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An Extensive Investigation of Liposomes

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ABSTRACT
The development of the liposomal drug delivery method has been hastened by the capacity of bilayer vesicles to carry drugs, vaccines, diagnostic tools, and other bioactive substances. The cytotoxicity of many potent therapeutic medications could also be decreased by using a liposomal product to achieve site avoidance and site-specific medication targeting therapy. This article's goal is to provide an overview of the liposomal drug delivery system. It has focused on the factors that affect how liposomes behave in a biological setting. The study also explored a number of subjects, including the production mechanism of liposomes and the stability and characterization of liposomal pharmaceuticals. Liposomes can be used as a therapeutic tool for tumour targeting, genetic transfer, immunomodulation, skin, and topical therapy, among other things.
KEYWORDS:cytotoxic formulation, bilayered vesicles, and drug encapsulation in percentage.

I. INTRODUCTION

Novel chemical entities (NCEs) having potential therapeutic effects on biological systems have been discovered as a result of advances in combinatorial chemistry. However, the majority of the NCEs discovered present a challenge to formulation scientists due to their physicochemical characteristics, such as poor solubility and permeability. Even though the aforementioned problems might be resolved, most compounds do not exhibit the intended therapeutic action in humans, hence there is no connection between in vitro and in vivo. [1,2] Most anti-neoplastic medications have an impact on normal cells in addition to being highly cytotoxic to tumour cells in vitro. This is so that the dose necessary to provide an anti-tumor effect won't be toxic to healthy cells (low therapeutic index, or TI). Such treatments must be directed to a specific area in order to reduce their risky effects on healthy tissues (diseased site). [3] To ensure that the greatest percentage of the delivered dose reaches the target site, an efficient drug delivery system is therefore required. Several carriers, including nanoparticles, microparticles, polysaccharides, lectins, and liposomes, can be used to deliver the medication to a particular location. [4-9] Due to its contributions to a number of industries, including medication administration, cosmetics, and biological membrane structure, liposomal drug delivery is becoming more and more well-known. [10] Liposomes have the potential to be used as therapeutic carriers for a variety of drugs. Colloidal carriers called liposomes have sizes that range from 0.01 to 5.0 m. In fact, bilayered vesicles are produced when phospholipids are overhydrated in aqueous media[11,12]. The advantage of liposomes is their capacity to encapsulate both hydrophilic and hydrophobic medications and transport them to the affected area of the body. [10] phospholipid and a liposome's bilayered vesicle structure. Bilayered vesicles can be used to encapsulate anticancer drugs, vaccines, antimicrobials, genetic resources, proteins, and macromolecules. [13] Liposomal technology has been used to successfully encapsulate paclitaxel[14], acyclovir[15], tropicamaide[16], arteether[17], chloroquine diphosphate[18], cyclosporine[19], and dithranol[20]. the small number of liposomal products that have been given human use approval. Liposome Formation Mechanism The central component of a liposome is made up of amphiphilic molecules called phospholipids (having a hydrophilic head and hydrophobic tail). While the hydrophobic portion is made up of two fatty acid chains, each having 10–24 carbon atoms and 0–6 double bonds, the hydrophilic portion is predominantly composed of phosphoric acid bound to a water-soluble molecule. [21] To form the spherical/vesicle-like structures known as liposomes, these phospholipids organise into lamellar sheets with the polar head group facing outwards to the aqueous region and the fatty acid groups facing each other. While the non-polar component is protected, the polar portion maintains contact with the aqueous environment (which is oriented at an angle to the membrane surface). [22] The hydrophilic/hydrophobic interactions between lipid-lipid and lipid-water molecules during the

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hydration of phospholipids in water lead to the production of bilayered vesicles, which enable the aqueous phase to achieve thermodynamic equilibrium. [23] The following are a few factors that affect the creation of bilayers. • By producing large bilayered vesicles, the high free energy difference between the hydrophilic and hydrophobic environments may be reduced, and maximum stability to supramolecular self-assembled structures can be achieved. Folding into tight concentric vesicles reduces the unfavourable interactions between the hydrophilic and hydrophobic phases.

Differentiating Liposomes

The literature reveals a wide range of sizes and forms for liposomes. They can be categorised according on their size, number of bilayers, composition, and manufacturing method. According to the size and number of bilayers, liposomes are classified as multilamellar vesicles (MLV), large unilamellar vesicles (LUV), and small unilamellar vesicles (SUV), as illustrated in Fig. 2. According to their chemical makeup, different forms of liposomes include conventional liposomes (CL), pH-sensitive liposomes, cationic liposomes, long-circulating liposomes (LCL), and immuno-liposomes. Reverse phase evaporation vesicles (REV), French press vesicles (FPV), and ether injection vesicles are the three different varieties dependent on how they are created (EIV). The division based on the dimensions and variety of bilayers.

Multilamellar vesicles (MLV)

MLVs are composed of two or more bilayers and have a diameter more than 0.1 m. Simple hydration of lipids or thin films with an excess of organic solvent is used in their manufacture. Even after extensive storage, they maintain their mechanical stability. They can be utilised to target RES organs because of their huge size and the speed with which reticulo-endithelial system (RES) cells eliminate them. [3] As measured by the proportion of aqueous to lipid volume, MLV have a moderate trapped volume. Increased drug trapping in the vesicles can be achieved through slower hydration and careful mixing. [24] Hydrating thin lipid films made of dry lipids can increase encapsulation effectiveness. [25] Lyophilization and rehydration with the aqueous phase allow MLV to be produced with an encapsulation effectiveness of 40%. (containing the drug). [26, 27]

Large unilamellar vesicles (LUV)

These liposomes are composed of a single bilayer and have a diameter more than 0.1 m. They have a higher encapsulation efficiency because they contain a big volume of solution storage space in their hollow. [28] Because of the substantial volume they have trapped, they can be utilised to encapsulate hydrophilic medications. LUV has the benefit of using less lipid to encapsulate a substantial amount of medication. They are eliminated by RES cells more quickly than MLV because of their larger size. [3,8] LUV can be produced using a variety of techniques, such as ether injection, detergent dialysis, and reverse phase evaporation. In addition to these techniques, LUV can also be prepared by slow swelling of lipids in non-electrolyte solution[32], freeze-thawing of liposomes[29,30], dehydrating/rehydrating SUV[31], and freeze-thawing of liposomes[29,30].

Small unilamellar vesicles (SUV)

SUV are smaller (less than 0.1 m) and have a single bilayer when compared to MLV and LUV. They are distinguished by a long circulation half life and a low entrapped aqueous volume to lipid ratio. SUV can be created by extruding or sonicating MLV or LUV under an inert environment such as nitrogen or argon to reduce their size, or by using solvent injection techniques (ethanol or ether injection methods)[33]. Sonication can be done with a probe sonicator or a bath sonicator. In order to achieve SUV, MLV can also be forced under high pressure via a small hole. These SUVs are susceptible to aggregation and fusion at low or no charge. [34]

Methods Of Preparation

Some of the typical methods for creating liposomes include solubilizing lipids in organic solvents, drying lipids from organic solutions, dispersing lipids in aqueous media, purifying the resulting liposomes, and analysing the finished product. [35] The simplest and most often used technique for creating liposomes is thin-film hydration. This method results in MLV with sizes ranging from 1 to 5 m. Drugs that are hydrophilic enter the aqueous buffer; those that are hydrophobic enter the lipid film. For hydrophobic medications, however, this method has a low encapsulation efficiency (approximately 5–15 percent). Hydrating the lipids in the presence of an organic solvent can increase the MLV's encapsulation effectiveness. [36,37] LUV can be

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produced using a variety of techniques, including solvent injection, detergent dialysis, calcium-induced fusion, and reverse phase evaporation. SUV can be created by sonicating or extruding MLV or LUV. These all need the use of organic solvents or detergents, which can be dangerous even in little quantities. There are also techniques to prevent this without using organic solvents or detergents, such as polyol dilution[38], bubble method[39], and heating approach[40]. The synthesis of liposomes is described in depth in the literature[21,35].

Typicality Of Liposomes

The performance of liposomes in vitro (for sterilisation and shelf life) and in vivo (for disposition) varies depending on the procedures used to create them. [41- 43] Rapid, exact, and repeatable quality control tests are required for characterising the liposomes after formulation and storage to ensure a predictable in vitro and in vivo behaviour of the liposomal medicinal product. [44,45] A liposomal pharmaceutical formulation can be described using some of the criteria listed below.

Size and the distribution of sizes

The size distribution is important when liposomes are intended for parenteral or inhalation delivery since it impacts both the in vivo fate of the liposomes and the therapeutic molecules they contain. [46-50] The size of the vesicles is measured by microscopy (optical microscopy[51], negative stain transmission electron microscopy[42], cryo-transmission electron microscopy[52], freeze fracture electron microscopy, and scanning electron microscopy[45]), laser light scattering and photon correlation spectroscopy[45], diffraction and scattering methods, and hydrodynamic methods (field flow fractionation[53], gel permeation[54] and ultracentrifugation).

Drug encapsulation percentage

The amount of drug encapsulated or trapped in a liposome vesicle is known as the percent drug encapsulation. The amount of medication that liposomes encapsulate can be calculated using column chromatography. [55] The medication is offered both free (unencapsulated) and enclosed. To calculate the precise amount of medicine encapsulated, the free drug is separated from the encapsulated one. The medication is then freed from the vesicles and released into the surrounding medium after the drug-encapsulated fraction of liposomes has been treated with a detergent to accomplish lysis. The amount of medicine that is encapsulated when this exposed drug is assessed using a suitable method produces the encapsulation efficiency. [56-59] The trapped volume per lipid weight method can also be used to determine the percentage of medication stored in a liposome vesicle. The most common unit of measurement is aqueous volume entrapped per unit quantity of lipid, or l/mol or g/mg of total lipid[41,43]. The confined volume can be determined using a variety of materials, including radioactive markers, fluorescent markers, and spectroscopically inert fluid[60]. Calculating confined volume frequently uses the radioactive technique. [41] It is determined by dispersing lipid in an aqueous solution containing a non-permeable radioactive solute like [22Na] or [14C] inulin[61]. Water soluble markers like 6-carboxyfluorescein, 14C or 3H-glucose, or sucrose can be used to estimate the confined volume. [45] Additionally, a novel technique for measuring intravesicular volume via salt entrapment has been made public. [62]

Surface charge

Because the charge on the liposome surface is so important for in vivo disposal, knowing the surface charge on the vesicle surface is crucial. The surface charge of the vesicle can be estimated using two methods: free-flow electrophoresis and zeta potential testing. Estimating the mobility of the liposomal dispersion in a suitable buffer (found using the Helmholtz–Smolochowski equation)[63] can be used to compute the surface charge.

Shape and lamellarity of the vesicle

The shape of the vesicles can be evaluated using a number of different electron microscopy methods. The number of bilayers present in the liposome, or lamellarity, can be determined by freezefracture electron microscopy[41] and 31P-Nuclear magnetic resonance analysis[64]. In addition to identifying the shape and lamellarity of liposomes, the surface morphology can be investigated using freeze-fracture and freeze-etch electron microscopy[64].

Phospholipid analysis and identification

Chemical components of liposomes need to be checked both during and after manufacture. [45] Thin layer chromatography, the Barlett test,

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and the Stewart assay are methods that can be used to quantify the amount of phospholipids present in the liposomal formulation. [67] A spectrophotometric method was used to quantify the intensity of the blue colour produced at 825 nm against water in order to calculate the total phosphorous in a sample. [68] Cholesterol levels can be determined using gas liquid chromatography methods[70], cholesterol oxidase tests, or the ferric perchlorate method[69].

Preservation of Liposomes

A key consideration while developing liposomal pharmaceuticals is the stability of the formulation that is created. The therapeutic efficacy of the medicine is determined by the liposomes' stability throughout the whole production process, including storage and administration. During the creation and preservation of stable dosage forms, the active molecule's physical stability and chemical integrity are maintained. A properly-designed stability study will include the assessment of physical, chemical, and microbiological parameters as well as the assurance of product integrity during the storage period. A stability methodology is therefore needed to examine the drug product's physical and chemical integrity over the course of storage.

physical steadiness

Bilayered vesicles called liposomes are created when phospholipids are hydrated in water. Different sizes of vesicles are produced as a result of this process. In order to create a thermodynamically advantageous situation, the vesicles tend to group together and expand in size while being stored. The physical stability of the liposomal drug product may be jeopardised if drug leakage from the vesicles occurs during storage as a result of vesicle fusion and breaking. In order to assess physical stability, vesicle structure, size, and size distribution are essential factors. [28] The visual appearance (morphology) and size of the vesicles can be estimated using a variety of techniques, including light scattering and electron microscopy[71].

chemical resistance

Chemically unsaturated fatty acids known as phospholipids are vulnerable to oxidation and hydrolysis, which jeopardises the stability of the medicine. A stable liposomal composition depends on factors such as pH, ionic strength, solvent system, and buffering species. Chemical reactions can be triggered by anything, including oxygen, heat, light, and heavy metal ions. Oxidation degradation is the term for the formation of cyclic peroxides and hydroxyperoxidases as a result of the production of free radicals during the oxidation process. Liposome oxidative degradation can be prevented by using antioxidants such alpha-tocopherol or butylated hydroxyl toluene (BHT), manufacturing the product in an inert environment (such as with nitrogen or argon present), or adding EDTA to remove trace amounts of heavy metals. [21,28] The hydrolysis of the ester bond at the carbon position of the glycerol moiety of phospholipids results in the formation of lyso-phosphatidylcholine (lysoPC), which increases the permeability of the liposomal contents. Controlling the amount of lysoPC in the liposomal pharmaceutical product is therefore crucial. This can be achieved by producing phosphatidylcholine-free liposomes. [21]

In Vivo Liposome Behavior

During liposomal formulation optimization, a number of physico-chemical parameters are altered to ensure that medications are properly biodistributed and taken up by cells. The factors listed below determine how well liposomes operate in vivo (biologically).[72]

Liposome size

Because the fraction cleared by RES is governed by the size of the vesicle, the in vivo fate of liposomes is determined by the size of the vesicle. [73] As the vesicle size develops, RES absorbs liposomes at a faster pace. Larger liposomes are more readily taken up (opsonized) by RES compared to liposomes less than 0.1 m. The size of the vesicle also affects liposome extravasation. Tumor capillaries are more permeable than healthy capillaries. Such a leaky vasculature allows for the passage of liquids and microscopic liposomes, which enhances the accumulation of drug-loaded liposomes in tumour tissue. Tiny liposome extrvasation is fueled by the difference between intravascular hydrostatic and interstitial pressure. [74]

Interfacial tension

The kind and amount of charge on the liposome surface can affect how lipids interact with cells. By charging the lipid content, the type and charge of the liposome can be altered. SUV liposomes' lack of charge can lead to aggregation, which reduces the liposome's stability;

nevertheless, neutrally charged liposomes hardly ever interact with cells. [75,76] The strong electrostatic surface charge of the liposome might help to improve lipid-cell interaction. Positively charged density changes the degree of lipid-cell interactions and boosts the intracellular absorption of liposomes by target cells. [77] On the other hand, positive-charged liposomes are eliminated more quickly following systemic administration. Contrary to negatively charged liposomes, cationic liposomes deliver their contents to cells via merging with the cell membrane. [78]

Surface moisture

RES cells are less likely to absorb liposomes with hydrophilic surface coatings because they are less likely to opsonize. This is because liposomes are unable to interact with the components of cells and blood because of their hydrophilic surface coating. [79-81] These sterically stabilised liposomes are more stable in the biological environment and have longer circulation half-lives than liposomes with hydrophobic coverings. The steric stability of liposomes is a result of hydrophilic groups such monogangliosides, hydrogenated phosphotidyl inositol, and polyethylene glycol. [82,83]

Fluidity in a bilayer

Lipid exists in a number of physical states both above and below the phase transition temperature (Tc). They are rigid and well-ordered below Tc, whereas fluid-like liquid-crystalline forms are present above Tc. Table 2 includes a list of the phase transition temperatures for numerous phospholipids. [3,21] Low Tc (below 37°C) liposomes are fluid-like in consistency and prone to drug content leaking at physiological temperatures. High Tc (above 37°C) liposomes are rigid and leaky at physiological temperatures. The phase transition temperature also controls the liposomal cell interaction. Liposomes with low Tc lipid content had a higher level of RES uptake as compared to those with high Tc lipid content. [80] Liposomes can become more stable at temperatures above the phase transition point when cholesterol is incorporated into the bilayer, which decreases membrane fluidity.

Therapeutic Uses For Liposomes

When a conventional dose form fails to have the desired therapeutic impact, new drug delivery methods are created. One of these systems that outperforms current formulations in terms of therapeutic effectiveness and safety is liposome technology. The most popular therapeutic applications for liposomes in drug delivery include the following.

Delivery that avoids the site

The cytotoxicity of anti-cancer medications to healthy tissues is brought on by their low therapeutic index (TI). In these situations, liposome encapsulation of the medication can minimise drug distribution to normal cells and improve the TI. Free doxorubicin has a high level of toxicity, but when it is contained in liposomes, the level of toxicity is reduced without impacting the drug's therapeutic effectiveness. [3, 84]

Targeting specific websites

Site-specific targeting can deliver a higher fraction of the medicine to the desired (diseased) site by reducing the drug's exposure to normal tissues. A safer and more efficient form of treatment is possible when drugs are encapsulated in liposomes for both active and passive drug targeting. [3] After systemic therapy, long circulating immunoliposomes have a higher selectivity for identifying and binding to target cells. [85,86] Monocyte tumoricidal activity was elevated when muramyl peptide derivatives were created as liposomes and administered systemically to patients with recurrent osteosarcoma. [87]

medication delivery inside cells

The liposomal drug delivery system can speed up the transfer of potent medications to the cytosol (where drug receptors are located).
[3] N-(phosphonacetyl)-L-aspartate (PALA) is often poorly assimilated by cells. When contained in liposomes, these drugs operate more effectively against ovarian cancer cell lines than free drugs do. [76]

Medication delivery with sustained release

To attain the highest level of therapeutic efficacy, medicines that need a prolonged plasma concentration at therapeutic levels can be delivered using liposomes. [3] Drugs like cytosine arabinoside can be liposomally encapsulated for longer release and an ideal drug release rate in vivo. [88] administered intraperitoneally Tumors that develop in the intra-peritoneal (i.p.) cavity may be treated with the medication. However, the drug concentration at the ill region is kept to a minimal because the drugs are promptly evacuated from the i.p. cavity. As opposed to free medicines,

liposomally encapsulated medications have a reduced clearance rate and can deliver the maximum amount of drug to the target site for a longer period of time. [89, 90]

vaccinations with immunological adjuvants

Immune response can be improved by delivering liposomal antigens. Depending on their lipophilicity, antigens can either be accommodated in the aqueous cavity or integrated into the bilayers. [3] As immunological adjuvants to enhance the immune response to diphtheria toxoid, liposomes were first used.[91]

II. CONCLUSION

Numerous incredibly potent drug candidates with constrained therapeutic applications can be delivered to the desired ill spot using liposomal drug delivery technology. Drug pharmacokinetics in liposomes can be significantly impacted. The success of the liposomal formulation depends on its capacity to distribute the therapeutic molecule to the desired area over an extended period of time while minimising adverse effects. The phospholipid bilayers that surround the pharmaceuticals should allow the drugs to gradually diffuse out of them. The formulation of liposomal drug delivery systems must take into account a number of factors, including drug concentration, drug to lipid ratio, encapsulation effectiveness, and in vivo drug release. Examples of technological advancements include the production of deformable liposomes and ethosomes, as well as the inhalation and ocular injection of drug-loaded liposomes. Because of this, the liposomal approach can be utilised to lessen the toxicity of a range of very potent drugs while also improving pharmacokinetics and therapeutic efficacy.

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