REVIEW ARTICLE

An Extensive Review on Novel Liposomes : Classification, Methodology, Characterization, Current Formulations

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ABSTRACT

Liposomes are vesicles consisting of a phospholipid, hydrophobic drug, hydrophobic tail, hydrophilic tail, cholesterol, targeting agents, positive and negatively charged lipids, and a drug encapsulated in the center of the phospholipid group having a spherical shape. The phospholipid consists of an equal number of aqueous membranes, making the liposomes an important nanocarrier for the drug delivery to the targeted site. Various liposome-based products have recently been approved and are in clinical trials. This review will discuss the structure, classification, types, and method of liposome preparation and various marketed liposomal products.

Keywords: liposomes, phospholipid, cholesterol, vesicles, nanocarriers, DSPG, EPC, Sphingomyelin, zeta potential,

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INTRODUCTION

Once upon a time in 1961Alec Bangham found liposomes and from then various researches have been made on liposomes.[1] Liposomes are made up of phosphatidylcholine (natural or artificial sources) and cholesterol. The cholesterol being a sterol, is used to maintain the stability of liposomes (Figure 1).[2] Liposomes are spherical vesicles in structure ranging in size from 50 to 500 nm in diameter particle size. The liposomes differ in size form 0.025 to 2.5 µm.

Structure of liposomes

In today's industries, liposomes are used in all aspects are: gene therapy, targeted delivery (as an antitumor, anticancer drug), sustained release, cosmetic products (such as face serum, liposomal creams), enhanced penetration, agriculture, etc.

Timeline representation of liposomes

The ideal physiological pH for liposomes is considered to be 7.4 and if the pH is even decreased till 6.5 then it starts to degrade and this has been observed in the treatment of targeted antitumors.[3] The major factor taken into consideration for any type of dosing or PK/PD system in that the medication should not have adverse toxic reactions.[4] Thus, the main target of the selected nanocarriers is to mask the pharmacokinetic and biodistribution of a drug by reducing it.[5,6] The various sorts if synthetic and natural materials are used to create the nanocarriers such as cholesterol, phospholipids, ceramic, etc. which create nanoparticles like liposomes.[7-9] In spite of the major development in the fields of the nanocarriers, there are major issues of drug degradation from the nanocarriers due to changes in temperature or due to the pH.[10] Although, in the form of nanocarriers, liposomes have proved to give good out-turn.

Therefore, liposomes have been extensively used as parenteral, oral, transdermal, ophthalmic, nasal routes of drug administration as they are used to DDS as small nanocarriers such as protein, nucleic and imaging agents[11-15] and also in the fields of cosmetics and cosmeceuticals.

The nanoemulsion-based liposomes are excessively used preparations in nanomedicines as they provide ease in biocompatibility, stability and drug loading capacity.[16-18] Normal tissues have tight endothelial junctions, while abnormal tissues have highly porous capillaries.[19] Liposomes easily permeate through the neo-vasculature and are detained at abnormal tissues.

A liposomal modification was done for the vascular endothelial growth factors, folic acid, CD44, CD13 and epidermal growth factors.[20-27] In the treatment of pulmonary arterial hypertension (PAH), nitric oxide can also be encapsulated in liposomes.

Drug encapsulation of liposomes reduces hepatic drug clearance and increases the bioavailability of the drug in systemic circulation.

The various marketed liposome-based products are DaunoXome for ovarian cancer and AIDS and then

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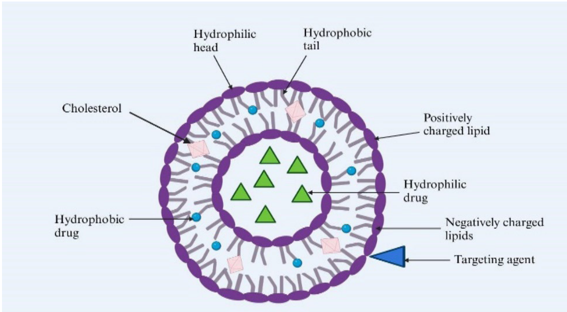

Figure 1: Structure of liposome

daunorubicin to treat HIV-associated Kaposi sarcoma.

Classification of Liposomes (Figures 2 and 3)[28-31]

- *Unilamellar vesicles*

The unilamellar vesicles have an onion shape, mono phospholipid bilayer, present between 2 to 5 concentric lipid bilayers

- *Large unilamellar vesicles*

lUVs size range of >100 nm and a lamellarity of 1

- *Small unilamellar vesicles*

SUVs range in size from 30 to 100 nm and a concentric lipid bilayer is 1.[32]

- *Giant unilamellar vesicles*

GUVs range in size of >1000 nm,[31] and lamellarity is 1.

- *Multilamellar vesicles*

MLVs range in size from >500 nm and lamellarity >5

- *Oligolamellar vesicles (Figure 4)*

OLVs have a size range of 100 to 1000 µm and a concentric lipid bilayer of 2 to 5.

- *Multivesicular vesicles*

It has an eccentric hydrous chamber gamboled by a single lipoidal bilayer film and have a hexagon type structure. And a size range greater than >1 µm.[30]
Various shapes of liposomes (Table 1).

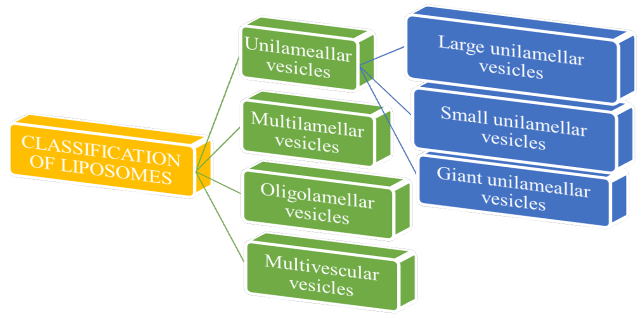

Figure 2: Classification of liposomes[28,29]

Composition of Liposomes

Phospholipids

The diacyl chain of phospholipids are used for self-assessing the drug in aqueous layer of liposomes.[38] The phospholipids in the liposome contain an aquaholic head and hydrophobe tail, which forms an amphiphilic structure.[39] The hydrophilic groups are zwitterions and hydrophobic groups are extended in acyl chain length they are symmetrical and show saturation.[40] The dipalmitoyl phosphatidylcholine is a saturated phospholipid that gives a strong, rigid and impermeable bilayer structure.[41] And the phosphatidylcholine, made from egg or soybean, is an unsaturated phospholipid they have low stable properties and are highly permeable.[41] The liposomes, which are made of phospholipids have multifunctional stimulus-sensitive, pH-sensitive, thermosensitive and targeted liposomes.[42] The various types of lipids used in liposomal preparations are:

Inorganic lipids

By modifying saturated organic phospholipids' polar and immiscible head there is a formation of inorganic phospholipids.[43] Most of the saturated synthetic phospholipids are made from palmitic fatty acids or stearic acids.[44]

Steroids

Steroids are hydrophobic lipids. Cholesterol is a steroid used for increasing the rigidity, stability and structure of liposomes.

Table 1: A brief description of the marketed products containing liposomes discussing about their structure, size composition and uses[33-37]

Product	*Structure*	*Size*	*Composition*	*Uses*
Doxil/Caeylix	SUV	100 nm	HSPC, Chol, MPEG-DSPE	Doxorubicin liposomal injection is used for ovarian cancer and Kaposi sarcoms (AIDS patience)
AmBisome	SUV	50–100 nm [33]	HSPC, DSPG, Chol	To treat variety of fungal infections
DaunoXome	SUV	40–80 nm [34]	DSPC, Chol	1st line cytotoxic therapy for advanced HIV
Arikaye	LUV	200–300 nm [35]	DPPC, Chol	Amikacin liposome inhalational suspension
Vyxeos	Bilamellar	110 nm	DSPC, DSPG, Chol	Daunrubicin is cytrabine liposomal injection
Myocet	MLV [34]	80–90 nm [36]	EPC, Chol	Liposomal doxorubicin
Mepact	MLV [37]	2.0–3.5 µm	DOPC, DPPG, Chol, triolein	Liposomal mifamurtide powder for dispersed infusion
DepoDur	MVL	17–23 µm	DOP, DPPG, Chol, triolein, tricaprylin	Morphine sulfate extended-release liposomal injection

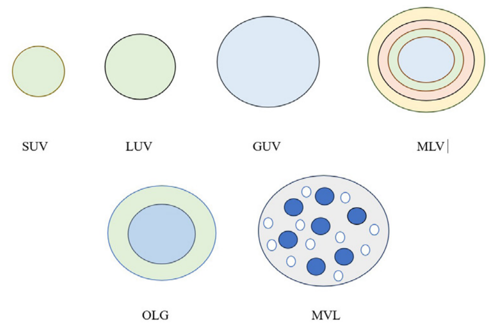

Figure 3: Structures of various shapes of liposomes

Cholesterol decreases the liposomal membrane fluidity, increases the zeta potential and decreases the DPPC phase transition T_m.[45] Cholesterol avoids the hydrolysis of QS21 at a specified ratio (2:1).[46] Cholesterol reduces the toxicity of AmBisome.[47] The effect of bilayer properties of cholesterol is concentration-dependent. Other than cholesterol, ergosterol, lanosterol, sterol was also found to enhance the membrane rigidity and stability.[48,4 9] During the time of liposome manufacturing the molecules of cholesterol arrange themselves according to the hydroxyl group, orienting towards the aqueous core region of the bilayer.[50]

Natural phospholipids

The natural phospholipids are mainly obtained from egg, soybean, etc.[51] but natural lipids are less stable as compared to synthetic phospholipids.[52,53]

Phospholipids can be classified as phosphatidylethanolamine, phosphatidylcholine, phosphatidic acid, phosphatidylinositol. These are composed of fatty acids such as hexadecenoic acid, heptadecanoic acid, cisoctadecanoic acid, egg yolk, etc.[54] The eggs contain phosphatidylcholine and it has 40% 1-palmitoyl2-oleoylphosphatidylcholine. The soyabean consist of 95% hexadecenoic acid, octadecanoic acid, cis-octadecanoid acid, linolic acid.[55,56]

Surfactants

Mostly used surfactants for the production of liposomes are: sodium chlorate, sorbitan monostearate, sorbitan oleate, polyoxyethylene sorbitan monostearate and polysorbate 80.[57,58] The use of surface active agents in the liposomes is done to enhance and stabilize the sustained release from the carrier by decreasing the surface tension between the insoluble phases.[59] These are single-chain amphiphiles which increases the nano-vessel deformability.[60] The surfactants based nano-vessels are ultra-deformable liposomes (transfersomes) are used in TDDS and thus they are good for topical administration of antihypertensive drugs.[61-68]

Glycerol-phospholipids

It consists of glycerol and it connects the hydrophobic fatty acid chains and hydrophilic polar heads.[69] It plays a significant role in the PK and PD properties of the liposomes *in-vivo*.[70] It affects the length, symmetry, intermol. Interaction, intra-mol. interaction, branching, and the unsaturation degree of chains which decides the thickness and the fluidity of the bilayers, phase T_m and the drug release profile.[71,72] Therefore, longer hydrocarbon chains and leads tight membrane packaging and increase the drug-persistence.[73,74]

The DSPG is used in the preparation of amBisome and vyxeos while the DSPG used in AmBisome has a negative charge but it can affect with the NH_3^+ group containing amphotericin B for the formation of a stabilized electrovalent bond complex and the DSPG is taken into consideration for vyxeos(cytarabine/ daunorubicin) reduces the liposome assemblage and gives a powerful abhorrent force.[75,76]

DSPC used in DaunoXome, onivyde and cytarabine/ daunorubicin liposomes have a alkyne charge also are inorganic lipids formed by stable amount of fatty-acid composition.

EPC is used in the Myocet and Visudyne, which is obtained from egg yolk and thus, it is of natural lipids. As the Phospholipids obtained from the natural are less stable and have a low production cost thus NPLs are not used mostly. Thus, EPC is also not taken into consideration.[77] However, myocet-containing EPCs have a release rate of drug after 24 hours and are not stable in the blood.[78] While, Visudyne containing EPC and DPMC are not stable in the presence of serum.

Sphingomyelin are similar to GP in structure, though in place of glycerol, there is a sphingosine. SM is utilized in Marqibo, which forms a bilayer membrane that reduces triglyceride catalysis in acetic habitat and prompts stabilization of liposomes.[79]

While liposomes containing both the SM and Cholesterol showed significant PK properties with increased circulation time and good targeted drug delivery.[80]

Nature of encapsulated drugs

Amphotericin B and imiquimod are less impermeable in water while indole has good water permeability. For designing a selection of good drug dosage form is necessary so that it can easily get loaded in the drug to retain inside the drug and for site-specific drug delivery.[81,82]

Membrane composition

Liposomes can trap aquaholic and aquaphobic medications.[83,84] Thus, Vincristine containing liposomes was manufactured by the replacement of egg N-acyl-4sphingenyl-1-O-phosphorylcholine with dihydrosphingomyelin in a ratio of 55:45 mol/mol, leading a significant growth in drug enclose and controlled release. While the modulation of molecular complexes leading controlled release and prevention of dose leakage from the vesicles.[83-85] By controlling the cholesteric level in the liposomal development of maximum level of encapsulation efficiency of sustained drug release.

Types of Liposomes

Conventional liposomes

When we increase the cholesterol concentration in a liposome, they have shown good rigidity and membrane fluidity of drugs and thus, they have also been used in anti-tumour drugs.[86,87]

The membrane rigidity was decreased in the HSPC-composed liposomes when there was an addition of liposomes.[88] Conventional liposomes have shown low blood circulation clearance from the mononuclear phagocytic system as they get rapidly accumulate in the spleen and liver, causing slow elimination rate from the body.[89] In conventional liposomes, there is low in-vitro stability.[90]

Charged liposomes

The charged liposomes are cationic and anionic liposomes they have good stability during storage and they are made of DOTAP and oleic acid.[91] The cationic liposomes are used for gene therapy and macromolecules such as DNA, RNA, and oligonucleotides as they have a negative charge and thus, they can encapsulate nucleic acids by electrostatic attraction and they avoid passive diffusion into the cells.[92] The cationic liposomes are also used in targeted drug delivery in tumor present in the angiotensin endothelial cells.[93] Cationic liposomes carry excessive positive charge on their surface, leading an electrostatic interaction with non-ionic species in the RBC which increases the liposomal aggregation and reduces the targeted site of action.[94,95] Thus, the surface of non-ionic liposomes is coated with polyethylene glycol, which keeps them safe from circulating protein.[96,97]

The anionic are not used in antitumor targeting as they are less stable in blood circulation but they are used in TDDS as they have good permeability from the skin's stratum corneum layer.[98]

Bubble liposomes

The bubble liposomes are made by entrapment of gas in the bubble, such as bioactive gases are used for delivering ultrasound-controlled drug release.[99] NO entrapment in bubble liposome is used to dilate blood vessels and regulate blood pressure and it avoids formation of microbubbles caused due NO. Then, oxygen bubble liposomes are prepared by entrapping oxygens into the bubble liposomes they contain an excessive amount of oxygen in them and are used for the treatment of high PO2 formation in the lungs.

Stealth liposomes

Stealth liposomes have extended flow time in the blood circulation and give targeted drug action, their surface is coated with glycoproteins, site-specific ligand receptors, polysaccharides and synthetic polymers due to which they give narrow distribution of drug and sit-specific action.[100] Stealth liposomes are also called as PEGylated liposomes as they are derived from PEG.[101,102] Doxils was the first PEGylated product and PVA, polyethylene glycol and hyaluronic acid are used as liposome steric protection.[103]

Actively-targeted liposomes

Actively-targeted liposomes give site specific action as the name suggest they directly bind to the targeted receptor cells and show the action by activating the receptor-mediated endocytosis.[104,105] Active targeting is done by indirect and direct covalent conjugation that binds with the targeting ligands

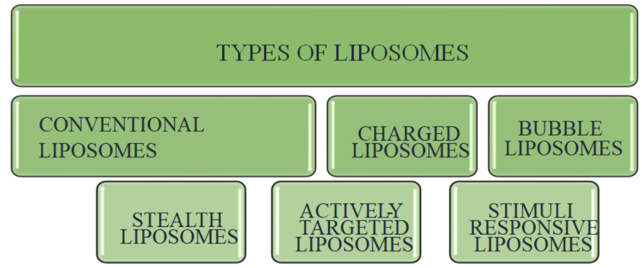

Figure 4: Types of liposomes

(binding with the overexpressed biomarkers of the targeting tumor cells) to the surface of the nanocarriers, though it has been mainly seen that direct conjugation binding has mainly caused disruption in the ligand or receptor recognition.[106,107] Active targeting nanocarriers are formulated to overcome challenges such as avoiding removing the drug from blood circulation, reducing the adverse effects caused due to the drugs and increasing the efficacy of the administered liposomal nanocarrier drug.[108,109]

Stimuli-responsive liposomes

The stimuli-responsive liposomes work by the mechanism of biochemical or physicochemical stimuli, which means that the drug is delivered from the liposomes after they receive signals from through the pH, temperature, redox, heat, etc (Figure 5).[110] Thus, the stimuli-responsive liposomes are classified into various types. Stimuli-responsive liposomes are also called as smart liposomes. Stimuli-responsive liposomes also contain local and remote induction.

Light sensitive liposomes[111,112]

It works on the fundamentals of modification of phosphatides of fatty acyl chains with photosensitive functioning groups (Figure 6). Light sensitivity work by the stimuli of ultraviolet, infrared radiation, and visible radiation.

Thermosensitive liposomes[113-116]

Thermosensitive liposomes are made by DPPC lipids which have temperature transition in the range of 40 to 45°C. These liposomal-based drugs show payload at a very high temperature. They have a stimulus of radiofrequency and microwave.

Redox potential liposomes[117,118]

Redox potential stimulus is ROS peroxides, hydroxyl group and dioxygen and oxidation liposomes amid their action when there is a difference between the non-cellular and endogenic occurring area, which comes under the biological activities. The reactive oxygen species leads in a high concentration of glutathione in antitumor cells.

Enzyme-responsive liposomes[119-121]

Enzyme-responsive liposomes work on the principle by releasing the drug is released based on the stimuli protease, amidase and esterase enzymes thus, they are named as enzyme responsive drug. Enzyme-responsive liposomes work by reducing the toxic effects, increasing therapeutic level and by enabling encapsulation of prodrugs.

pH-sensitive liposomes[122-124]

The pH-sensitive liposomes work according to the stimuli pH change. It is built of CHEMS and1,2-Dioleyl-sn-glycero-3-phosphoethanolamine

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Methods of Liposomal Preparation (Figure 7)[162,163]

This is the general method of liposomes preparation.[9,10]

Passive Loading Techniques

In this method, the drug is entrapped in the aqueous core, which is embedded with covalency, Ionian, geostatic, nonbonding interactions or steric interactions between the drug granules. AmBisome, DepoDur, Depocyte, and Visudyne are marketed products that are formulated by passive loading technique.

Mechanical dispersion technique (Figure 8)

- *Freeze thawed liposomes*

When we grow the lipid content and cationic power of the media there is a formation of unilamellar vesicles as there is amalgamation of small uniflagellar vesicles all over the process of freeze and defrost.[125-127] Thus, 20 to 30% entrapment efficiency was determined.[128]

- *French pressure cell: extrusion*

This method is mainly used in the processing of reduction of liposomes in combination methods. However, this method has many drawbacks such as intense heat is hard to maintain and small capacity are relatively small.[129-131] But it has many advantages over the sonication method. This method is attained by extrusion over large MLVs through small orifice.

- *Extrusion*

Out of all the other methods combination methods used in the preparation of liposomes size and reduction extrusion is the most specifically used method. Various marketed products such as marqibo, vyexos and onivyde are formulated using the extrusion method. Thus, the steps followed in this procedure are (Figure 9).[132,133]

- *Thin film hydration technique*

It is used for lipophilic drugs, everolimus drugs, double drug filled liposome containing enclosed resveratrol and 5flurouracil was prepared in polyethylene glycol and gemcitabine was also formed using thin layer hydroxylation process. Other commercial products formed by these methods are Visyudine, Shingrix, AmBisone.[134-136] The thin film hydration follows the following steps:

- In an RBF, the phospholipids and water-repellent drug were dispersed in a natural solvent.[137]
- Then the natural solvent is vaporized under lower pressure to obtain a thin layer.
- Then the acquired thin layer is hydrous with used lipid over the transition temperature with an aqueous buffer solution.[138]
- The aquaphilic drug is filled in the aqueous core of the carrier is actually contained inside the hydrated solution.[139]

Sonication (Figure 10)

- For the preparation of SUVs, infrasound is taken into consideration and for manufacturing of MLVs also, bath or probe sonication is taken into consideration under passive conditions.[140] Sonication is done using
- Probe sonicator:

The probe sonication follows following steps;

- First, the apex of probe sonication is dipped in a scattered liposome.
- And then there is a local hotness in the tip due to the energy coupling, thus the vessel in which the liposome dispersion is kept should be kept in a ice bath.
- In this process more than 5% of lipids are de-esterified until the sonication goes on for 1 hour.
- Bath sonicator:

The process followed in bath sonication is as follows;

- The liposomal dispersion is putted in a cylindric which is positioned into the ultrasonic cleaner.
- While the material for sonication is perhaps easily safeguarded in this aseptic container or beneath an inert environment.[141]

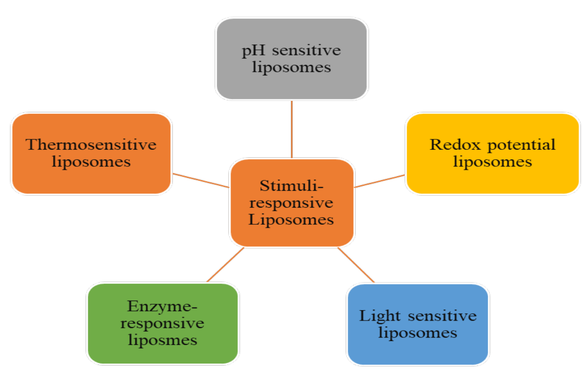

Figure 5: Various types of stimuli- responsive liposomes

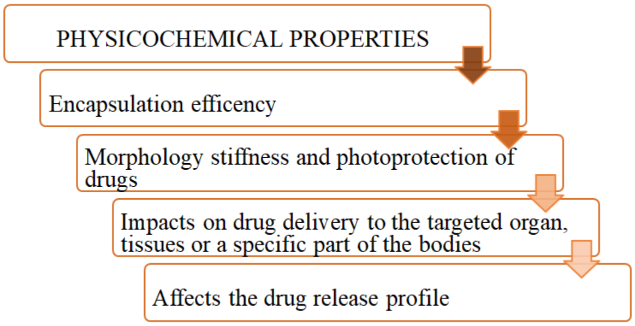

Figure 6: Brief description of the physicochemical properties of the lipsomes

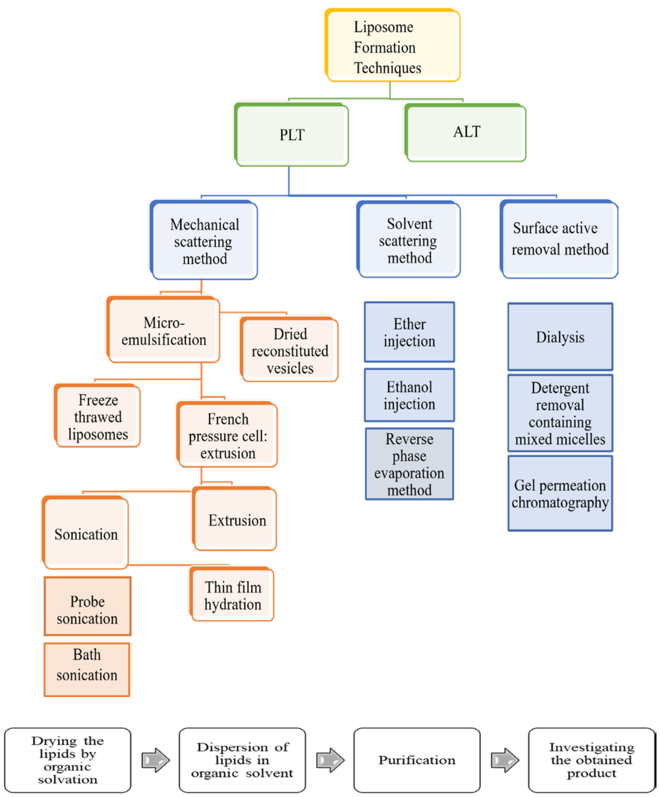

Figure 7: A brief description of the various methods used in the preparation of liposomes[162,163]

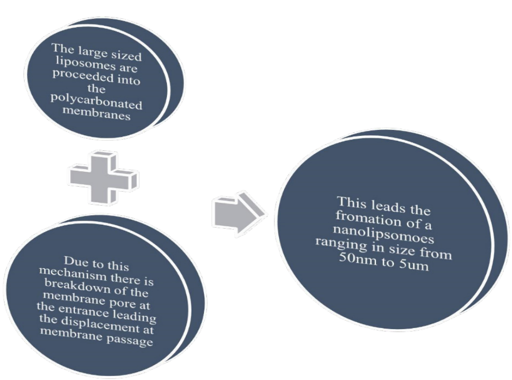

Figure 8: A general method of liposomes preparation[9,10]

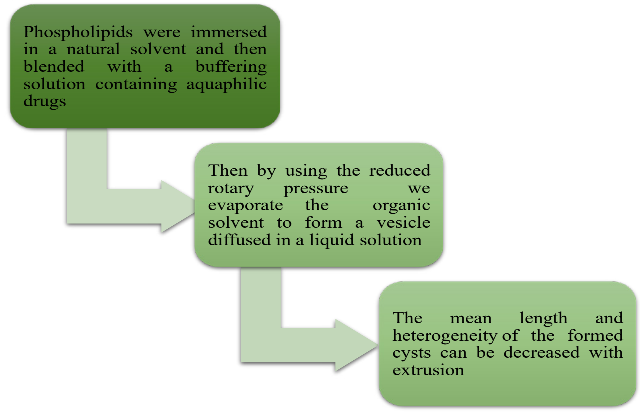

Figure 9: A brief description of the steps followed in an extrusion procedure[132,133]

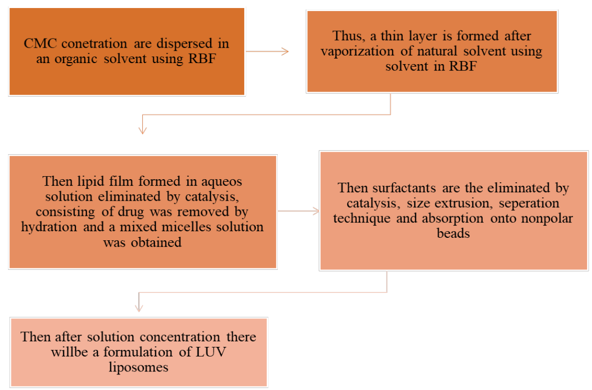

Figure 10: A brief description of the steps followed in the counter phase vaporisation process[147,148]

Solvent Dispersion Technique

Ether injection

The process of solvent injection using ethanol injection is as followed:

- First, an organic solvent was prepared by dispersing lipids and hydrophobic active agents and then injected quickly in the aqueous phase.[142]
- The ethanol was when used for the injection, it needed 10 to 20 folds of aqueous phase and evaporation in ethanol was done at sub-atmospheric pressure through rotating vacuum evaporator, catalysis or filtration.[143]
- It yielded liposomes with high polydispersity index thud they are considered potent.

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Counter phase vaporization process

It is applied as substitute in place of thin layer hydroxylation process. This method works by the formation of w/o suspension.[146] The process of counter phase vaporization process is as follows.[147,148]

Detergent removal process

It works for the formation of LUV liposome.[149-152] The process of detergent removal method is as follows[153-156]

- *Dialysis*

Catalysis or dialysis is the procedure in which critical micellular concentration is taken in to consideration for the distribution of the phospholipids or fats.[157,158] Thus, the detersive is removed by dialysis. Due to this the critical micellular concentration goes off with phospholipids and then combines to form LUV. LipoPrep device is used to detach the detergents and it is a dialysis system.

- *llDetergent removal containing mixed micelles*

XAD-2 and bio beads SM2 are organic polystyrene adsorbers are used for the adsorption of detergents by shaking the mixed micelles with these beads. The advantage of these beads is that they can eliminate the detersive with extremely depressed critical micellular concentration.

- *Gel permeation chromatography*

In gel permeation chromatography, detersive is drained by volume special chromatographic. Sephadex G-50, Sephadex G-100, sepharose 2B-26B and sephacryl S200-S1000 is used for gel clarification as liposomal carriers cannot penetrate the orifices packed in the column and filter over intercalation areas. A substantial amount of amphiphilic lipids because of the swollen polysaccharide lipids.

Active Loading Technique

It is also known as isolate drug filling. The ALT follows the process that after the formation of empty liposomal shells, the drug is encapsulated inside the liposome within 5-30 minutes due to which a high efficiency drug loading can be achieved. Doxil is prepared by this process.

SUMMARY

The review on liposomes has focused on the discovery of liposomes in the ongoing liposomes clinical trials from the 1960s to the 2020s. Then, the structure of liposomes and the composition of liposomes containing the steroids, phospholipids, polar heads, targeted site, positively and negatively charged lipids and uses and properties have been encoded in the review. Then the classification of liposomes (ULV, MLV, OLV, SUV, MVL, GUV, etc.) and their types, such as conventional, charged, stealth, stimuli-responsive, and actively-targeted liposomes have been elaborated in detail. The methods of liposome preparation and physicochemical properties have been described in detail

CONCLUSION

This was an extensive review on the recent advancement of liposomes with various regulatory and manufacturing aspects, which also discussed the current procedures involved in the development of liposomes. Liposomes were studied successfully and their therapeutic and toxic effects were determined. Because liposomes are multifunctional drug carriers they are used in every aspect of drug dosage forms. Thus, there are many liposomal products that are approved in the market with high therapeutic effects and they are also the ones that have shown magnificent benefits of the drugs especially as anticancer and antitumor targeted drug delivery systems.

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