REVIEW ARTICLE

Liposomes in Medicine: An In-depth Analysis of Preparation Methods and Applications

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ABSTRACT

Liposomes are the targeted drug administration system that offers the potential to improve the medicinal value of drugs by boosting the concentration of medication the duration of time in intended cells, thereby reducing side effects. Liposomes, which are sphere-shaped vesicles built from phospholipids and cholesterol, are being investigated extensively as a means of increasing the bioavailability and delivery of therapeutic medications. Liposomes have both hydrophilic head and hydrophobic tail and exhibit eminent properties, including reduced toxicity, better biocompatibility, easily biodegradable, easy to function and enhanced sustained release of drugs with increased therapeutic efficacy. Liposomes are said to be an ideal drug-carrier system as they enhance the delivery of anticancer drugs at the tumor site. Since liposomes are amphiphilic carriers that may be modified to have various functional characteristics, they are seen as a potential technology for a range of pharmacological and industrial uses. As a potential way to carry drug delivery across cell membranes, liposomes help medications to target specific disease sites. Liposomes are employed to deliver genetic material, such as DNA fragments, to specified cells so they may synthesize certain proteins. Thus, liposomes are investigated as adaptable nano-vesicular vehicles with potential medical applications for medicinal and diagnostic purposes. The future of liposomal formulations is projected to be a multipurpose use of imaging capabilities and medicinal components in a single liposome for diagnostic and actual-time therapy. The most promising approach for topical administration is liposomal drug delivery since it is compatible with living systems and can accommodate simultaneously hydrophobic and hydrophilic medicated substances because of the amphipathic feature of phospholipids.

Keywords: Liposomes, Amphiphilic, Hydrophobic, Cancer therapy, Applications, Targeting technique.

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INTRODUCTION

In 1964, British haematologist, Dr. Alec D. Bangham gave a talk at Cambridge's Babraham Institute regarding liposomes.[1] The term "liposome" originates from the Greek concepts "Lipos" (fat) and "Soma" (body), which are combined to form the term.[2] Drugs can be embedded into liposomes and administered to treat cancer and other illnesses.[3] Membranes are typically constructed from lipid bilayer, with a head group in addition to a tail group. Water repels the long hydrocarbon chain that makes up the tail, whereas the head is attractive to it. Lipid structure is found in secure two-layer (bilayer) covering in nature. A lipid two-layer of natural or synthetic lipids surrounds the aqueous core of a liposome. They have little inherent toxicity and are made of biologically inert, weakly immunogenic, natural phospholipids.[4] Liposomes are hydrophilic bilayer vesicles coated with phospholipids. Due to their suitability for confining a variety of medications that may be hydrophilic or lipophilic, they have been the subject of extensive research over the course of the last few decades as a fancied vehicle for the portage of therapeutic medicines.[5] Drug compounds included in liposomes improve bioavailability and adsorption rates due to their vesicular structure.[6]

Liposomes are also used as drug delivery methods that protect encapsulated substances against enzymatic, chemical, and environmental alterations as well as variations in pH, temperature, and ions. In order to selectively localize active drugs at disease areas like tumors and inflammatory sites, liposomes are used as agent drug carriers. Other excellent qualities can be obtained by harmonizing the components of the lipid bilayer.[7] A prolonged vascular half-life (stealth lipid bilayer), a tendency to formation of aggregate with nucleic acids that mediates delivery of gene or genetic monitoring, and the capacity to transport encased components to the cytosol by means of the lysosomal channel. The main advantages

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in cancer therapy of extended vascular duration, increased absorption by cells and freight build-up at the tumor location can be accomplished with liposomes by releasing the stimuli-sensitive carrier after lysosomal breakdown at the desired location using the proper surface functionalization and alteration techniques.[1]

Clinical evidence supports the use of four liposome-based medications used in the therapy of breast cancer are: Myocet liposomal, Lipodox®, Doxil®/Caelyx®, and Lipusu®. Doxil®/Caelyx®, the first clinically utilized chemotherapeutic nanosystem, is a PEGylated nano-liposomal drug transportation system that contains DOX HCl on account of the main ministrations of Kaposi's sarcoma associated with AIDS, multiple myeloma, refractory ovarian cancer, and metastatic breast cancer. Myocet is often employed as the first line of treatment for HER2+ metastatic breast cancer due to it being able to reduce drug-related cardiotoxicity while enhancing anti-tumor activity.

In the USA, Lipodox®, an entirely distinct PEGylated DOX HCl encompassing liposomal composition, served as a stand-in during an incredibly restricted supply of Doxil® in 2012. PTX is incorporated within Lipusu®, a non-PEGylated lipid bilayer system. PTX, a chemotherapeutic derived from the Pacific Yew tree, is commonly administered in the Kolliphor-EL solubilized form of Taxol® for the curative purposes of ovarian and breast cancers.[8] Numerous advantages of liposomes include biocompatibility, minimal immunogenicity, cell structure, safety, and effectiveness. Antifungal and anticancer chemotherapeutic medication liposomal formulations for tumor targeting have demonstrated significant advantages over conventional therapy in attaining customized therapeutic action of pharmacological substances.[9]

Additionally, liposomes can increase the encapsulated medications, pharmacokinetic features and bio-distribution, which will improve their therapeutic index. The interiors of these "liposomes" are safeguarded by an obstacle that is impervious to the salts of bile, gastrointestinal fluids, alkaline environments, stomach and mouth enzymes, reactive oxygen species, and human intestinal flora. As a result, until the liposomes are transported to the precise gland organ, along with the system where they will be used, their contents are shielded from the oxidative process and destruction.[10] The goal of the design is to minimize side effects while achieving the best potential curative outcome by administering the appropriate dosage of medication to diseased areas at the right duration.[11]

Composition of Liposomes

Liposomes are globular lipid bilayers with sizes ranging midst 50 to 1000 nm that are advantageous portage mechanisms in the contemplation of biologically active compounds.[1] Liposomes are particularly beneficial in healing conditions that impair the phagocytes of the body's defense system because they have a tendency to collect in the phage cells, which are such as foreign assailants. Liposomes are composed of both structural as well as non-structural compositions. The components regards to liposomes are:

Phospholipids

Phospholipids hold the fundamental constituent and building elements of liposomes. Vesicular liposomes are encased in a bilayer of phospholipids. A significant portion of a lipid bilayer structural component endures phospholipids. The relatively common kind of phospholipid utilized in lipoid preparation is phosphatidylcholine. Phosphocholine, a pair of hydrophobic acyl hydrocarbon chains, glycerol connections and a hydrophilic polar head component generates the amphipathic molecule referred to as phosphatidylcholine.

Natural occurring substances chemical composition twin acyl chains, preferably saturated or unsaturated, are joined by a glycerol moiety in phosphatidylcholine.[12] The array of the bilayer molecule's hydrocarbon linkage depends on the lipoid wall stability. In nature, phospholipids are widely distributed, and choline-containing phospholipids are employed to produce lipoid structures. Instances of phospholipids endure phosphotidylcholine (Lecithin), phosphotidylserine, phosphotidylglycerol, and phosphotidylethanolamine (Cephalin).

Being insoluble in water followed by an aqueous environment, phosphatidylcholine firmly aligns its own tightly in flattened bilayer film to reduce the unfavorable interaction between the bulk watery facet along with extensive fatty hydrocarbon series.[13] Glycerols, which include phospholipids, the content that is utilized in the formation of liposomes, make up over 50% of the load of the lipid in cellular layers as shown in Figure 1. There is a temperature when the fluidity of all lipids changes. The transition temperature (TC) is another name for this temperature. The acyl chain's length directly relates to the TC; the protracted the chain, the greater the TC; moreover, the stiffer the membrane. Supplementary stiff membranes limit leakage by keeping medications that are trapped inside. The TC is crucial because it can influence how the membrane responds to agglutinate with other lipid bilayers, sturdiness, agglomeration, and pervious, and likewise, how the liposomes respond when in contact with biological systems.[7]

Cholesterol

A further integral portion of the liposome's structural configuration is cholesterol. It is a periodically used sterol. Sterols are added, which modifies the function of stability and stiffness and lengthens the period that blood is in circulation. It cannot create a bilayer structure on its own. Phosphatidylcholine, along with cholesterol in phospholipids, exhibits a molar proportion of 1:1 or 2:1, signaling that it is extraordinarily concentrated.[14,94]

The computation of cholesterol to the lipid bilayer improves durability and results in a highly structured, hard membrane. Cholesterol escalates the unsteadiness and stability of the cellular membrane and diminution the previous molecules, affirming water fluent. Cholesterol hindered the interaction and destabilization of liposomes. In the membrane, cholesterol occupies a position where its hydroxyl portion faces the watery expanse, along with its aliphatic series is collateral to the acyl part in the midway of the bilayer. Although hydrophobic and

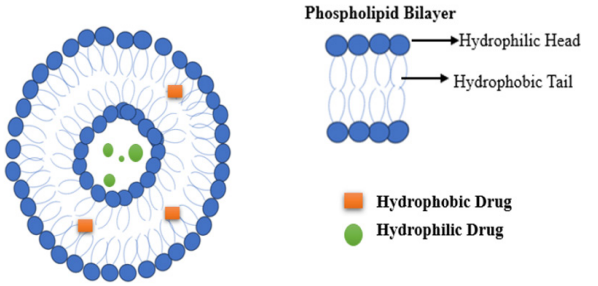

Figure 1: General structure of liposome

defined head group interactions have been associated with increased cholesterol solubility in phospholipid liposomes, the configuration of cholesterol in the membrane is unclear.[7]

Mechanism of Liposome Formation

When skinny lipid layers are hydrated and swollen, lipid vesicles are created. Throughout whisking, the drenched lipid sheets disparate into massive MLV, which prevents water from interrelating along the margins of the bilayer's hydrocarbon core. Once created, the particles are extruded or sonicated to minimize their size.[7]

Endocytosis

A receptor, ligand, or antibody serves to attach the liposome to the cell surface before the cell membrane absorbs it. Liposomes release the drug that they have been holding after their absorption by cellular engulfment, preventing the drug's first-pass metabolism.

Content transfer

Human cell membranes and liposomes share a similar structure. Therefore, after engaging with the lipids of the cell membrane, the phospholipids in liposomes carry the medication they contain through the cell laminate.

Fusion

This contraption results in the transfer of medication material through liposomes to cells by fusing the liposomes' phospholipids with the cell membrane's phospholipids. The lipids of the liposome, in addition to the cell membrane, merge to form a single membrane during this fusion procedure.

Adsorption

This procedure involves exculpating the medication to explode liposomes furthermore, the absorption of the unmodified medicament by the cells. Liposomes attach to the cell membrane's surface due to attraction forces between them.

Classification of Liposomes

The liposomes can be categorized and positioned on a numeral of factors,[15] as given in Figure 2 including;

Grounded on vesicle formation

Uni-lamellar vesicles

- Small uni-lamellar vesicles (SUV) (Size: 40–80 nm)
- Medium uni-lamellar vesicles (MUV) (Size: 40–80 nm)
- Large uni-lamellar vesicles (LUV) (Size: 100–1,000 nm)

Oligolamellar vesicles- Ten to twenty lipid bilayers encircled by an interior quantity make up an OLV.

Multilamellar vesicles- They contain lipid bilayers. MLVs developed in a distinctive manner. The design resembles an onion's layers. LUV/MLV make up the majority of the centre part.[16]

Based on method of preparation

- Reverse phase evaporation method (REV)
- Stable plurilamellar vesicles (SPLV)
- Frozen and thawed method (FATMLV)
- Extrusion technique (VET)
- Dehydration-rehydration method (DRV)

Based on the composition

- *Conventional liposomes (CL)*

Composed of phospholipids and neutral along with negatively charged cholesterol.

- *Fusogenic liposomes*

Reconstituted Sendai virus enfold (RSVE).

- *Cationic liposomes*

Cationic lipid escort dioleoyl phosphatidylethanolamine (DOPE)

- *Immuno liposomes*

Monoclonal antibody or recognition sequences linked to CL or LCL

- *pH-sensitive liposomes*

Phospholipids thus DOPE or phosphatidylethanolamine (PE).

- *Long circulatory (Stealth) liposomes (LCL)*

They have polyethylene glycol (PEG) derivate ardent to their facet to reduce the phagocyte system's ability to recognize them. PEG is added to liposomes, which inhibits their departure from the bloodstream and extends their presence in the body. Another name for the PEG attachment procedure is PEGylation.[17]

Liposome Characterization

to certify their accomplishment *in-vitro* conjointly *in-vivo*, liposomes need to be thoroughly defined in order to assess their physical moreover chemical properties. Size, size distribution, surface charge (as measured by zeta potential), sculpture, lamellarity, phase behavior, sheath efficiency, also *in-vitro* drug liberate are the key features of liposomes that have received the most research.

Size and poly-dispersity index

The foremost attributes in liposome description are their acreage and poly-dispersity index. Minimal liposomes can circulate in the body for a longer amount of time than massive liposomes, which are eliminated from the arterial stream more promptly.[18] Generally speaking, liposomes with sizes between 50 and 200 nm are the best for delivering medications. A scaled, immeasurable poly-dispersity index can have values allying

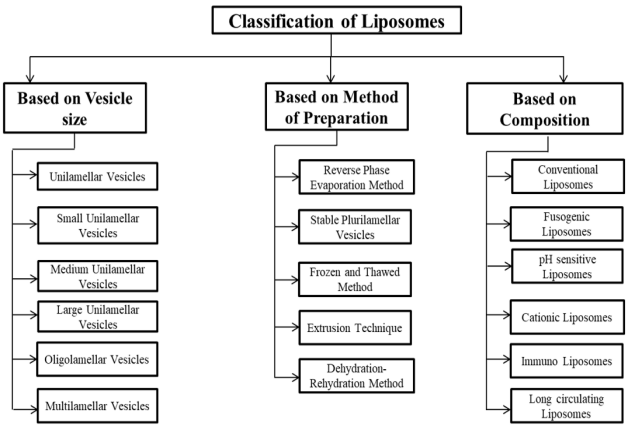

Figure 2: Classification of liposome

0 and 1. A PDI estimate of 0.3 or below implies a suitable and homogenous liposomal population in drug transport prosecution utilizing liposomes, whereas a high PDI estimate is linked to an extremely broad variance in size (heterogeneity) or probable countless liposomal populations in the sample.[19] The parameters used to calculate PDI are particle size, solvent refractive index, measuring angle, and distribution variance.

Zeta potential

The absolute net potential of the particles generally appears as the surface or zeta potential.[20] This property of liposomes is thought to be an important physical property in controlling the electrical interactions between suspended particles.[21] The head group of lipids, followed by the ligands connected to them, which may be neutral, positive, or negative-are significant determinants of the liposome's net charge. Zeta-potential measurements are used to estimate the longevity of colloidal frameworks, including liposomes, in the liquid around them. Since there is no force to stop flocculation, liposomes with low zeta potential or those without charge are more prone to consolidate over time. The identification of the surface charge enables the measurements of changes in the intensity of scattered light caused by the movement of particles in a suspended state as a result of being exposed to an electric field.[22]

Shape

Examining the physical attributes of liposomes – more especially, their shape – is crucial for a precise description.[23] The TEM approach has certain limitations when it comes to specimen handling because it needs to remove the liposome's native environment. Repeated examinations are not possible with it because of its lengthy processing time. Furthermore, this technique might lead to improvements in the structure of liposomes, including possible vesicle growth, shrinkage, and the creation of artifacts in the image that is generated. Using a liquid nitrogen flash freezing phase followed by direct observation of the liposomes in a controlled setting, this approach minimizes shape distortion or shrinkage while maintaining the liposomes close to their native form.[24]

Lamellarity

Due to its impact on the EE as well as the medication release profile, lamellarity is another property that may affect future liposomal uses. The most used approach, cryo-TEM, provides useful statistics on liposome lamellarity comprehend bilayer thicks*et al*ong with inter-bilayer distance.[25]

Phase behavior

The lipid bilayer's fluidity can be affected in significant ways by the crystallization temperature. Because the inconstancy of the lipid layer enhances the pervious of the lipid bilayer to hydrophilic medicines that are entrapped, phase behavior is heavily considered for drug delivery applications. Differential scanning calorimetry is a vital, frequently utilized technique for studying and arbitrating the TC. A liposomal membrane's phase behavior also affects fusion, aggregation, stability, and protein binding, among other liposomal properties.[26]

Encapsulation efficiency

The encapsulation efficiency of a drug can be outstandingly affected by the composition of the liposome, the method used to produce the liposome, rigidity of the bilayer membrane. In the field of medical applications, the most important thing is to load the right amount of drug to make it effective as a treatment.[25] The encapsulation efficiency is the proportion of the medication contained in enclosed liposomes (the encapsulated drug) to the sum-up amount of medication utilized in liposomal preparation. The non-encased medication concentration in the eluted is the focus of the indirect method, which subtracts this concentration from the integral drug coalescing used in the liposomal composition. The direct procedure can be used to determine EE by directly disrupting liposomes accompanying an organic solvent along with quantifying the extricated material.[24]

In-vitro drug release

Under dialysis conditions, the *in-vitro* medication extricate outline can be evaluated. The drug specifications should guide the selection of the membrane for the dialysis bag. The liposome sample is hermetically sealed, captivating the dialysis bag with a particular molecular weight cut-off. The tubing layer approach is placed in a release medium that simulates a physiological fluid, typically buffered saline, escorting pH of 7.4. To manifold an *in-vivo* environment, the complete arrangement is continuously stirred and kept at 37°C. When designing liposomes for the restrained exonerate of drugs, the findings of the *in-vitro* extricate study are customarily taken into narrative.[27]

Method of Preparation of Liposomes

Y. Johnson invented the first artificially manufactured liposomes in the 1940s and licensed the process for advantage in the pharmaceutical sector (I. G. Farbenindustrie AG). When various fats or fatty oils were assimilated with numerous water solutions, the so-called "depots" were formed.[28]

According to FDA industry guidelines on liposomes, lipid information must be supplied for new medication applications.

The high standard for lipid excipient quality results in a high barrier for liposomal medication R&D. It is feasible to produce liposomes utilizing different procedures, including film dispersion, injection, freeze-drying, high-speed shearing, ultrasonic scattering, and extrusion.[29] The essential excipients of liposomes are pharmaceutical-grade phospholipids. The lipid materials substantially influence the physical and biological characteristics of liposomes. For instance, the establishment of industrialized lipid synthesis is the key R&D stage for cation liposome-based mRNA delivery.[30] However, the therapeutic applicability of liposomes is limited by their complicated composition and manufacturing process.[31]

Parameter for Liposome Preparation

Determination of technique to form the liposome relies upon the accompanying boundaries;[9]

- The components of the material to be encased, both through chemical processes and physically, as well as the liposomes' constituent parts.
- The type of media employed to disseminate the lipid vesicles
- The optimal proportion and potential toxicity of the enclosed component
- Furthermore, steps taken through the application or transport of the vesicles
- The vesicles' appropriate size, poly-dispersity, and shelf life for the desired use.
- Consistency from lot to lot and the potential for mass production of proven and successful liposomal products.[18]

Various techniques are used to formulate liposomes & drug loading processes in liposomes as mention in Figure 3:

Passive Loading Technique

Mechanical dispersion method

- *Lipid hydration method (Bangham Method)*

The process of making phospholipids being dissolved in organic solvents, for instance, dichloromethane, chloroform, ethanol, and chloroform-methanol combination, MLV can be produced (2:1 v/v; 9:1 v/v; 3:1 v/v) 4,5,13 is known as the in thin-film in hydration In procedure. When solvent evaporates under a vacuum at a temperature between 45 and 60˚C, a thin, homogenous lipid layer is created. The residual solvent is entirely removed using nitrogen gas. Distilled water, a buffer with phosphate, phosphate saline buffer at pH 7.4, and normal saline buffer 2 to 4 solution are employed in the hydration process. The hydration procedure took between one and two hours at a temperature of 60 and 70˚C. The liposomal suspended state is kept for the time being at 4°C to guarantee the most extreme lipid water intake. All types of lipid mixtures can be hydrated using the thin-film approach. The approach's major limitations are poor encapsulation, challenges with scaling up, and a heterogeneous size distribution.[32,33]

- *Micro emulsification*

For the large-scale production of liposomes, micro fluidization, or micro emulsification approach is used. The method for creating antimicrobial liposomes was described by Bolti *et al*. It involves thin-layer water content, micro-fluidization to achieve partial blending, and sonication using a bath-type sonicator. Micro fluidization produces liposomes with good aqueous phase encapsulation and is repeatable.[32,34]

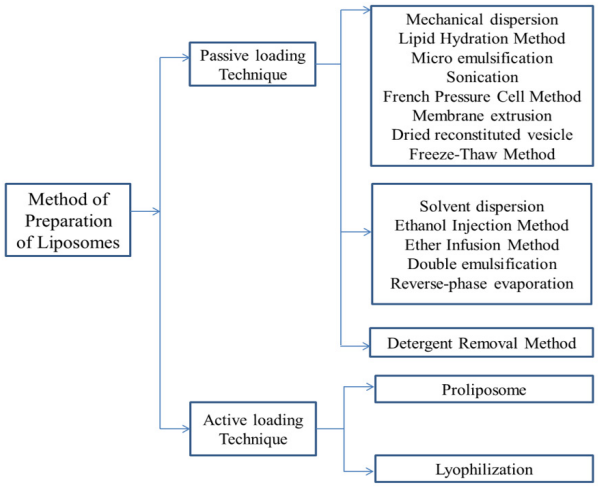

Figure 3: Method of preparation of liposome

- *Sonication*

This is the most popular technique for making SUV from MLV, which is made using both the handshaking method and the rotary evaporator method. In the process of preparing SUVs, two different sonication techniques are used. Perhaps the most popular strategy for concocting SUV is sonication. Here, MLVs are sonicated utilizing a probe sonicator or a bath-type sonicator in an apathetic ambiance. This technique's key constraints incorporate its very low inner volume/embodiment viability, conceivable corruption of the chemical substances to be typified along with phospholipids, the expulsion of enormous molecules, metal deterioration of the probe tip, and the concurrence of MLV and SUV.

- *Probe sonication method*

This technique involves directly dispersing the titanium probe's tip into a liposome dispersion to produce SUVs. Heat is produced in this process because of the large energy input. Liposome dispersion is kept in the ice bath to regulate temperature. During the sonication procedure, over 5% of the triglycerides may become desterilized for up to an hour. Titanium will also slough off with the probe sonicator and contaminate the fluid. The biggest drawback of this procedure is that the titanium fragment contaminates the solution and makes it into sludge.[35]

- *Bath sonication*

This technique involves placing a container containing liposome dispersion on the sonication bath. For the manufacturing of SUVs, this process is more practical than probe sonication since the temperature can be easily managed. It is possible to obtain the sterile liposome and there is no contamination with titanium. The stuff that was sonicated can be stored in

neutral surroundings or a sterile container, in contrast to the probe units.[36]

- *French pressure cell extrusion*

MLV is propelled through a French pressure cell through a small opening. The proteins seem less inflated during processing when using the French press vesicle approach as opposed to sonication. It's interesting to note that French press vessels seem to retain encased solutes for a lot longer than SUVs do, whether they were formed by sonication or detergent elimination. The approach entails cautiously handling irrational materials. Comparing the process to the sonication strategy, there are significant improvements. The outcome liposomes are a bit bigger than SUVs that have been sonicated. The disadvantages of the approach are the laborious in maintaining the elevated temperatures and the small functioning amount (the maximum is 50 mL).[35]

This technique transforms unsteady MLVs into SUVs along with LUVs, bypassing them with a tiny opening of apparatus. This process produces more dependable liposomes than the sonication method because it has better stability. This method's disadvantages include a tiny working capacity of only 50 mL at most and a difficult-to-manage high temperature.[37]

- *Membrane extrusion*

Extrusion *via* membranes with pores span in size betwixt 1 mm to 25 nm constitutes the extrusion technique. A heating block is positioned encircling the protrusion to accomplish the extrusion surpassing the phospholipid's phase-inversion temperature. After many runs through the polycarbonate membrane filters, it is feasible to generate (narrow-size distribution) LUV liposomes with measurements near the diameters of the membrane inlet. Many research investigations carried out on various lipid formulations show that this procedure enables a repeatable result of the final liposome outcome.[38]

- *Dried reconstituted vesicles*

Before being transported to a specific site, natural extracts are generally destroyed by cellular oxidation and other chemical processes. A common technique used in the creation of many pharmaceutical goods is freeze-drying. These goods are largely made by lyophilizing basic water solutions. There are still many instances in which pharmaceutical products are made utilizing a method that involves freeze-drying from organic co-solvent systems, even though water is usually the sole solution that needs to be removed from the solution using the procedure. Freeze drying, sometimes called lyophilization, is the process of extracting water from frozen materials at very little pressure.[33]

Typically, the process is employed for airing items that heat drying would harm. Since they are labile to heat. The method offers a tremendous quantity of prospects for tackling long-term stability issues in relation to liposomal durability. According to studies, entrapped components may escape throughout the freeze-drying process and during reconstruction. Recent research has demonstrated that when freeze-dried liposomes with sufficient trehalose (a carbohydrate frequently present in high concentrations in organisms), they can maintain up to 100% of their unedited contents. It demonstrates that trehalose is a superb cryoprotectant (freeze-protectant) to obtain liposomes. Pharmaceutical equipment vendors sell a variety of freeze-driers in sizes ranging from compact laboratory models to enormous industrial machines.[35]

- *Freeze-thaw method*

Here, SUVs created via the sonication approach are slowly and constantly frozen and thawed, leading to the development of LUVs as a result of SUV assemblage throughout the thawing stage. By using this technique, encapsulation efficacies rise by 20 to 30%.[39]

- *Solvent dispersion method*

In these procedures, the lipids initially dissolve in an organic solution before appearing in interaction with the phase of water, which contains the substances that will be enclosed inside the liposome. The phospholipids orient to form a monolayer at the affiliate of the organic and hydrophilic phases, which is a crucial stage in the development of the liposome's bilayer.[40]

- *Ethanol injection method*

This technique includes rapidly infusing an overabundance of saline or one more fluid medium with an ethanol arrangement of the lipids through a fine needle. With this method, a high amount of SUVs (approximately 25 nm in diameter) are produced.[34] Additionally, it is challenging to remove ethanol from a solution, which raises the possibility of inactivating physiologically active macromolecules.

- *Ether injection (Solvent Evaporation) method*

This approach includes continuously infusing lipid broken down in a diethyl-ether or ether-methanol mixed within a hydrated media containing drug at a temperature of 50 to 65°C or governed diminished pressure. Liposomes are shaped when ether is eliminated under a vacuum. The making of a heterogeneous populace of liposomes (70–200 nm) and the openness of liposomes to high temperatures during exemplification, which can disable liposome dependability, are the fundamental downsides of this technique.[41]

Double emulsification method

This technique involves dissolving the drug in a watery phase (w_1), followed by emulsifying the essential w_1/o emulsion in a chemical solvent of a polymer. A $w_1/o/w_2$ double emulsion is created by combining this main emulsion with an emulsifier that contains an aqueous solution (w_2). Microspheres are as yet present in the fluid persistent stage after the dissolvable has been wiped out, and they are recuperated by centrifuging or separating.[42]

Reverse phase evaporation method

Once the lipid mixture is in a round bottom flask, the solvent is extracted at low pressure using a rotary evaporator. In the organic phase, the lipids are re-dissolved once the system has been nitrogen-purged. In this phase, the reversal stage, spheres

will form. As solvents, diethyl ether addition to isopropyl ether, is commonly used. After the lipids in this phase have been re-dispersed, a phase of water containing the drug to be encapsulated is incorporated.[93]

The dual-phase system is sonicated as long as the combination turns into transparent one-phase dispersion while the system is kept in a constant nitrogen environment. Before the non-encapsulated material is removed, an organic solvent is extracted from the mixture using a rotary evaporator once the gel has formed.[43]

- *Detergent removal method (removal of non-encapsulated material)*

The simplest way to eliminate detergent is to dilute it with a buffer (by 10–100-fold). After a buffer is added to the water-based solution of a blended lipid-detergent framework, the size and polydispersity of the initial micelles increase. Using a detergent solution and the detergent removal process, the lipids are moistened (and solubilized). When the detergent mixes with the phospholipids, it creates mixed (lipid/detergent) micelles that shield the hydrophobic parts from coming into close proximity with the watery phase. As the detergent is gradually (successfully) removed, the mixed micelles generate unilamellar vesicles and rise in lipid content. High CMC detergents are commonly utilised, such as sodium cholate, Triton X-100, sodium deoxycholate, and alkyl glycoside. There are various techniques for getting rid of detergent.[38,44]

Active Loading Techniques

Formation of liposomes from pro liposomes

A soluble carrier is used to encapsulate medications and lipids in a pro-liposome, producing a free-flowing particulate substance that, when nourished, transforms into an isotonic liposomal solution. The pro-liposome method might enable low-cost mass production of liposomes that include, in particular, lipophilic medications.[45]

Lyophilization

The process of eliminating water from items that are frozen at very low pressure is called lyophilization (sometimes referred to as freeze drying). Drying materials that are heat-labile and susceptible to harm from heat drying is a common application for this sort of technique. This approach holds out a great lot of hope for the long-term resolution of liposomal stability problems. Leakage of components that have been trapped may occur both during the freeze-drying process and upon reconstitution.[46]

Drawback of conventional methods of liposomes preparation

The creation of liposomes by conventional techniques has various drawbacks, including poor mono-dispersibility, decreased stability, excessive residual levels from organic solvents, and hazardous adverse consequences. Additionally, the chemical solvents employed in previous techniques have negative environmental effects, are hazardous to humans, and frequently cause drug degradation. The production of liposomes using organic solvents or detergents runs the risk of denaturing drugs or proteins and changing the properties of the membrane.[47] Unfortunately, there are a number of issues with those traditional preparation techniques that fall into the following four categories.[48]

- Post-processing granulation is required because the particle dimensions of liposomes are disproportionately big or widely dispersed.
- The organic solvent that is still present in the finished product is also a significant problem since it has a negative impact on the clinical treatment but also has a detrimental effect on the durability of several protein or polypeptide medications.
- Because many lipids are temperature-sensitive, sterilizing liposomal formulations can be challenging. As a result, techniques for devising that can be done in an ultraclean atmosphere are preferred. Conventional approaches, meanwhile, don't always meet this need.
- Careful monitoring may be required in some operations, and this subjective technique may affect reproducibility.

Novel methods of liposome preparation

according to Pattni *et al*. (2015), the key reason for researching innovative liposome preparation techniques is to make it easier to scale them up for commercial manufacturing and use them with a variety of phospholipids and medications. The Wagner method of cross-flow injection[49] and layer contactor equipment and enhancement of the ethanol injection method.[50] are instances of unique approaches based on modifications or enhancements to traditional methods.

Another simple way to avoid the usage of dissipative processes is the immediate hydration of lipid components after the sonication process. In order to produce liposomes, supercritical fluid (SCF) techniques have also been investigated. These processes make use of a fluid that can be maintained at supercritical temperatures and pressures, such as carbon dioxide.[51] Novel methods of liposome preparation are mentioned in Figure 4.

Supercritical fluid technology

Supercritical fluids constitute non-condensable liquids that become extraordinarily dense at temperatures as well as pressures above their thresholds. Supercritical fluids have a missing liquid-gas phase boundary, which sets them apart from ordinary fluids. Unexpectedly, supercritical carbon dioxide ($scCO_2$) performs better than organic solvent substitutes. Despite being inexpensive, it is neither toxic nor flammable. It also has a critical temperature and pressure that are quite low (31ºC and 73.8 bar), and its dissolving characteristics are similar to those of nonpolar solvents.[35]

Along with the fundamental creation of liposome formation, more innovative novel techniques have been implemented in the production of liposomes. They can be applied to solve some issues brought about by novel methods of preparation. The need for industrial-scale uses in pharmaceuticals is a simple operation, excellent consistency, and adaptability for industrial-scale up will additionally be aided by the novel applications of emerging technology. More liposome

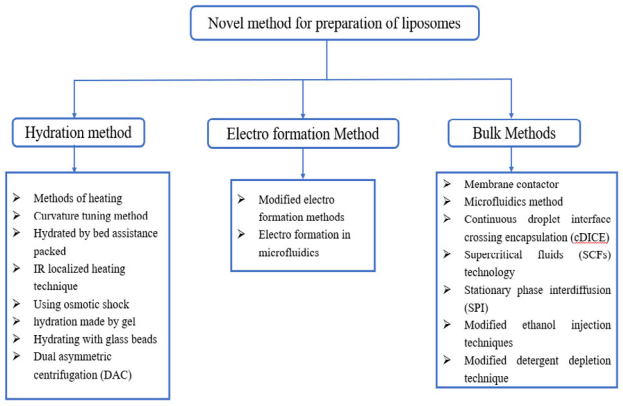

Figure 4: Novel method of liposome preparation

formulations appropriate for industrial-scale production and even more potent therapeutic uses will be created in the lab as a result of the introduction of new technologies.[38,51]

Targeting techniques of liposomes

The most crucial functional distinctive feature of liposomes as medication transportation arrangement is the particular targeting. Targeting in these certain areas hence concentrates on creating new diagnostic tools as well as enhancing the medicinal agents' efficacies. Currently, there are primarily two methods for targeting liposomes; passive and active targeting.[52]

Active targeting

The tethering of receptor-specific ligands to the liposomal surface for cell engagement is known as active targeting.

Paul Ehrlich, an eminent scientist, first proposed the idea of active targeting in 1906 by outlining the need for a "magic bullet" to control the distribution of a specific medicine within the body. Afterward, researchers worldwide have been searching for the "magic bullet" that might accurately hit particular cells, thus simplifying the process of identifying and treating them.[53] Targeting ligands that have been used to actively target cells include antibodies, nucleic acids (aptamers), peptides, whole proteins (like transferrin), and small molecules (like vitamins).[54]

Choosing an intended ligand involves taking into account many parameters such as the extent of covering of the molecule of interest, selective expression, level of overexpression or on the address, and specific cell absorption of the ligand-targeted composition. Additionally, it is important to choose these ligands so that they can bind to the target cells with the least amount of healthy cell binding.[55]

For enhancing liposomes effectiveness, usually, there are three approaches[56];

- The first strategy involves first binding the specific ligand of choice to a lipid and then mixing it with additional lipids.
- In the dual approach, just after the preparation, the necessary targeting ligand gets incorporated into the liposomes.
- In the third approach, modified lipid are incorporated into already prepared liposomes.

Passive targeting

Through the use of molecular drive inside liquids, liposomes are transported and delivered into the cancer interstitium *via* defective tumor capillaries in a process known as passive targeting.[57] The mechanical and physical characteristics of liposomes play a role in this passive targeting. However, it is more significant that liposomes remain attached to the dysfunctional lymphatic system when they penetrate the tumoral tissue. As a result, the drug included in liposomal carriers can exert its curative impact after being released.[58]

The formulation of cationic and stealth liposomes is preferred for the passive targeting of liposomes. In stealth liposomes, surface alterations are done using PEG to prolong the circulation time, while cationic liposomes bind to the negatively charged head group of phospholipids that is present on tumor endothelial cells by electrostatic interactions.[59, 60]

Application of Liposomes in Various Treatments

In anticancer therapy

Surgery and chemotherapy are the major methods used to treat and control cancer. Chemotherapy is particularly unpleasant since it affects both healthy and tumor cells because of biodistribution and the action of anticancer drugs. Anticancer chemotherapy is not particularly stable or permeable, has poor plasma retention duration, shows adverse side effects, and lacks selectivity towards the target location.[61] Through appropriate surface modification, anticancer medicated-loaded liposomes can be selectively specific to malignant regions, decreasing the consequence of chemotherapy on normal cells and, hence, the accompanying discomfort. Furthermore, liposomes can promote permeability, impart biocompatibility, minimize immunogenicity, and boost drug retention at the targeted site, making them an effective carrier for chemotherapeutic agents.[62]

Different liposomal formulations have been produced & approved by utilizing multiple compositions for the supervision of many appearances of cancer, including breast cancer, lung cancer, or tumor-targeted malignancy as mentioned in Table 1. The initial liposomal dosage licensed for the management of AIDS-related Kaposi's sarcoma malignancy was Doxil®, doxorubicin HCl liposome injection.[63] Anthracycline category drugs have potent action towards the cancerous cells by stopping and killing the growth of rapidly dividing cells, but these drugs also have hazardous effects on the body. In some cases, toxicity has been reduced to half by encapsulating drugs in liposome formulation.[64]

A different DOX citrate-encapsulated liposomal formulation that works well as the initial remedy for breast cancer is called Myocet®. As compared to Doxil®, it has a shorter circulation time & reduced cardiotoxicity.[65,66] Cisplatin is preferred as the initial-line chemotherapy drug for numeral cancer and, hence has serious adverse effects such as renal damage, nausea as well as emetic, ototoxicity, and allergic reciprocation.[67] Cisplatin encapsulated liposome formulation lipoplatinumis extends the duration of drugs, enhances cell pervious, and assembles drugs in tumour tissue.[68,69]

Table 1: Liposomal-approved drug used in anticancer therapy[65-71]

Product name	*Drug*	*Liposome composition*	*Cancer targeted*
Doxil	Doxorubicin	HSPC:CL:MPEG 2000-DSPE	AIDS-associated Kaposi's sarcoma, ovarian carcinoma
Thermodox	Doxorubicin	DPPC:MSPC:MPEG2000DSPE	Hepatocellular carcinoma, Colon cancer, childhood cancer, liver tumors, pancreatic carcinoma, breast carcinoma
Lipo-Dox®	Doxorubicin	DSPC:CL:MPEG 2000-DSPE	AIDS-associated Kaposi's sarcoma, ovarian cancer, breast cancer, and multiple myeloma
Lipoplatin	Cisplatin	SPC-3: DPPG: CL: MPEG2000-DSPE	Cancerous effusions in the pleura
Zolsketil®	Doxorubicin	HSPC:CL:MPEG 2000-DSPE	Multiple myeloma, AIDS-related Kaposi's tumor, and metastatic carcinoma of the breast
Mepact®	Mifamurtide	DOPS: POPC	Osteosarcoma
Onivyde®/Nal-IRI	Irinotecan	DSPC:CL:MPEG 2000-DSPE	Pancreatic cancer
PLM60	Mitoxantrone	HSPC:CL:MPEG 2000-DSPE	Small-cell tumors in the lungs, progressive carcinoma of the liver, and non-Hodgkin's lymphoma

Abbreviation: DSPC (distearoylphosphatidylcholine), DOPC: dioleoylphosphatidylcholine; DOPS: dioleoyl-phosphatidylserine; HSPC (hydrogenated soy phosphatidylcholine); DPPC: dipalmitoylphosphatidylcholine; DPPG:dipalmitoylphosphatidylglycerol; MSPC: monostearoylphosphatidylcholine; SPC-3, soy phosphatidyl choline

A lengthy circulating liposomal injection called Onivyde™ is used to manage pancreatic ductal adenocarcinoma. It functions as a topoisomerase inhibitor, preventing cancer cells from replicating their DNA.[70] It has been demonstrated that EndoTAG-1TM, a paclitaxel-loaded cationic liposome formulation, predominantly targets angiogenesis endothelial cells. As a result, EndoTAG-1TM can lessen cancer growth and metastasis by reducing the blood supply to the tumor. According to a category of clinical trials for many liposomal-based formulations, patients with endometrial or lung cancer that has metastasized or recurred may benefit from aerosolized liposomal camptothecin.[72,73]

In infectious disease

In parasitic illnesses such as Kala azar (black fever), monilia disease, mycotic pneumonia, histoplasmosis, high hematocrit, infection of the intestine with protozoa, sharp fever and tuberculosis, liposomes act as an efficient drug-delivery carrier.

Recently, in the COVID-19 pandemic, patients were also affected by black fungus developed an infectious disease known as mucormycosis. The most effective drug used in the treatment of mucormycosis is amphotericin B. In its free form, because it induces dose-related adverse effects such as ionosphere poisoning and neurotoxicity, amphotericin B has a limited penetration rate and is less efficient. The increased medication penetration and absorption rate at the target site of the liposomal amphotericin B formulation has greatly increased its effectiveness as a treatment. Moreover, it allows for lower drug dosage, thereby reducing the occurrence of dose-related adverse effects. Its ability to lower drug toxicity is also noteworthy. Additionally, this formulation can help to minimize the occurrence of drug resistance.[74, 75]

The main downsides of antimalarial drug molecules include failure to treat owing to *Plasmodium* species resilience and reduced bioavailability because of poor water solubility, bio-stability, and opacity. Malaria is endemic in many countries across the world. Due to liposomes' dual hydrophilic and hydrophobic characteristics, a drug-delivery framework utilizing them can administer water-soluble as well as fat-soluble antimalarial medicines simultaneously to lower the prevalence of *Plasmodium* species resilience.[76] This can get around the constraints of antimalarial medication. Furthermore, liposomes are a viable delivery route for antimalarial drugs due to their low toxicity, sustainability, and biocompatibility.

Depending on how severe the condition is, local or systemic chemotherapy may be used to treat the parasitic disease leishmaniasis. Antimonial, amphotericin B, pentamidine, paromomycin, and miltefosine are some of the drugs used in conventional drug therapy; however, these drugs are less effective and have a high level of toxicity in addition to a number of negative side effects.[77, 78]

Due to their particular affinity for the parasite, dinitroanilines are curiously used to treat leishmaniasis. On the other hand, their limited water solubility along with unstable, have hampered their management as antiparasitic agents. These barriers were removed by the drug's encapsulation in dimyristoyl PC- plus dimyristoyl phosphatidylglycerol-based liposomes, which made it possible to distribute the medication effectively, as shown in animal models and provide therapeutic benefits.[79] Buparvaquone, a drug used to treat visceral leishmaniosis that has undergone substantial research, is another medication being studied for liposome administration. In the context of a hamster model, it was discovered that buparvaquone, which impacts host cells immuno-modulatory, was highly effective in eliminating *Leishmania infantum* parasites when given to macrophages in the form of phosphatidylserine (PS)-exposing liposomes.[80]

In respiratory diseases

When addressing respiratory illnesses, a liposome-based nano vesicular drug delivery system can be more effective because of its enhanced site specificity and dosage precision at the target locations, reducing systemic toxicity and irritation of the lungs.[81]

The ailment of asthma, which embraces wheezing, shortness of breath, chest tightening, and decongestant, is diverse

along recurrent and is caused by exceeding inflammation, airway remodeling, plus airway hyper-responsiveness. Bronchodilators, as well as other anti-inflammatory (ICS) drugs, should be taken regularly to manage asthma.[82] The goals of current asthma treatments are to manage symptoms and avoid asthma flare-ups. However, his method has a number of negative side effects and necessitates lifelong therapy.[78]

Pneumonia, the condition of the air sac, is usually treated and managed with antibiotics. The curative effect of medications can be improved by using liposomes to prolong their duration in the lungs and raise their concentration in lung tissue.

Liposomes have the ability to entrap molecules at the bilayer junction or in the water-filled center of the phospholipid bilayer, depending on the type of medication. The therapeutic index of encapsulated medications, including amphotericin, doxorubicin, levodopa, and several other pharmaceuticals, was expanded by liposomal delivery, which lowered the toxicity effects of those treatments.[83] As compared to conventional aerosol formulation, liposomal aerosols offer sustained release, minimize local irritation & toxicity, and enhance stability. For inhalation during nebulization, either the liquid or dry form is used. Spray drying or milling are both used to create drug powder liposomes.

Liposomes as vaccines adjuvant

Liposomes have the potential to be widely used as adjuvants in vaccines to boost the therapeutic efficacy of medicines due to their high biocompatibility and adaptability, high loading capacity, versatile nature & targeting properties. Emulation of viral membrane-bound proteins or peptide antigens within liposomes has been shown to elicit immune responses that are both humoral and cell-mediated. Liposome also offers robust and persistent immunity against the pathogenic microbe.[84, 85]

Through the integration of immunopotentiators and targeting agents with sophisticated vaccination tools, liposomes have rapidly evolved into a versatile vaccine adjuvant-delivery arrangement (VADS). As a result of this interaction, liposomes activate target immune cells plus even cellular structure, facilitate lysosome evasion, and stimulate Ag cross-conferral, significantly improving the therapeutic effect of vaccines.[86]

Numerous investigations have demonstrated the MHC class-I display and the generation of cytotoxic T lymphocytes (CTLs) by liposome-synthesized antigens, which are widely used.

Liposomes in brain targeted drug delivery

Liposomes are small vesicles made of lipids that can carry drugs inside them. By breaching the barrier between the blood and the brain, they may be able to deliver drugs to the brain (BBB), a protective layer that blocks most chemicals from entering the brain. Liposomes can be enhanced by adding distinct molecules on their surface to target specific cells or receptors in the brain through different routes, such as intravenous injection, intranasal administration, or direct injection into the brain. Liposomes can be attached to antibodies or other molecules that can attach to particular receptors on the BBB or the brain cells, allowing them to cross the barrier and reach the specific site. One of the most studied nanoscale drug delivery methods is liposome delivery, which can be used to administer a range of medications, such as anti-inflammatory, anticancer, neuroprotective, gene therapy vectors, or imaging agents. Through increased stabilization, liposomes can both maximize the effectiveness of therapy and reduce the adverse effects of these medications.[87]

Yagi and his co-workers formulate a liposome using sulfatide and monoclonal antibodies as a sensory device to tramp over the blood-brain barrier to reach human gliomas. It increases the target efficiency of liposomes via bridging the cerebral blood obstruction. In rat brain tumors, egg PC liposomes covered with CHP were likewise considerably collocated (Ochi *et al*. 1990). The Fisher-344 strain of rats implanted with 9L-gliosarcoma was given carotid injections of CHP-coated lipid tagged with [14C]-DPPC.[12]

After injecting the liposome, specific tissue (tumor, ipsilateral and contralateral brain, liver, spleen, kidney, and blood) was removed 30 minutes later. Investigations were made into the liposome's tissue distribution with and without the CHP covering. The distribution of the CHP-coated liposome was shown to be 4.5 times more in the tumor, four times smaller in the spleen, and 2.1 times greater in the ipsilateral brain when compared to the control lipid particles.

In ophthalmology/ocular preparation

The majority of traditional dosage forms, such as eye lotions, ointments, plus suspensions, are used for the treatment of various ocular illnesses. These dosage forms have low ocular absorption because the eye has a number of pathophysiological and anatomical constraints. Liposomes were employed for the treatment of ocular disorders, particularly those affecting the anterior and posterior regions.[88]

It was first noted in 1981 that idoxuridine-encapsulated liposomes had improved effectiveness in treating corneal ulcers caused by herpes simplex infection in rabbits. It was demonstrated in 1985 that the application of liposome carriers can either help or impede the ocular transport of pharmaceuticals based on the physical and chemical characteristics of the medications and the lipid combination utilized. For an array of ocular circumstances, including fungal infections of the eyes, corneal transplant rejection, endophthalmitis, uveitis, and proliferative vitreoretinopathy, liposomal formulations have been developed in large quantities.[89]

Eye drops containing the antibiotic ciprofloxacin (Ciprocin) are available commercially and effective as opposed to dual aerobic gram-positive plus gram-negative bacteria. The Albino rabbit was used as an animal dummy to compare ciprofloxacin eye drops and liposomal formulations. The highest aqueous humor concentration (Cmax) from the liposomal formulation was determined to be 3.87 µg/mL, which was almost the same as the Tmax of 2.68 µg/mL for ciprocin eye drops. As an outcome, encapsulating ciprofloxacin in a liposomal formulation increases bioavailability and residence duration,

reduces the dose of the antibiotic, and increases the possibility of penetration through the ocular tissue.[90]

Montmorillonite (Mt), an ion-exchange drug carrier with a large surface area, and bexolol hydrochloride (BH), an efficient treatment for glaucoma, were integrated and mixed to generate liposomes (Mt-BH-LPs) for use as an ocular drug delivery system. Comparatively, the betaxolol hydrochloride specimen revealed a quick ransom of about 100% within 2.5 hours, whereas Mt-BH-LPs (50.3%) indicated a longer release.[91,94]

These investigations concluded that liposomal ocular formulations could lengthen the period that drugs remained on the eye's surface while reducing their removal.

CONCLUSION

Liposomes are the most commonly used therapeutically authorized nanocarriers for anticancer medicines. Liposomes have been praised for their beneficial properties; they offer a plethora of therapeutic pharmaceutical uses as drug delivery vehicles, especially in the treatment and diagnostics of cancerous cells. The ability of long-circulating liposomes to improve the administration of medication to target tissues is generating attention and getting clinical approval. Furthermore, the biocompatibility and lower medication clearance of liposomes make them appealing. As previously said, considerable efforts have been made since the introduction of Doxil® in 1995 to bring these adaptable nanomaterials to the laboratory; despite this, modifications to their structure are still necessary to fix some challenges. Liposomes' physical and chemical fragility is one of the most serious issues they face. Despite the fact that liposomes are usually benign and have little intrinsic toxicity, research on the biological and toxicological consequences of prolonged liposome exposure in humans and animals should be conducted. The critical necessity for more-durability liposomes has a substantial effect on their medical applicability.

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