Chapter 1: Advances in liposomal drug delivery: Formulation and optimization

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ABSTRACT:

This abstract discusses advances in the field of liposome research, focusing on their nano-details, functionalization, and commercialization. Liposomes, which are bio-degradable and bio-compatible phospholipid bilayer vesicles, are favored carriers for conveying both hydrophobic and hydrophilic bioactives. Clinically, liposomal medicate conveyance frameworks are utilized to treat cancer, contagious contaminations, and viral contaminations, with many advanced clinical trials. The development of liposomes has evolved to meet clinical and industrial demands, including ligand acquisition, long circulation, and stimuli responsiveness. This chapter will focus on liposomes. We will consider how liposomes are formed and the various strategies for preparation and recognize important examples of their applicability in the real world. Liposomes are of particular interest for their clinical applications in multiple contexts, including imaging, vaccination, and gene delivery. Moreover, we will focus on the physical and chemical factors that influence liposome stability, and will highlight materials that could be used to coat liposomes to enhance stability for use in pharmaceuticals and food products.

Keywords: Liposomes, Formulation, Optimization, Advancement.

Introduction:

Nanotechnology has positively influenced drug sciences in many ways, especially with the improvements that have been made in nano-sized systems castoff for drug transport arrangements. Liposomes are nano-sized vesicles that are sphere-shaped, made from cholesterol + phosphor-lipids. They possess properties that allow them to be highly safe, biocompatible and biodegradable, which allow them to serve well as drug carriers. Liposomes were initial introduced as non-natural lipid vesicles in 1965 by Mr. Bangham, working with phospholipids. Since then, they are widely used within medicine and nutrition delivery systems. Liposomes have lipid bilayers that suitable in carrying both hydro-philic and hydro-phobic molecules. Liposome is made of phosphor-lipids that have hydrophilic heads and hydrophobic tails, making them amphiphilic. Liposome structure and some modifications are shown in fig.1 The favourable aspects of liposomes include their high surface area, capable of growing the solubility, absorption and stability for drugs or genes. [1]

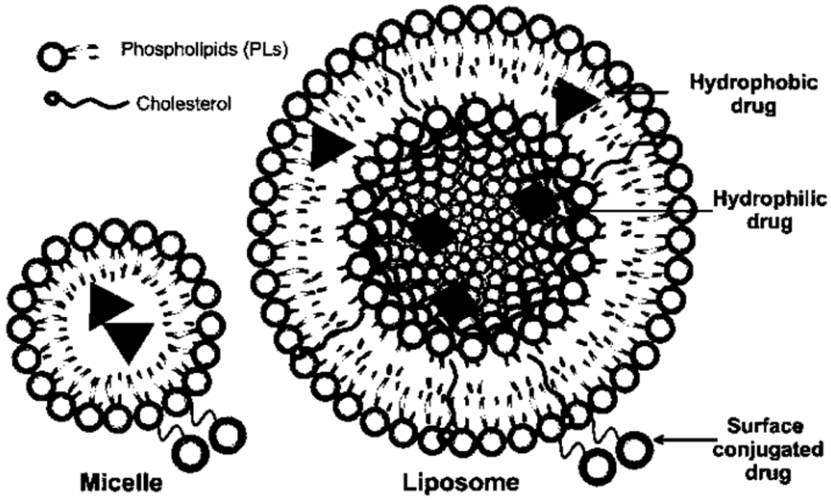

Figure 1: Liposome structure and some modifications.[18]

Liposomes are round structures composed of 1 or extra lipid bilayers nearby an watery core and are usually composed of phosphor-lipids containing a hydro-philic cranium and a hydrophobic_tails. theyare amphiphilic, and so in water they'll form solid bilayers where the heads face outward, and the tails face inward. Liposomes may be classified as either uni- (one bilayer) or multi- (multiple bilayers) lamellar. For intravenous (IV) packages, uni-lamellar liposomes are normally preferred due to their smaller size and ability to flow into longer time in the frame. Multilamellar liposomes have a wider size range and shorter circulation but can be converted to unilamellar forms through high shear processes like sonication and extrusion. [9]

It covers important factors like liposome size, size distribution, encapsulation efficiency, and scalability. The review aims to help researchers choose suitable methods for their needs, emphasizing that liposome size is crucial for drug delivery effectiveness. Advances in the past 15 years have led to new liposome applications in pharmaceuticals, optimizing drug delivery by temporarily increasing permeability and targeting specific areas. [8]

Formualtion Aspects:

1. Designing liposomes to achieve optimized properties:

Designing liposomes to optimize drug loading and release is crucial due to several challenges with first-generation liposomes. Early issues included difficulty retaining entrapped molecules, with serum proteins affecting drug release. Incorporating cholesterol or sphingomyelin into the bilayer, or using a solid-phase bilayer, helped reduce leakage. Hydrophilic drugs had low permeability, while hydrophobic drugs had high permeability, posing retention problems. Advances like drug loading via transmembrane pH gradients improved retention, particularly for weak bases. Drug retention varies, with doxorubicin showing excellent retention and ciprofloxacin being more challenging. Enhancing drug precipitation within liposomes or converting drugs to weak base prodrugs can improve retention. Optimizing drug release rates is essential for therapeutic efficacy, as bioavailability increases. [4]

2. Nature of drug associated in formulations of liposoms [7]

Liposomes they are good carriers for medicines, enzymes, and vaccinations, increasing their therapeutic potential. The drug's characteristics affect how it loads into liposomes. A study on encapsulating the hydrophobic molecule ß-caryophyllene, for instance, revealed that although large drug levels resulted in poor loading and release, low drug amounts enhanced loading capacity and cytotoxicity. Various loading strategies, such as transmembrane loading, have been effective in improving release patterns and enhancing drug retention. As demonstrated by FDA-approved commercial liposomal medications, the drug's kind and quantity are essential for the best liposome composition.

3. Structural Elements Include: [13]

- The phospholipids Phosphatidic acid is the source of these. Glycerol moieties serve as the molecules' skeleton. The most often utilised component of liposome formulations, phospholipids that contain glycerol, account for more than 50% of the weight of lipid in biological membranes. A phosphoric acid ester is formed at the C3 OH group. Phospholipids include, for instance, phospholipids such as phospholipid choline (Lecithin), phospholipid ethanolamine (Cephalin), phospholipid serine (PS), phospholipid inositol (PI), and phospholipid glycerol (PG) Use of saturated fatty acids results in stable liposomes. In general, unsaturated fatty acids are not used.
- Sphingolipids- It is the sphingosine backbone or a similar base. These are the crucial elements found in both plant and animal cells. Three defining components make up this.
  - Fatty acids in a mole
  - A sphingosine mole
  - A head group can range from incredibly complex carbohydrates to basic alcohols like choline. Sphingomyelin is the most popular sphingolipid
  - Lipid glycosphingoid.
  - Gangliosides—Located in the grey matter and employed as a liposome miner component.

  This molecule has one or more negative charges at pH neutral because it holds multifaceted carbohydrates with Sialic acid deposits and their polar-head groups. A layer group with a charged surface was added by the liposome.[13]

Drug loading techniques used in the synthesis of liposomes: [7]

Drug loading in liposomes mainly accomplished by two unique methods such as passive loading and active loading. A diagrammatic representation of drug loading into liposomes (Figure 2) explains the development and characterization of solvent-assisted active loading technology for liposomal loading of poorly water-soluble compounds. Drug loading techniques in liposomes are shown in fig. 2. On other hand Active loading refers to hydrophilic or hydrophobic medications becoming trapped in the aqueous core or bilayer of liposomes. Numerous research effectively described how to produce liposomes using active and passive drug loading strategies.

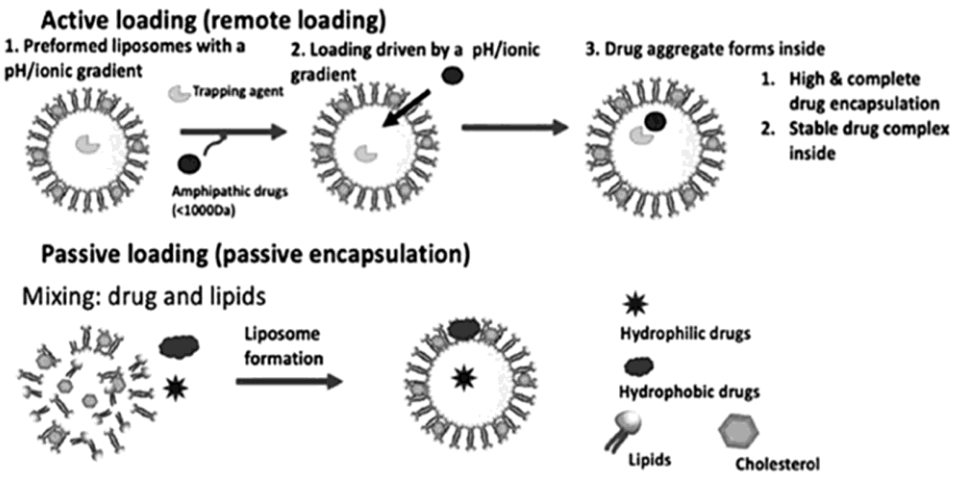

Figure 2: Drug loading techniques in liposomes

This paper explains how formulation and process parameters affect drug loading and release in doxorubicin liposomes, with an emphasis on active loading and optimizing the drug-to-lipid ratio for optimal efficacy. Combining tiny volume loading with remote loading, it investigates how to improve gemcitabine loading. It points out that remote loading requires chemical modification and drug diffusion in order to keep the drug inside liposomes. To optimize the concentration gradient across the liposomal membrane, small volume loading employs high external concentrations. Combining these techniques produced a high loading capacity of about 10% w/w with continuous release, according to the study. The research underscores the importance of pre-loading techniques for better encapsulation efficiency and discusses formulating these liposomes into a hydrogel. [7]

The thin layer evaporation approach was used to manufacture conventional liposomes using a 7:3 ratio of hydrogenated soy phosphatidylcholine to cholesterol. BBG-250 was enclosed by the lipid combination, which was dissolved in a chloroform-methanol solution. Rota evaporation was used to create a liposomal suspension after the lipid film was hydrated using a phosphate-buffer solution (pH 7.4). The study assessed the shape, size, stability, release patterns, blood compatibility, cytotoxicity, and drug entrapment effectiveness of the liposomes.[12]

Therapeutic Applications of Liposomes: [3]

New drug delivery systems are intended to function when traditional dosage forms are unable to produce the intended therapeutic effect. One such technology that offers

better medicinal effectiveness and safety than current formulations is liposomes. Among the main therapeutic uses for liposomes are:

Site-avoidance delivery: The narrow therapeutic_index of anti-cancer agents is responsible for their cytotoxicity. Here, encapsulating the medicine in liposomes can minimize drug distribution to normal cells, improving the therapeutic index. Cardiovascular toxicity is a serious adverse effect of free doxorubicin; however, when liposomes were created, the toxicity was decreased without affecting the therapeutic efficacy.

Site-specific targeting:

By limiting the drug's acquaintance to vigorous tissues, site-specific targeting can deliver a high % of the medication to the intended (diseased) location. For a more secure and effective delivery method, encapsulated liposomes are utilized for both active and passive targeting. Long-circulating immunoliposomes can more precisely identify and attach to target cells after systemic injection.

Intracellular drug delivery: It can be castoff to upsurge the delivery of powerful medications to the cytosol, which contains the drug's receptors. Cells do not absorb N-(phosphonacetyl)-L-aspartate (PALA) well. Compared to free medicines, these medications exhibited increased action against ovarian carcinoma cell lines when encapsulated in liposomes.

Types of Liposomes[6,15]

Long liposomes: in circulationATP is the main obstacle to delivering liposomes to a possible location. Numerous attempts have been attempted to circumvent the reticuloendothelial system uptake and binding cassette of liposomes by altering their surface or increasing their size, which also increases their circulation time. In order to increase their circulation duration, the surface of second generation liposomes—a form of modified liposome—is altered with glycoprotein, oligosaccharides, polysaccharides, and synthetic polymers.

The PEG-modified Liposomes:

According to reports, PEGylated liposomes can boost loading capacity by up to 90%. Because PEGylated technology contains a variety of hydrophilic and hydrophobic active compounds, it is acknowledged as a potential drug delivery technique. PEGylated liposomes, coated with the biocompatible polymer PEG, enhance blood circulation by avoiding opsonin recognition. However, pegylation integrity can reduce upon systemic injection, affecting long circulatory behavior. Novel PEG-dendron-phospholipid constructions were created in order to overcome this issue, resulting in ultra-smooth liposomes with better antitumor activity, improved bio-distribution, longer circulation times, and good stability. Wolfram et al. (2014) found that

PEGylated liposomes maintained constant zeta potential and stability in serum for up to 12 days, unlike non-PEGylated liposomes.

New-generation liposomes[16]

The range of contemporary methods for employing liposomes as medicinal carriers is demonstrated by the following few instances. Virosomes are an additional pathway for liposome formation. Fusogenic viral envelope proteins163 were used to alter the liposome surface in these applications, which seek to improve tissue targeting. Virosomes were originally designed to transfer DNA and medications intracellularly.

magnetic Liposomes: Using liposomes loaded with a medicine and a ferromagnetic substance is an intriguing method of a magnetic field. In one instance, hamsters with osteosarcoma were given doxorubicin-containing magnetic liposomes intravenously. The medication concentration increased fourfold after 60 minutes of field application when the tumor-implanted limb was positioned between two poles of a 0.4 Tesla magnet.

Stealth liposomes[15]

Sterically stabilized liposomes, or second-generation liposomes, are coated with a low concentration of biologically inert polymer molecules to create a resistant layer on their surface. This coating reduces Van der Waals attraction, preventing proteins from approaching too closely. The protective polymers used are non-toxic, flexible, affordable, biocompatible, and have low immunogenicity, with good and favorable excretion kinetics. These polymers can be natural, like oligosaccharides and glycoproteins, or synthetic. PEG is the preferred polymer for steric shielding, and PEGylated liposomes are known as stealth liposomes. Doxil® was the first successful PEGylated liposome-based product. Stealth liposomal medications have impressive outcomes, such as a longer half-life in circulation and a decrease in drug clearance [24]. Stealth liposomes, which are smaller in size and shape, aid in preventing drug extravasation. When combined with these unique properties, the vascular permeability rises as the targeted medications build up in the tumor tissues.

Targeted liposomes

Targeted liposomes are developed to decrease off-target effects on healthy tissues in cancer therapy. Anticancer medications are transported to polyps by targeted liposomes using both targeting. The spontaneous buildup of liposomes at the tumor location is necessary for passive targeting but frequently fails to produce more efficacy than normal liposomes. The ligands subsequently bind to receptors that are overexpressed on cancer cells, which increases drug uptake and accumulation into the cancer cell while limiting the nonspecific distribution. This is achieved with better specificity and efficacy than normal liposomes.

Stimuli liposomes

Results from studies have demonstrated that classic and stealth liposomes show slow releases of drugs and do not fuse properly with endosome membranes. As a fix to this, stimuli-responsive liposome have come into existence. These liposomes respond to local stimuli derived from target tissues such as local pH, local redox reaction or local enzymatic hydrolysis, by releasing their cargo. pH sensitive liposomes are stable (do not release drug) at the physiological pH (7.4) of the body, and release drug due to destabilization in the acidic environment of a tumor. Oscillating on mast these liposomes when they are destabilized they lose stability, exit the liposome and fuse, efflux out of the tumor. Enzyme-responsive liposomes utilize either enzymatic hydrolysis of amides or miscible esters by certain enzymes (such as proteases or esterases) to release drug. That's a big deal because only the local region will feel the change, provide both localized relief and reducing side effects for some delivery systems by protodrug loading or any of those stresses experienced my pre- and pro-behavioral drug speificent. Redox-sensitive liposomes utilize the, high> glutathione in tumors to disrupt the liposomes. Light-sensitive liposomes, controlled by factors such as exposure time and wavelength, are typically activated by UV or visible light. Thermosensitive liposomes release drugs at high temperatures and are used against tumor cells, exemplified by the commercial formulation Thermodox®. Near-infrared sensitive liposomes are also considered ideal for effective tissue drug release.

Bubble liposomes[5]

Bubble liposomes are a type of advanced liposome developed to meet the need for the fast, effective, and precise release of therapeutic agents. These liposomes, introduced by Maruyama and his colleagues, are encapsulated with gases and therapeutic agents, including PEGylated liposomes loaded with plasmid DNA and the ultrasound imaging gas perfluoro propane. Bubble liposomes are effective for delivering nucleic acids-based therapeutics both in vitro and in vivo. Parameters to be evaluated for Liposomes and the related Techniques shown in Table 1: Nucleic acids can be entrapped in the core or functionalized on the surface, lipid bilayers for co-delivery with therapeutic nucleic acids. Cationic liposomes, known for their biodegradability and biocompatibility, are particularly suitable for in vivo administration.

Table 2: Parameters to be evaluated for Liposomes and the related Techniques **[10]**

Parameters	Evaluation Techniques
1) Vesicle shape	Electron microscopy
2) Lamellarity	Freeze fracture electron microscopy and nuclear magnetic resonance spectroscopy
3) Vesicle size and distribution	Light microscopy, fluorescent microscopy, electron microscopy, laser scattering photon correlation spectroscopy, and permeation technique
4) Surface morphology and size of vesicles	Cryo-transmission electron microscopy
5) Encapsulation efficiency	Mini column centrifugation method and protamine aggregation method
6) Phase response and transitional behaviour	Freeze fracture electron microscopy and differential scanning calorimetry
7) Drug release	In vitro diffusion cell

Manufacturing Hurdles:

Current Practices and Future Innovations [11, 14]

The manufacturing process for liposomal drug delivery systems faces challenges that affect scalability, reproducibility. It is often problematic to accomplish constant liposomal formulations due to the variability in lipid composition, size, and drug encapsulation efficiencies from batch-To-batch. Furthermore, the current lipid fabrication process used by many liposomal drugs is expensive due to the excessive purification steps during production. Another significant issue related to quality manufacturing revolves around ensuring sterility without compromising stability. Meeting the Quality and regulatory agency standards adds another layer of complexity regarding consistent liposomal drug manufacturing. Continuous manufacturing methods including microfluidics (although unproven) show promise for better process control and less batch- to-batch variability. These challenges must be addressed if liposomal drugs are to be successfully applied as a therapeutic in humans. The stability of liposomes as drug formulations will be exaggerated by numerous issues like, temperature, lipid composition, pH, and size. Stability is also relevant to the half-life of drug products as well as the connection with encapsulation efficiency of liposomes. Standardized and reliable methods for liposomal characterization must be established to aid in optimizing drug delivery and drug imaging.

Fresh developments and innovations in liposomal delivery[17]

Smart liposomes are built to respond to their environments by releasing their payloads in appropriate conditions so the release happens at specific target site. E.g pH-responsive lipo-somes release their drugs only in the slightly acidic and mildly hypoxic conditions of tumor tissues. Likewise, temperature-sensitive liposomes will release their payloads in a situation of hyperthermia, which is also unique to the tumor microenvironment, thereby selectively releasing drugs in tumor tissues. Enzyme-sensitive liposomes_are designed to publication drugs in the occurrence of precise enzymes in target tissues, while light (e.g., UV and visible laser)-sensitive or magnetic-responsive (external magnetic force) liposomes provide a mechanism for precise drug-release with external stimuli. Smart liposomes are designed to take advantage of known environmental cues for stimulus-triggered drug release, potentially providing an effective therapeutic intervention while avoiding systemic side effects..

A Brief Summary of Clinical Trials for Liposomal Drugs and Their Current Developments [11]

There is substantial momentum developing liposomal drugs, particularly in oncology. Both doxorubicin (DoXil) and irinotecan (Onivyde) are examples of liposomal formulations that effectively deliver drugs to tumor sites with less exposure to systemic toxicity. Other applications of liposomal drugs such as antivirals, focused on maximizing stability, bioavailability and direct target drug-delivery, have potential promising outcomes extending to antiretrovirals. Liposomal vaccines have attracted extensive attention aimed toward enhancing desired immune responses for infectious diseases and cancers. In general, regulatory bodies, for example the FDA, marking the growth of liposomal-drug development, are now developing procedures for validating liposomal drugs' safety and effectiveness and are opening doors for licensure activities. Even in early-phase studies, we are seeing liposomes being explored for gene therapy to treat inherited genetic disorders: the potential is remarkable. While the optimization of liposomal products have many challenges, future directions will involve focusing on consistent liposomal formulations, evaluating liposomal products as part of combination therapies, and the utilization of advances in nanotechnology and drug formulations for clear therapeutic indications. The current enthusiasm for liposomal formulations indicates that we are just beginning to determine the breadths of their applications across an array of therapeutic areas.

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