure cell approach uses an extrusion technique that forces multilamellar vesicles to sonication because the resultant liposomes are bigger and it retains encapsulated solutes for longer than SUVs. The production process,however necessitates extremely high temperatures, therefore there is a limit. Liposome prepared by this method are less likely suffer from structural defects and instabilities as observed in sonicated vesicles and in the range of 20000-40000psi pressure is applied [13].

- **Membrane extruction method:**

It's used to process both LUVs and MLVs. Membrane filter extruction liposomes are liposomes made using the membrane extruction process. The extruction method has an advantage over the homogenization and sonication methods in that it produces liposomes with a variety of membrane hole sizes. Because that it relatively simple and quick to create, as well as being free solvent and surfactant contamination, this method is commonly utilized in liposome sizing. It just takes 3-10 cycles to get a a homogeneous liposomal preparation [14].

- **Freeze -thawing method:**

The procedure of freezing and thawing is useful for increasing the trapped volume of MLVs preparation. MLVs are made up to concentric bi-layers separated by tiny aqueous gaps and have a low encapsulation capacity. Decrease familiarity employing a number of procedures such as freeze thaw, dehydration-rehydration, and reverse phase technique can result in greater trapped volumes encapsulation efficiency of 20-30%. The freeze thaw method limited by concentration of phospholipids and ionic strength of the medium [15].

II) **Solvent dispersion method:**

- **Reverse phase evaporation method:**

The reverse phase evaporation method of liposome synthesis has several advantages over other methods, including a 65% encapsulation rate and the ability to encapsulate valuable water-solublecomponents. Such as protein, nucleic acids and other biochemical reagents. The organic phase containing the dissolved lipid and the aqueous phase are mixed together in the first step of the process using sonication to generate a water in oil emulsion. Diethyl ether, isopropyl ether or a combination of isopropyl ether and chloroform are the most common organic solvents employed. Inverted micelles occur within the emulsion as a result of this. The organic solvent is extracted using rotary evaporation at reduced pressure to remove any remaining solvent, at this stage, the surplus phospholipids form the second half of the bilayer, resulting in the formation of liposomes [16].

- **Ethanol injection method:**

This process is suitable for incorporating hydrophilic (e.g. Cytarabine; Ara-c) and lipophilic (e.g. Beclomethasone dipropionate;BDP) drugs into SUVs in order to administered via the pulmonary route .Encapsulation of hydrophilic drugs results in hydration of lipids hydrophilic drugs mixture and encapsulation of hydrophobic drugs through solubilizing of drugs in the organic solvent and phospholid .In this process an ethanolic solution of lipid is fast injected into an aqueous medium through a needle, dispersing the phospholipids throughout the medium and help the vesicle formation at the temperature 55°c -65°c,with spontaneously stirring by using a magnetic stirrer.Ethanol was evaporated by rotary evaporator. 30-110 nm size ranges liposomes are formed. In this process complete removal of ethanol is difficult because azeotropic mixture is formed with water [17].

- **Ether injection method:**

The lipids are dissolved in diethyl ether or a combination of ether and methanol in the ether injection method .This lipid solution is mixed with an aqueous solution that contains the medicine to be encapsulated .The addition is carried out a very slow rate ,roughly 0.25ml per min ,using a mechanical motor .The addition must

be carried out under reduced pressure and at a temperature above the other's boiling point of 55°c-65°c.After thatthe ether is removed under vacuum resulting in the creation of liposomes (70-200nm) [18].

III) Detergent removal method:

In this method detergent (like cholate, alkyl glycosides, Triton-100) are used to solubilize the phospholipid in aqueous phase at critical micelles concentrations. The structure formed are called as micelles. The detergent can be removed by dialysis, dilution, gel chromatography, bio bead method etc.But retention of traces of detergent within the liposome [19].

- ## Devices used in liposomal pulmonary drug delivery:

There are four different type of inhalation devices like pressurized metered dose inhaler, Dry powder inhaler, medical nebulizer, soft mist inhaler used pulmonary drug delivery system.

1. ## Dry powder inhaler device:

Dry powder inhalers are a dry, free flowing powdered formulation of liposomal medication that can be made by spray drying micronizing a mixture of the drug and inert carriers such as mannitol, sucrose, lactose, glucose sorbitol, trehalose. Dry powder inhalers are more stable than aqueous formulation because they don't need to be kept in a cold storage facility and reconstitution of powders and shows promising result [20].

1. ## Pressurized metered dose inhaler:

Pressurized metered dose inhalers are widely used for the administration of anti-asthmatic drugs . In this device drug solution or suspension are incorporated to liquified propellant (hydrofluorocarbons) instead of chlorofluorocarbons [21]

1. ## Soft mist inhaler:

Soft mist inhalers are metered dose inhalation devices with no propellant that produce slow-moving water aerosols for deep lung deposition. SMIs that can only deliver a little volume of aerosol are more suited to treating disorders that require low dosages of the therapeutic ingredient [22].

1. ## Medical Nebulizer:

In comparison to other inhalation devices, a nebulizer can produce high amounts of respirable aerosol without the requirement for drying operations or the use of propellant. The most popular inhalation device for the delivery of liposomes is a nebulizer. Air jet nebulizers, ultrasonic nebulizers, and vibrating mesh nebulizers are the three types of nebulizers [23].

- ## Liposomal pulmonary drug delivery applications:

  - **Asthma treatment:**

Budesonide is an inhaled corticosteroid that is used to prevent asthma attack. However, frequent dosing and adverse effects remain a serious concern when using budesonide. Longer retention period and lower biodistribution throughout the systemic circulation are possible with a liposomal sustained release formulation of BDS in the lungs. It will also be the best medicine for preventing bronchospasm for 6-8 hours while patients sleep, leading in better asthma treatment. Budesonide liposomes were created using the lipid film hydration method,suitablecryoprotectants such as lactose, mannitol, maltodextrin, and trealise must be added to avoid leakage of the encapsulated medication from liposomes following the freeze drying /rehydration cycle, preventing fusion,aggregation and leakage of encapsulated compounds [24].

  - ## Lung'scancer:

Many antineoplastic medicines on the market like paclitaxel, have drug delivery limitations due to poor solubility and low bioavailability. for e.g., Paclitaxel is a lipophilic medication that has broad anticancer effect when encapsulated in liposomes and combined with cyclosporine A via pulmonary administration

because of nano size,controlled release rate as well as decreasing normal tissue damage and systemic side effects [25].

- **Diabetes treatment:**

Aerosolized insulin -loaded liposomes were injected into the lungs of rats and the effect on insulin delivery was measured by changes in plasma glucose levels.Liposome containing FITC(Fluoressein-5-isothiocyanate)dextranand dipalmitoylphosphatidylcholines increased pulmonary insulin delivery in rats by expanding the epithelial cell gap in the pulmonary mucosa without causing mucosal cell injury [26].

- **Microbial infection:**

Ciprofloxacin-loaded liposomes were created using a gradient of ammonium sulphate and ciprofloxacin was incorporated into monosylated liposomes that were utilized to treat respiratory intracellular microbial infections. In vitro release was sustained in the optimised liposome, which has a high encapsulation efficiency and average particle size. The pulmonary route of administration improves drug targeting efficiency [27].

- **Coronavirus infection:**

Remdesivir is among the primary medicine to be approved by the government agency FDA for covid-19 therapy in 2019.It is now given as an injection the bloodstream. This means that the medicine circulates throughout the body,but only a little portion of it reaches the disease location,which is the lungs. The lungs are most affected by coronavirus. As a result, regular dosage is required, which necessitates the use of specialized people and many hospital trips,as well as the risk of adverse effects. Because of liposomes passes unique characteristics such as 99.79% encapsulation efficiency, nano size, avoid first pass metabolism and negligible cytotoxicity due to facilities direct delivery to the main site of infection via pulmonary delivery and in-vitro study shows that the aerosolized nanoliposome carriers of remdesivir are effective for covid 19 treatment.

Lung infection in covid 19 can change total pulmonary surfactant composition, diminishing the availability of phospholipids, lowering pulmonary function and causing alveoli collapse without surfactant during normal breathing, resulting in decreased lung compliance. Lactoferrin, is a globular macromolecule actas antiviral and antibacterialactivity,Lactoferrin liposome is 100 nm in size, but when it is via the airways, it shrinks to 50 nm in size. It helps to reaches deep inside the lungs and releases lactoferrin in a controlled manner at the targeted spot. The liposome may interact with the target tissues endogenous surfactant and phospholipid may have a powerful anti-inflammatory impact [28].

- **Cystic fibrosis treatment (gene therapy):**

Gene delivery can be carried out using cationic liposomes shows better result than neutral or anionic liposomes. A IIb clinical trial research published in 2015 found that the DNA packaged into liposomes could administer a gene replacement therapy for cystic fibrosis treatment is safe with little side effects [29].

- **Conclusion:**

Administration of antibiotic, anti-asthmatic,antiviral,cytotoxicdrug,protein and peptide,gene transfer through systemic route is replaced by liposomal pulmonary drug carrier. because of vesicles may be modified, they can be used to treat a variety of diseases. liposomes have a high solubilization capacity and prolonged persistence time due to biodegradability without causing any adverse effects make them useful tool of delivery of drug through pulmonary route.

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