Optimizing Drug Design: Balancing efficacy & Reduced Toxicity of Novel therapeutics in liposomes

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ABSTRACT: The aim behind the selection of this topic is to study the development of novel therapeutics in liposomal formulation, which has garnered significant interest due to its potential to enhance drug delivery while minimizing toxicity. This article reviews the strategies for optimizing liposome drug design, focusing on balancing therapeutic efficacy and safety profiles. Important considerations include liposomal composition, drug encapsulation method, targeting strategy, and pharmacokinetic properties. This review article aims to understand the essence and concept of the balance between efficacy and reduced toxicity of new liposomal therapeutic products. It further aims to massively review the existing literature on balancing efficacy and reduced toxicity of novel therapeutics in liposomes. This conceptual study is completed with the help of secondary data. It presents the thoughts of the authors and researchers who contributed to this study.

KEYWORDS*: liposomes, drug design, efficacy, toxicity, cholesterol, phospholipid*

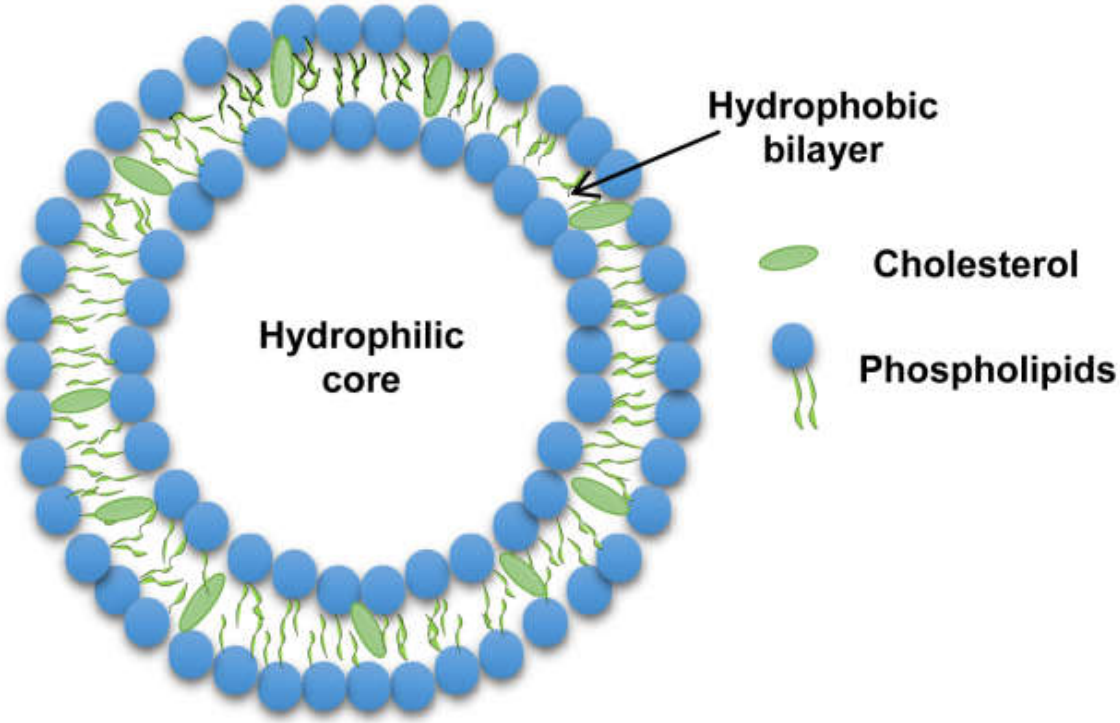
INTRODUCTION: Liposomes, spherical concentric vesicles, are a combination of the Greek words "lipos" meaning fat and "soma" meaning body.Liposomes are phospholipid molecules contained within round sacs.Liposomes encapsulate water droplets, especially when artificially formed to deliver drugs to cell membranes. Liposomes are nanoparticles of 100 nm size.**[1]**When Bangham first described liposomes in 1961, it was a serendipitous discovery: He dispersed phosphatidylcholine molecules in water and found that they formed a closed bilayer in which the aqueous portion was trapped within the lipid bilayer.**[2]** Liposomes have a medicinal or other use and function as many drugs. Drugs are targeted in specific areas using a variety of environments, such as lectin, liposomes, polysaccharides, nano particles, and fine particles. Due to its contributions to several fields including drug delivery, cosmetics, and biological membrane structure, liposome drug delivery is becoming increasingly popular.**[3]**

Definition of liposomes: Liposomes are spherical vesicles composed of one or more phospholipid bilayers, closely resembling the structure of cell membranes. The ability of liposomes to encapsulate hydrophilic or lipophilic drugs has enabled these vesicles to become useful drug delivery systems.

Toxicity: The degree to which a substance possesses the potential to inflict damage upon humans or non-human animals. Acute toxicity pertains to the detrimental consequences experienced by an organism resulting from a singular or brief exposure. Subchronic toxicity refers to the capacity of a toxic agent to induce effects that persist for a duration exceeding one year but falling short of the entire lifespan of the affected organism.

Novel therapeutics: By collaborating at the nexus of engineering, biology, chemistry, and medicine, the new medicines delivery program promotes the creation of breakthrough technologies and methods for the delivery of current or novel therapies. You may rapidly and effectively rework and rephrase your content with the help of uillBot's paraphraser, which takes your sentences and makes adjustments!

Structure of liposomes:



Schematic representation of liposomes.

1. **Phospholipids** • Liposomes include naturally occurring phospholipids: Phosphatidylethanolamine for example

   o Choline Phosphatidyl
   o Serine phosphatidylserine

   • Dioleoyl phosphatidylcholine and Other Synthetic Phospholipids The liposomes contain the following synthetic phospholipids:
   Phosphatidylethanolamine dioleoyl

2. **Cholesterol**
   The membrane of phospholipids contains cholesterol to phosphatidylcholine molar ratios as high as 1:1 or 2:1. Cholesterol is an amphipathic molecule that enters the membrane with its aliphatic chain parallel to the acyl chains in the middle of the bilayers and its hydroxyl group pointing toward the aqueous floor. Additionally, it removes the typical hydrogen bonding and electrostatic interactions and lengthens the distance between choline head structures.**[4]**

Liposomes can be classified into four groups based on their size and number of bilayers: multivesicular vesicles (MVVs), multilamellar vesicles (MLVs), large uniferal vesicles (LUVs), and small uniferal vesicles (SUVs). In the multilamellar structure, liposomes resemble an onion, whereas in the uniflagellated structure, liposomes contain a single phospholipid bilayer. Since multiple unifellar vesicles are created within larger liposomes, the MVV forms a multilayer configuration with concentric phospholipid

spheres.**[5]** Only for hydrophilic substances, the efficiency of liposome encapsulation increases with increasing liposome size and decreases with increasing number of bilayers.**[6]** An important element influencing the circulatory half-life of liposomes is the size of the vesicles. The amount of drug contained depends on the size and amount of the bilayers. If liposomes are used for medication, the doubt of the veline generally has a 50-150 nm amount. Many ideas explain how liposomes interact with the cell membrane that contains food action, local fusion (adhesive), selective (mediation by receiver) or non -specific absorption of cell membranes. Liposome-cell interactions are influenced by many parameters, including composition, liposome diameter, surface charge, targeting ligands on the liposome surface, and the biological environment**.[7]** Drug delivery systems (DDS) have the potential to improve the therapeutic index of drugs by increasing drug concentration and residence time in target cells and reducing side effects.**[8]** Liposomes are the most studied nanocarriers for targeted drug delivery systems. When natural or synthetic lipids are emulsified in an aqueous solution, spherical lipid vesicles known as liposomes are formed, consisting of one or more lipid bilayers. Their diameter is usually between 50 and 500 nm.**[9]** Liposomes are spherical vesicles with an aqueous core and a phospholipid bilayer. While lipophilic molecules can be trapped in the lipid bilayer, hydrophilic molecules can be confined in the aqueous core. Therefore, the amphiphilic properties of this lipid carrier make it suitable for loading drug compounds with different physicochemical properties**.[10]** Since liposomes are made from biocompatible lipids, they are generally nontoxic. Importantly, the pharmacokinetics and biodistribution of drugs and other encapsulated compounds can be controlled by deliberately altering the composition, size, surface charge, and modifications of liposomes. This has notably led to the use of liposomes to modify drug biodistribution and extend half-life, which has been shown to improve the safety and efficacy profiles of several important anticancer drugs**.[11]** Phospholipids, such as synthetic diallyl and trialkyl lipids from soybean phosphatidylcholine, are major components of liposomes. The incorporation of cholesterol into liposomes is important because cholesterol alters fluidity in the presence of body fluids such as blood and plasma, regulates membrane permeability, and enhances bilayer stability**.[12]** Targeted nanoparticles (NPs) have been developed to improve the systemic toxicity and therapeutic efficacy of several drugs, including anticancer drugs**.[13]** To create effective ligand-targeted nanoparticles, targeting moieties with high binding affinity and selectivity must be used in combination with an appropriate conjugation method, the latter of which has been shown to have adverse effects on ligand orientation and structure**.[14**] Many chemotherapy include harmful side effects, prompt metabolism, and stability development, and may limit its usefulness. To avoid these restrictions, we use liposomes and other nanoparticles to enhance the treatment efficiency of some chemotherapy. Long circulation duration, improved pharmacokinetic properties of encapsulated drugs, passive targeting and distribution to tumors and sites of inflammation are just some of the advantages offered by liposomes.The effectiveness of many chemotherapeutic drugs can be limited by rapid metabolism, severe side effects, and the development of resistance. Nanoparticles such as liposomes are used to improve the treatment efficiency of specific chemotherapy to avoid these restrictions.

The transparency of liposomes and the improved mechanism of the maintenance (EPR) enables passive targeting and exclusion in the tumor and inflammatory area, and the better drug objective characteristics and long -term circulation of the encapsulated drugs. They can also reduce the systemic toxicity of free drugs.In addition, liposomes can increase the solubility of drugs and provide a gradual and continuous release of the encapsulated drug. However, it is important to remember that although liposomes and other nanoparticles are effective in treating various malignancies, they still retain some potential toxicity and lack some targeting and clearance properties**.[15]**

HISTORY OF NOVEL THERAPEUTICS OF LIPOSOMES:

Dr. Alec D. Bangam, a British blood scholar, made Lipo's first discovery in the mid -1960s. Using an electron microscope, he found a liposome when searching for a cell membrane composition. Like the cell membrane, the liposom is a small spherical structure composed of lipid vica. The foundation for understanding and creating liposomes for a variety of purposes was laid by Dr. Bangham's pioneering work, and their potential for drug delivery was recognized over the following decades. Pharmacology has been transformed by the ability to encapsulate drugs and deliver them to specific locations in the body. Now, thanks to liposomal drug delivery technology, many medications can be released in a controlled manner, with reduced side effects.**[16]** Since then, liposomes have been used in areas such as gene therapy, food technology, cosmetics, and drug delivery. To maximize their effectiveness in specific applications, researchers have created a variety of liposomes with different sizes, compositions, and surface modifications. Liposomes remain important tools in the fields of biotechnology and medicine today, with ongoing research aimed at improving their efficiency and adaptability in the delivery of therapeutic agents and other bioactive chemicals**.[17]**

Methods for preparation of liposomes:

1. **Thin-Film Hydration Method:**

One of the most popular ways to make a liposome.

countermeasure:

• To create a lipid solution, dissolve lipids (cholesterol and phospholipid) in organic solvents such as methanol and chloroform. • To create a thin lipid coating on the walls of a glass vial or round-bottom flask, the solvent is evaporated at lower pressure.

• To create multilamellar vesicles (MLVs), the lipid film is hydrated by adding an aqueous solution (e.g., buffered water or distilled water) and apical or sonication. • It is optional to use sonication or extrusion to reduce MLVs into smaller uniflagellar vesicles (SUVs).**[18-19]**

2. **Reverse Phase Evaporation Method:**

Crafting high-efficiency liposomes is a breeze with this nifty technique! First, you'll dissolve your lipids and the material you want to encapsulate in an organic solvent. Then, you'll create a water-in-oil emulsion by evaporating the solvent at a lower pressure. Voila! The liposomes carrying your precious cargo will remain, ready to be put to work. It's a simple yet effective way to package and deliver your desired materials. Give it a try and see the magic happen! **[20]**

3. Extrusion Method

Alright, listen up! This technique produces liposomes with a uniform size and a tight size distribution. Here's how it works:

First, you make some multilayer vesicles (MLVs) using the thin-film hydration method. Then, you pass the liposome suspension through a series of polycarbonate membrane filters with specific pore sizes, using either a high-pressure or hand-held extruder.

The end result? You get smaller, evenly-sized liposomes that are ready to rock and roll**!**

4.Sonication Method:

This method is commonly employed to produce tiny uniflagellar vesicles (SUVs) or diminish the dimensions of liposomes.Procedure:The production of liposomes can be carried out using the thin-film hydration method or any other appropriate technique.To reduce the size of the liposomes, sonicate the liposome suspension or apply high-frequency ultrasound.**[21]**

5. Detergent Removal Method:

This method is utilized to produce liposomes incorporating hydrophobic substances.Procedure:Utilize a detergent solution to break down the hydrophobic material and lipids.In order to produce liposomes with the encapsulated material, the detergent can be eliminated through techniques such as chromatography or dialysis.**[22]**

6. Freeze-Thawing Method:

This method is employed to enhance the stability of liposomes.Instructions:Reduce the temperature of the liposome suspension before freezing, typically below the phase transition temperature of the lipid.Continue to repeat the process multiple times to gently thaw the frozen suspension at an elevated temperature.This process reduces leakage and securely encases materials within liposomes**.[23]**

Methods for increasing efficacy:

Passive techniques such as enhancing permeability and retention, or PEGylation, can have a significant impact.Incorporating targeted molecules onto the surface of liposomes through active methods.The latest advancements in liposome technology have been focused on highlighting bioconjugation methods.The commercial impact of liposomes boosted by advanced drug encapsulation methods is significant.

Methods for reducing toxicity:

Phospholipids, cholesterol, and PEG are some of the key components typically found in liposomes, each playing a role in influencing their functionality. Phospholipids, the main components of liposomes, are diverse molecules with numerous isomers. Sphingomyelins, along with egg and soy lecithins, are commonly used phospholipids when producing liposomes. The level of saturation, whether saturated or not, varies significantly. Variations in the level of saturation in phospholipids and the length of the hydrophobic acyl tails can commonly influence the oxidation properties and the way drugs are released from liposomes.**[24]**

Preparation of Liposomes.

Adria Laboratories, located in Columbus, Ohio, graciously supplied the doxorubicin, with Sigma contributing the PtdCho phosphatidyl-serine (PtdSer) and cholesterol (Chol). Silica gel TLC was employed to assess the purity of each chemical, using a mobile phase of chloroform/methanol (90:10 vol/vol) and chloroform/methanol/water (65:25:4 vol/vol). It was found that in some PtdCho samples, the lipids were either pure or had small amounts of Chol traces. In previous testing conditions aimed at distinguishing Dxn from its metabolites and breakdown components like Adriamycin, Adriamycin one, and aglycone, Dxn was successfully identified as pure through silica gel TLC. The dried lipid was mixed with a drug solution containing 0. 077 M NaCl in a 1:2 molar ratio (Dxn/PtdCho), resulting in the formation of a complex between Dxn and PtdCho. The mixture was gently sonicated with a needle

probe-type solicitor (Braun Sonic, 1410) at 100 degrees Celsius for 5 minutes per milliliter under a nitrogen atmosphere. Liposomes with anionic properties, containing PtdCho, PtdSer, and Chol in specific molar ratios, were then employed to encapsulate the resulting complex formed by Dxn. As mentioned earlier, it was recommended to use this liposome suspension at 100 W for 5 minutes per milliliter. By employing gel filtration on a Sephadex G-50 column, liposomes were separated from the unincorporated Dxn using an elution buffer containing 0. 154 M NaCl. Upon following this process, approximately 5 to 10% of the original Dxn may be captured or trapped. As per electron microscopy, the vesicles exhibited an average diameter ranging from approximately 800 to 1000 angstroms.

Organ Distribution.

Moravek Biochemicals, located in City of Industry, CA, produced [3H] Dxn with custom labeling, boasting a specific activity of 600 mCi/mmol. It is worth noting that 1 Curie is equivalent to 3. 7 x 10^10 becquerels. The previously mentioned TLC techniques showed that the substance was pure after being incubated at pH 6. 4 and 8. 4. Swiss mice from Simonson Laboratories in Gilroy, CA, weighing approximately 20 g, received injections of either free or entrapped [3H] Dxn through the tail vein. Mice were euthanized at one and four hours after receiving the medication. The brain, heart, liver, lungs, and spleen were carefully prepared for liquid scintillation counting using Protocol as a tissue solubilizer, and then analyzed in a Beckman LS-9000 device. The distribution of [3H] Dxn in each organ studied was indicated as a percentage of the overall radioactivity measured.

High-Dose Cardiac Toxicity Study.

Swiss mice weighing approximately 20 g each received weekly tail vein injections, often at a dosage of 5 mg/kg. The study examined groups consisting of drug-entrapped in anionic liposomes, free drug combined with empty liposomes (having the same drug to lipid ratio), free drug on its own, and saline. New Dxn solutions and liposomes were prepared for each injection. The mice were administered either a total of 20 or 40 mg/kg of dosage, with treatment sessions conducted either four or eight times. In order to support the recuperation of bone marrow depression, animals were given a two-week break from treatment between the fourth and fifth injections over the course of the eight-week trial. Mice were sacrificed by cervical dislocation either 12 weeks after a low-dose injection or 13 weeks after a high-dose injection. The atrial and ventricular sections of the hearts were carefully separated, with the latter being preserved in a solution containing 3% paraformaldehyde, 2% glutaraldehyde, and 1. 6% cacodylate at pH 7. 3. Later, the tissue was fixed in either Vertosol or glycol methacrylate, and slices measuring 1. 0–1. 5 micrometers were cut using a Sorvall JB-4A microtome. After being stained with 0. 1% toluidine blue, the slices were observed under light microscopy at magnifications of X 400 and X 1000, while being immersed in oil. The heart was gently separated into two parts and placed on separate slides. In order to facilitate impartial observation and assessment, random coding was applied to each slide. Two separate reviewers assessed in a blinded study utilizing the cardiac phytotoxicity criteria outlined by Bertazzoli et al. 18. Cardiac lesions were evaluated based on both their size and severity. Two types of severity values are identified: degree 1, encompassing cellular edema or sarcoplasmic macrocylizations; and degree 2, covering all criteria of degree 1 along with cellular necrosis and sarcoplasmic macrocylizations. The extension values ranged from 0 to 5: 0 indicated an absence of lesions, while 5 signified that 50% of the visible cells were affected. The toxicity score was calculated by multiplying the severity and extension values.

In Vivo Antitumor Activity.

T. Khwaja from the Animal Tumour Resource Facility at the University of Southern California Comprehensive Cancer Centre graciously supplied the collection of ascitic L-1210 or P-388 tumor cells.

Female DBA/2 mice received intraperitoneal injections of tumor cells (105 cells suspended in 0. 25 ml of RPMI-1640 tissue culture media) from Simonson Laboratories in Gilroy, CA. Treatment commenced the day after tumor cells were inoculated. The treated animals received three doses of Dxnin at 5 mg/kg each, either in its entrapped form with empty liposomes or as the drug alone. New liposome and Dxn solutions were prepared for each injection. The controls were only administered isotonic saline. Tumor-bearing mice L-1210 and P-388 underwent treatment at intervals of 5 and 7 days, respectively. The effectiveness of free and liposome-encapsulated drugs in treating leukemia was evaluated by comparing their average survival times with those of the control group. Animals that lived over 30 days post-vaccination were categorized as long-term survivors. Statistical analysis involved the use of the one-tailed Student t-test.**[25]**

Methods for increasing stability:

There are various methods to enhance liposome stability such as applying a protective coating, modifying the liposomal membrane structure, incorporating cryoprotectants prior to liposome lyophilization, and utilizing surfactants or different types of polymer gels**.[26]** Cryoprotectants like polyampholytes, sugars, disaccharides, glycerol, and dimethyl sulfoxide commonly improve the stability of liposomes when undergoing lyophilization. These substances help preserve the integrity of liposomes during storage at low temperatures by preventing the formation of harmful ice crystals.**[27]**

The layer-by-layer electrostatic deposition technique offers an effective method for modifying the surface of liposomes and enhancing their stability in the challenging GT surroundings.Various types of polymers such as chitosan, pectin, alginate, and others have been utilized to create a coating on liposomes, influencing their effectiveness.Extending the circulation time boosts the flow and distribution of beneficial components.The dense polymer layer serves to minimize oxygen contact, thus preventing lipid oxidation.The decrease in the leakage of the loaded component.**[28]**

Liposome composition:

Lipids and phospholipids used for liposomes

In water-based solutions, diacyl-chain phospholipids come together to naturally create spherical or multilayered vesicles called liposomes. The hydrophilic head and hydrophobic tail of the bilayer phospholipid membrane work together to create an amphiphilic structure. Liposomes can be produced using either synthetic or natural phospholipids. The size, stiffness, fluidity, stability, and electrical charge of liposomes are key aspects greatly affected by the types of lipids used in their composition. As an example, liposomes crafted from naturally sourced unsaturated phosphatidylcholine, like the one present in eggs or soybeans, exhibit a restricted shelf life and enhanced permeability. Nevertheless, liposomes that rely on saturated phospholipids, such as dipalmitoyl phosphatidylcholine, developed rigid bilayer structures that were almost impermeable**.[29]** The lipids utilized in the preparation of liposomes belong to one of the specified categories.Biological fatsThe membrane bilayer of regular cells mainly consists of glycerophospholipids. Phospholipids are crafted by linking a glycerol unit with two fatty acid molecules and a phosphate group (PO42-). Furthermore, the phosphate group has the ability to interact with the small yet essential chemical compound known as choline.**[30]** Phospholipids are distinguished into groups based on their polar head components, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidic acid (PA). Due to the unsaturated structure of the hydrocarbon chain, natural phospholipids exhibit lower stability compared to synthetic phospholipids during the preparation of liposomes.**[31]** Natural phospholipids consist of saturated fatty acids like

palmitic acid (hexadecenoic acid, H3C-(CH2)14-COOH) and margaric acid (heptadecanoic acid, H3C-(CH2)15-COOH), along with unsaturated fatty acids like oleic acid or 9Z-octadecenoic acid, which can be located in egg yolk lecithin.**[32]** Egg-derived phospholipids and PCs consist of a combination of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and arachidonic acid (C20:4) in their fatty acid compositions. The majority - over 92% - of the total fatty acid composition is made up of these fatty acids, with polyunsaturated fatty acids such as C 20:4 (n-6) and C22:6 (n-3) commonly present in egg phospholipids. Approximately 40% of the total composition of egg PC consists of 1-palmitoyl-2-oleoylphosphatidylcholine. The predominant saturated acid found in the other lipids was palmitic, while stearic acid was prevalent in PE and PS. The fatty acid composition of soybeans contains approximately 95% of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3).**[33]** Phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylcholine (PC) should contain unsaturated fatty acids at both the α- and β-positions of the glycerol component as they contribute to over fifty percent of the total acids.The alpha and beta positions of the glycerol moiety in these phospholipids**.[34]**

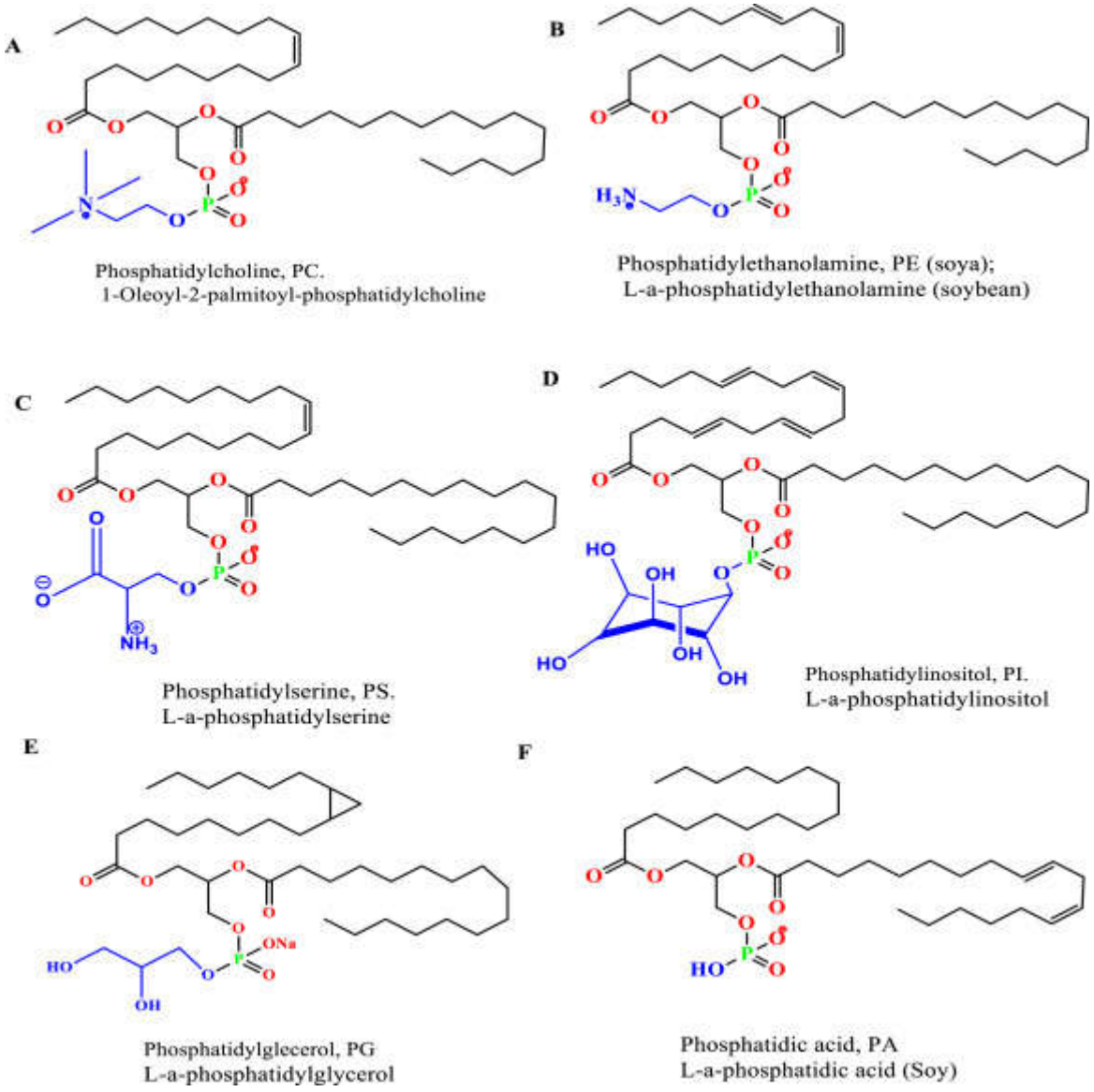


Fig:[34]

Phospholipids:

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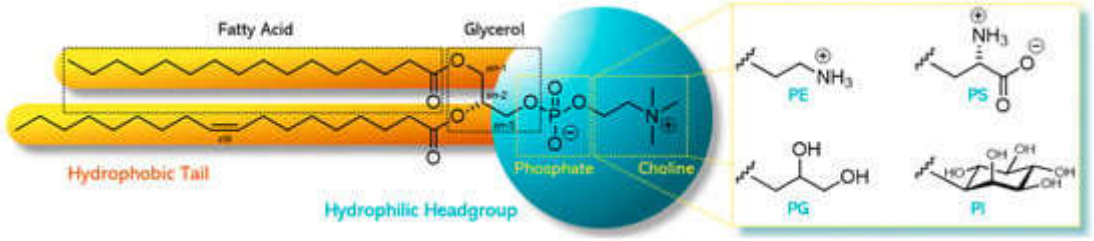

Fig:[35]

Illustration 3. An example of a phospholipid's chemical structure is 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). Additional headgroups with varying alcohol types are phosphatidylethanolamine (PE) with ethanolamine, phosphatidylglycerol (PG) with glycerol, phosphatidylserine (PS) with serine, and phosphatidylinositol (PI) with inositol.Fatty acids with varied lengths and saturation levels are known to attach to the glycerol backbone at positions sn-1 and sn-2 in phospholipids. Phosphoric acid is employed to esterify the leftover sn-3 position, followed by the use of an alcohol for the same purpose.**[35]** Phospholipids can be categorized as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), or phosphatidylserine (PS) based on the composition of the alcohol component.**[36]** Chirality arises from the specific and non-random placement of substituents across the sn-1, sn-2, and sn-3 positions of glycerol. At pH 7, phosphatidylglycerol (PG), phosphatidylinositol (PI), and phosphatidylserine (PS) carry negative charges, whereas phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are zwitterionic and remain neutrally charged. This is determined by the structure of their polar headgroups and the pH of the surrounding environment. The chemical structure of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and its various forms with different headgroups are depicted in Figure 3.Phospholipids play diverse roles in the body to support various physiological functions. An instance of this is when phospholipids, mainly PC, serve digestive and metabolic functions in bile (as monoacyl-phospholipids or lyso-phospholipids), besides their essential role in cell membranes. These functions involve dissolving the fatty and cholesterol components found in food, as well as lipophilic medicinal compounds.**[37]**

Phospholipids play a vital role as components of lipoproteins, aiding in the transportation of fat from the stomach to the liver. They also serve as a source of acetylcholine in PC, as well as providing energy and essential fatty acids. Moreover, a specific phospholipid, named 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), can be identified in lung surfactants.**[38]**

The surface tension of the alveoli in the lungs decreases at the interface between air and water. In the process of bone development, phosphatidylserine is included in the lipid-calcium-phosphate complex.**[39**] and plays an active role in regulating both apoptosis and blood clotting.**[40]**

Cholesterol

Cholesterol, a type of lipid molecule, is essential for human well-being and is key in forming cell membranes. It plays a key role in producing bile, essential for digestion, and serves as a building block for hormones such as estrogen and testosterone. It is imperative that our nerve cells retain their

mobility and proper functionality. Mammalian cells naturally produce cholesterol through the mevalonate pathway, with the liver serving as the key organ involved in maintaining the correct cholesterol levels in humans. It is distributed in varying ways across human tissues and organs. The central nervous system (CNS) contains a higher amount of cholesterol compared to any other organ in the body, despite the presence of fatty acid esters in organs such as the testes, ovaries, and adrenal glands. Many studies have detailed the utilization of cholesterol and cholesterol-based carriers in drug delivery due to the extensive presence of cholesterol throughout the human body.

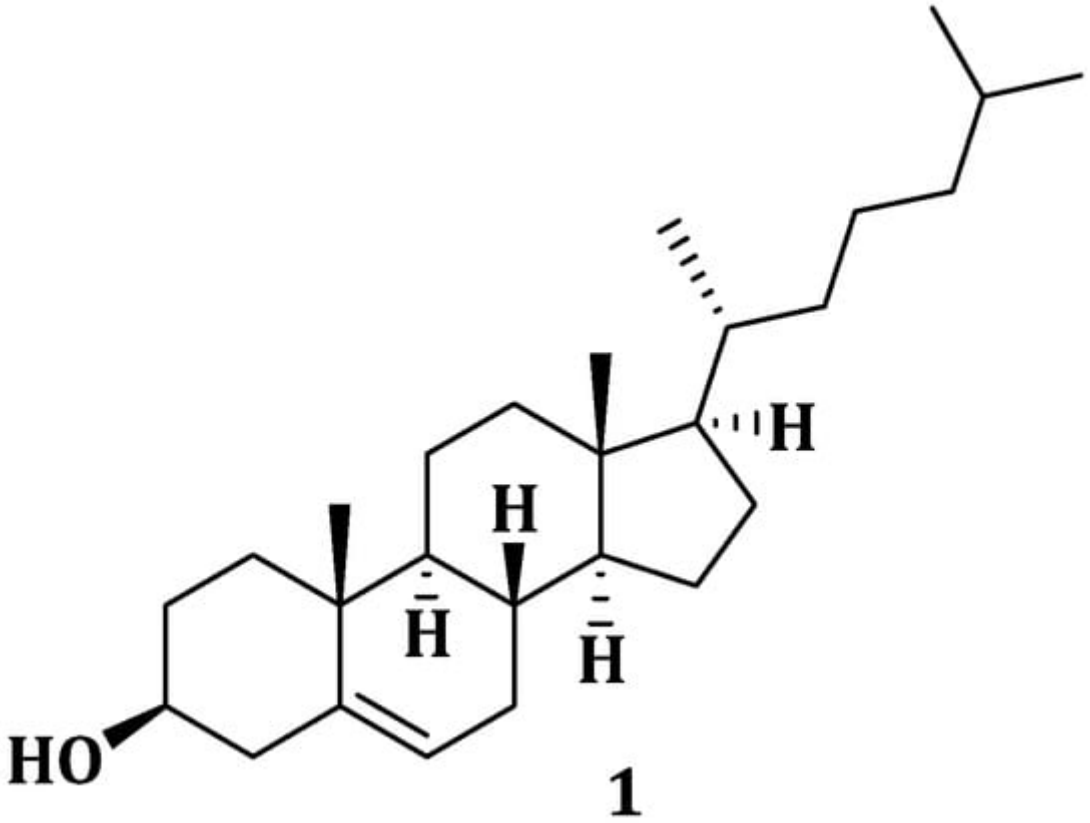

Figure 4. Structure of cholesterol.[41]

Cholesterol, being the most famous sterol, displays an amphiphilic nature attributed to the presence of both a hydrophilic 3-hydroxy group and a hydrophobic hydrocarbon domain. Cholesterol has been a popular choice as a starting material in numerous chemical syntheses over the years, thanks to its accessibility, affordability, and the ease of modifying its functional groups. Several chemical modifications have been carried out by combining cholesterol with established pharmacophores in order to produce molecules with enhanced biological efficacy. Cholesterol-inclusive drug carriers, such as liposomes, micelles, and nanoparticles, have been designed for efficient delivery purposes. It has been noted that incorporating cholesterol into a liposomal formulation enhances the formulation's stability by preventing the phase transition of phospholipids.Cholesterol's growing application as a carrier for medicines that are difficult to dissolve in water and high in fat content has expanded the horizons of drug delivery system design to a fascinating new level. The unique qualities of cholesterol make it a favored choice for delivering medications to various organs. This comprises its excellent biocompatibility, ability to stabilize membranes, ease of functionalizing the hydroxyl group, capacity to form rigid liposomes aiding fusion, and various other advantages. Research has also explored the

potential of connecting cholesterol to medications to enhance oral drug absorption, avoid the first-pass effect, and reduce gastrointestinal tract discomfort.**[41]**

PEGylation

As mentioned earlier, PEG is currently considered the top choice for protective polymer coatings, and "PEGylation" refers to the addition of a PEG corona to a medication or drug delivery device. Both coarse-grained and all-atom models have been employed in the examination of liposome PEGylation. This chapter will demonstrate the significance of employing a multiscale modeling approach that integrates all-atom and coarse-grained modeling in tight collaboration with experimental research. This method serves as a blueprint for utilizing computational modeling as an effective tool in investigating drug delivery research.The PEG monomer is composed of a non-polar ethylene group (C2H2) along with a polar oxygen. Dinç et al. have pointed out that PEG is soluble not only in polar solvents but also in a range of non-polar solvents. Moreover, PEG has been incorporated in batteries to serve as a polymer electrolyte. It forms loops around cations, with each cation binding to about 5% of electronegative oxygen atoms. The PEG polymer has been approved by the US Food and Drug Administration (FDA) for internal use because of its favorable excretion kinetics, low level of toxicity, and its ability to swiftly clear from the body with minimal structural changes. PEG has demonstrated notable efficacy as a stealth sheath, significantly prolonging an LDS's circulation time in the bloodstream from approximately one hour to 1-2 days. PEGylation of an LDS can be achieved by modifying the phospholipid headgroup in the liposome membrane. For example, when it comes to Doxil®, some of the DSPC molecules are replaced with DSPE-PEG. This change occurs as the choline group of the headgroup is modified to an ethanol group linked to PEG. Optimal results are achieved when using a molar density of approximately 5% DSPE-PEG versus others. District Schools Press Conference. Approximately 45 monomers constitute the 2 kDa PEG, commonly known as "PEG2000," in Doxil®. Moreover, it has been recommended to consider the utilization of extended 5 kDa PEG. Conjugating PEGylation covalently to small therapeutic molecules and protein-based "biologic" medicines serves a similar purpose as the LDS. In 1977, Davies and Abuckowski conducted a pioneering study showcasing the initial application of PEGylation. They achieved this by attaching PEG to two cow proteins, namely bovine liver catalase and bovine serum albumin. They were successful in showcasing an improvement in blood circulation duration as well as a reduction in immunogenicity. Lately, PEGylation has been implemented on solid nanoparticles, small medicinal molecules, and various proteins.It is essential to understand how the cutting-edge technique of PEGylation, achieved through phospholipid functionalization, impacts the behavior of liposomal drug delivery systems in the blood, extending their circulation time. This knowledge serves as a crucial step towards strategically enhancing stealth liposomes through a rational design process. Once this foundation is set, it provides us with a starting point to compare and contrast the actions of other options with it. The additional molecules composing the lipid membrane of the liposome will engage with the PEG corona. The combination of all these elements will determine the surface characteristics of the liposome. The outer layer of the liposome formed will engage with the salt ions present in the bloodstream, influencing the overall charge of the liposome. The way liposomes interact with bloodstream proteins, especially those related to complement activation, is influenced by the specific features of the liposome surface, such as its effective charge.One of the most noticeable and straightforward ways in which PEGylation prevents complement activation is through general resistance to protein binding. Adding PEG groups could potentially affect the activation of the complement system by altering the binding affinity for different proteins. This alteration may increase the affinity for certain proteins in the bloodstream while decreasing it for others, which in turn can hinder complement activation.**[42]**

Case study:

Liposomal Curcumin Nanoparticles

Liposomal nanoparticles can also be utilized for curcumin-conjugated liposomes. Turmeric extraction is where we primarily obtain curcumin, a naturally occurring polyphenolic and hydrophilic substance abundant in the Curcuma longa plant.Currently, the anti-cancer potential of curcumin has been shown to effectively combat various types of tumor cells such as those found in prostate, liver, and breast cancers.**[43]** The primary mechanism through which curcumin acts against cancer cells is by disrupting the translation of proteins such as Bcl-xl. It also influences apoptosis by modulating its process, regulating the release of cytochrome and reactive oxygen species (ROS), and controlling molecular factors like cyclin that impact the cell cycle. Nonetheless, curcumin has the potential to damage the nuclear and mitochondrial DNA structures of liver cancer cells, thus compromising their functioning.**[44**] Liposomal curcumin lowers the required dosage to target tumors and enhances pharmacokinetics and pharmacodynamics in comparison to free curcumin. Matheus Andrade Chave and colleagues. Explored the use of curcumin-containing liposomes by incorporating curcumin molecules into MLV liposomes.**[45]** Chapter 10 discusses the composition of curcumin and the production process of liposomal curcumin.Moreover, a diverse range of methods are available to produce liposomes suitable for use in therapeutic research endeavors. For example, Qiao Wang and colleagues. We developed daidzein long-circulating liposomes (DLCL) using the lipid film-hydration method along with ultrasonication**.[46]**

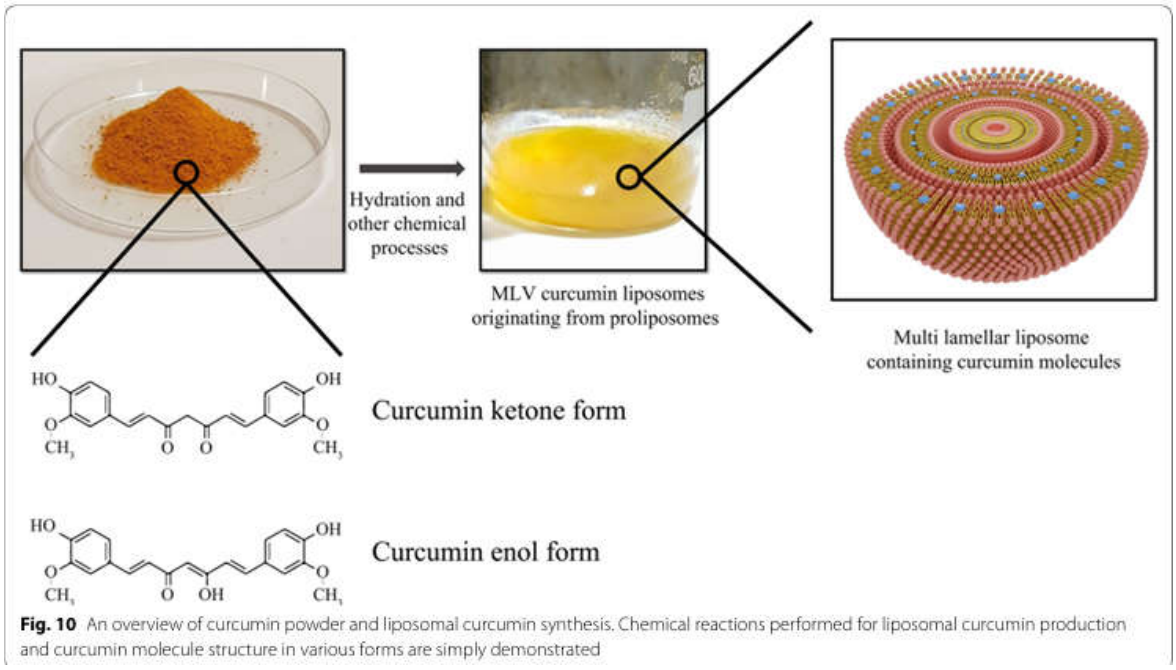

Fig. 10 An overview of curcumin powder and liposomal curcumin synthesis. Chemical reactions performed for liposomal curcumin production and curcumin molecule structure in various forms are simply demonstrated

Fig. [45]

Xiaoyuan Ding and colleagues successfully synthesized the Morin pH-sensitive liposome, which was modified using the aptamer and Au-NPs, referred to as Apt Au. By employing the film hydration technique. Their research revealed that the liposomes showed exceptional biocompatibility and minimal toxicity, positioning them as a favorable option for targeting tumors specifically. [47]

Conclusion:

The review highlights the significance of optimizing liposomal formulations to enhance drug delivery while minimizing toxicity, emphasizing the need for a careful balance between therapeutic efficacy and

safety. By exploring various strategies and existing literature, the study aims to advance the understanding of liposome-based therapeutics in improving treatment outcomes.

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