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An overview of lipid based vesicular systems: stability and regulatory considerations

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Abstract

Lipid based drug delivery systems are used to encapsulate the active substance within or interior space of the lipid bilayer. The lipid-based drug products are designed to improve the stability in-vivo and pharmacokinetics behavior of active substances. However, understanding of the factors that affect the stability is critical for rationale development of lipid based drug delivery systems with desired pharmacokinetics and bio-distribution. Lyophilization is one primary approach to improve the stability of lipid based systems. The article presents an overview of the characteristics of the lipid based drug delivery systems as drug carriers, particularly in relation to stability, regulatory considerations. Further, the present review emphasizes the formulation problems and marketed formulation of lipid-based systems.

Key words: Lipid based drug delivery systems; Lyophilization; Liposomes; Vesicular systems.

1. Introduction

An increasing number of new chemical entities (NCE's) in the pharmaceutical development pipelines are poorly soluble and permeable[1–3]. This low solubility and permeability of these NCE's drug molecules leads to variable pharmacokinetics and poor oral bioavailability[4,5]. The conventional formulation approaches such as tablets and capsules are not enough to improve the bioavailability related issues [6]. The advanced systems such as amorphous solid dispersions[7,8], lipid based drug delivery systems have been developed to address the solubility and bioavailability issues. Different type of lipid based systems (Liposomes[9], solid lipid/polymeric nanoparticles[4,10–14] , liquid crystalline nanoparticles [15] nanocrystals [16], are presented in the form of advanced lipid based drug delivery systems for bioavailability enhancement and targeted delivery of drugs. Continuous efforts have been made in the development and clinical application of these drug delivery systems because of specific drug targeting and delivery, biocompatibility, increased efficacy and safety, reduced toxicity, improvement of pharmacokinetic properties, reduced frequency of administration can be expected with these drug delivery systems[17–19]. In recent years, Hot melt extrusion is an innovative technology for the production of a variety of dosage forms offering several advantages over traditional processing techniques in continuous production of lipid based drug delivery systems[2,7,20–22].

Lipid based systems, especially, liposomes are spherical vesicles having an interior aqueous chamber and a lipid bilayer[23]. Phospholipids or artificial amphiphiles are included as liposome structural elements with sterols, such as cholesterol, to affect membrane permeability. The most popular approach for making liposomes is called thin-film hydration, which involves dissolving the lipid components in an organic solvent with or without a medication[18]. The film will next be rehydrated in an aqueous solvent after the solvent has been rotary vaporized. Reverse-phase evaporation, freeze-drying, and ethanol injection are some of the additional techniques. To manage the size and size distribution, processes such membrane extrusion, sonication, homogenization, and/or freeze-thawing are used[24].

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Different sizes, compositions, charges, and lamellarities of liposomes can be created through formulation and processing[25]. The current review describes an overview of the lipid-based drug delivery systems in relation to stability, regulatory considerations with the emphasis on the formulation problems and marketed formulation of lipid-based systems.

2. Stability challenges

Lipid based drug delivery systems such as liposomes and solid lipid nanoparticles are lipid bilayer vesicles in which both hydrophilic and lipophilic drugs are encapsulated to protect them from degradation[26]. The lipids used in these systems contains un saturated acyl chains that are prone to oxidative degradation, which may lead to changes in phase transition temperature and further affecting the stability of vesicular systems. Generally, the more unsaturated lipid contents in the vesicular drug product, the faster or easier the product is prone to oxidation, and thus shorter the shelf life or long-term stability of drug product. When compared with the unsaturated lipids, the saturated lipids offer the improved oxidative stability. In terms of stability requirement, the degree of unsaturation should be kept as low as possible for these vesicular systems. Along with oxidative degradation, the hydrolytic degradation of these vesicular systems are common stability issue. Several other factors responsible for this hydrolytic degradation includes pH, temperature, buffer species, ionic strength, acyl chain length and head group[27–29].

The original function or integrity of liposome may deprive due to the degradation of the lipids or disintegration of the lipid bi layer. Therefore, it is important to know the extent of degradation of the components of vesicular systems especially lipids and their impact on quality of liposome drug product. During long-term storage, fusion or aggregation and leakage of active substance from liposomal product can be observed which leads to changes in particle size distribution, loading efficiency and further affect the quality attributes of liposome drug product.

The liposomes' stability is maintained by taking a meticulous approach to procedures such as preparation, lipid selection, storage conditions, which are directly and indirectly related to liposome stability [30]. The liposomal instability risks include hydrolysis and oxidation of the lipids utilized in preparation, liposomal fusion, drug leakage, and aggregation formation during formulation development and storage[31]. The stability of a liposome drug product can be evaluated by confirming the corresponding evaluation criteria such as a) physical and chemical stabilities, b) size and structure protection, c) encapsulated drug integrity, and d) the impact of the biological fluids [32].

The number of approaches has been explored to improve the stability lipid-based formulations. Of which, modulation of lipid composition, surface coating and freeze drying are the majorly explored formulation approaches during liposomes production[33]. The main steps involved in the lyophilization process are freezing, primary drying and secondary drying. The development of freeze-dried lipid based formulation requires an understanding of several formulation properties including properties of the excipients, critical temperatures (glass transition and or collapse temperature) of the formulation [34,35].

Table 1 Details of formulation components, characterization parameters and stability challenges of lipid based drug delivery systems [24,36–38]

Pharmaceutical carrier system	Examples	Formulation components	Characterization parameters	Stability draws back
Vesicular systems.	Liposomes, Solid lipid nanoparticles, Exosomes.	phosphatidylcholine cholesterol, lecithin, sphingomyelin, Glyceryl behenate, Tween, Span, Poloxamer.	Particle size distribution, Morphology, surface charge, thermodynamic properties of liposome membrane (fluidity, homogeneity of lipid bilayer membrane), in vitro release, osmolality, pH, aggregation, impurities, physical state of encapsulated active substances by electron microscopy.	Aggregation, Leakage of the encapsulated active substance over the time, oxidative degradation of lipid components.

3. Regulatory considerations

The intense interest in the area of advanced drug delivery technologies has been translated into an increasing number of New Drug Applications (NDAs) and Abbreviated New Drug Applications (ANDAs) for these advanced drug delivery systems from the United States Food and Drug Administration (FDA). Currently FDA has approved eight liposomal drug products on the US market. Over the last few years, USFDA, European medicine agency (EMA) publish guidance documents containing recommendations on liposome-based products[39,40].

The first guidelines published by USFDA is on ''Guidance for industry on liposome-based drug products'' in August 2002. Further, product-specific guidance to ANDA for injectable pegylated liposomal doxorubicin formulations was published in February 2010 due to the fact that the earlier published guidance did not provide information about bioequivalence assessment methods.

To address the stability of drug loaded and unloaded liposome drug product in terms of physical and chemical stability, the FDA guidance dictates that stability of the encapsulated active and the lipid excipients used during manufacture liposomes should be evaluated for stability[41]. During sterilization process, the physical and chemical complexity of liposome drug products can present unique challenges to the sterilization process and a careful evaluation of the physical, chemical complexity and possible degradation of liposome drug product should be undertaken throughout the stability duration[40,42,43]. To demonstrate the chemistry, manufacturing, and controls (CMC) and human pharmacokinetics and bioavailability issues, the FDA issued a guidance, ''Liposome Drug Products Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation'.

4. Marketed formulations

Liposomes are most common type of lipid-based drug delivery system for both targeted and personalized delivery system, accounting for nearly 30% of the marketed nanotechnology products in the US market. Currently, there are number of approved liposomal drug products available for therapeutic use (Table 2). Of which majority of liposomal formulations are intended to be administered as intravenous and intramuscular injections. Of the marketed formulations, the challenges associated while formulating the liposomal formulation Doxil®) is discussed in this section. (Doxil®) is based on the nanotechnology mediated PEGylated liposome[44–46]. Doxorubicin hydrochloride is the main active constituent in the Doxil® liposomal formulation developed by Sequus Pharmaceuticals, USA in 1995 as an intravenous injection for the treatment of advanced ovarian cancer, multiple myeloma and HIV-associated Kaposi's sarcoma[47].

Doxil® available as single-dose vial containing 20 mg/10 mL and 50 mg/25 mL of doxorubicin hydrochloride administered through intravenous infusion at a rate of 1 mg/ min. Doxil® drug product composed of phospholipid hydrogenated soya phosphatidylcholine (HSPC), N-(carbonyl methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt and cholesterol in a molar ratio of 56:5:38. Remote drug loading approach followed for encapsulating the doxorubicin into the liposome lipid bilayer. This loading approach provides high and stable drug to lipid ratio, which allows high drug retention with less efflux and acceptable tissue distribution. The drug load (entrapment) and particle size of Doxil® was 90% and 80-100 nm respectively[18,48].

4.1. Advantages of Doxil®

Prolong the circulation time of doxorubicin. Use of PEGylated nano-liposomes avoid the uptake by reticuloendothelial system due to steric stabilization effect. High remote drug loading driven by transmembrane ammonium sulfate gradient allows almost 100% drug loading, which also favors drug release at the tumor site. Because of the high melting point liquid order phase (DSPE and cholesterol) in the lipid bilayer of liposome helps in minimizing the drug leakage during storage and also prevent burst release in the blood. Due to nanosize range of Doxil® liposomal formulation, offers a benefit such as enhanced permeability and retention effect and accumulate at the tumor site.

Table 2 Marketed liposomal formulations (The details presented in Table 2 was captured from Drugs@FDA)

Product name	**Active**	**Excipients**	**Dosage form**
Doxil®	Doxorubicin	Hydrogenated soy phosphatidylcholine, Polyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (PEG-2000-DSPE), Cholesterol	Liposomal suspension
DaunoXome®	Daunorubicin	Distearoylphosphatidylcholine, Cholesterol	Injectable suspension
Depocyt®	Cytarabine/ Ara-C	Dioleoylphosphatidylcholine, dipalmitoylphosphatidylglycerol, Cholesterol and Triolein	Injectable suspension
Myocet®	Doxorubicin	Egg phosphatidylcholine, Cholesterol (supplied as a three-vial system)	Lyophilized- Vial 1 - doxorubicin HCl is a red lyophilised powder. Vial 2 - liposomes is a white to off-white, opaque and homogeneous dispersion. Vial 3 - buffer is a clear colourless solution.
Mepact®	Mifamurtide	Dioleoylphosphatidylserine, palmitoyloleoylphosphatidylcholine	Lyophilized
Marqibo®	Vincristine	Sphingomyelin, Cholesterol (supplied as a three-vial system)	Vial 1- Vincristine sulfate injection Vial 2- Sphingomyelin/ Cholesterol Liposome Injection Vial 3 -Sodium phosphate injection
Onivyde™	Irinotecan	Distearoylphosphatidylcholine, Distearoyl-sn-glycero-phosphoethanolamine, methoxy polyethylene glycol	Injectable suspension
Abelcet®	Amphotericin B	Dimyristoyl phosphatidylcholine, Dimyristoyl phosphatidylglycerol	Injectable suspension
Ambisome®	Amphotericin B	Hydrogenated soy phosphatidylcholine, Distearoylphosphatidylglycerol, Cholesterol	Lyophilized
Amphotec®	Amphotericin B	Cholesteryl sulphate	Lyophilized
Visudyne®	Verteporphin	Dimyristoyl phosphatidylcholine, egg phosphatidylcholine	Lyophilized
DepoDur™	Morphine sulfate	Dioleoylphosphatidylcholine, dipalmitoylphosphatidylglycerol, Cholesterol and Triolein	Liquid injectable liposome
Exparel®	Bupivacaine	Dipalmitoylphosphatidylglycerol, dierucoylphosphatidylcholine, , Cholesterol and Tricaprylin	Injectable suspension liposome
Epaxal®	Inactivated hepatitis A virus (strain RGSB)	dioleoly-sn-glycero-phophoethanolamine, dioleoylphosphatidylcholine	Injectable suspension

Inflexal® V	Inactivated hemaglutinine of Influenza virus strains A and B	dioleoly-sn-glycero-phophoethanolamine, dioleoylphosphatidylcholine	Lyophilized - Suspension for injection

4.2. Formulation

The active ingredient in the Doxil® is anthracycline antibiotic (doxorubicin) obtained from Streptomyces peucetius var. caesius. The active concentration of Doxil® is 2mg/mL and total lipid content is 16mg/mL. The composition of Doxil® comprised of three lipid components namely, HSPC, which is a high phase-transition-temperature phospholipid with a melting point of 52 °C helps in providing a rigid lipid bilayer. The hydrophilic PEG chains covalently bound to the DSPE and extends into the inner and outer water phase thereby increasing drug retention and circulation time. The following characteristics of Doxil® liposomal formulation, including composition, size distribution, electrical surface potential or charge, and in vitro drug leakage, are considered to be critical to ensure liposome integrity and consistent drug delivery to tumor sites. In-vitro drug leakage testing demonstrates the physical state of the lipid bilayer and encapsulated doxorubicin. The drug loading and its leakage is depending on the internal environment of the liposome, including its volume, pH, sulfate and ammonium concentration. These are key parameters to maintains the precipitated state of doxorubicin[18,49,50].

4.3. Formulation problems

Despite the popularity of Doxil® liposomal formulation, developability and stability are the key challenges. The majority of formulation issues are due to the complexity of lipid bilayer. The lipids used in the Doxil® formulation contains un saturated acyl chains that are prone to oxidative degradation, which may have led to changes in phase transition temperature and further affecting the stability of vesicular systems. Along with oxidative degradation, hydrolytic degradation is another common stability issue of vesicular Doxil® liposome. Several factors responsible for hydrolytic degradation includes pH, temperature, buffer species, ionic strength, acyl chain length and head group [51,52].

The original function or integrity of liposome may be deprived due to the degradation of the lipids or disintegration of the lipid bilayer. Therefore, it is important to know the extent of degradation of the components of Doxil® liposomal formulation, especially lipids and their influence on quality of liposome drug product. During long-term storage, fusion or aggregation and leakage of active substance from liposomal products can be observed which leads to changes in particle size distribution, loading efficiency and further affect the quality attributes of liposome drug products.

Numerous approaches have been explored to improve the stability liposomal formulations. Of which, modulation of lipid composition, surface coating and freeze drying are the majorly explored formulation approaches during liposomes production. Doxil® liposomal formulation has got great attention due to their unique characteristics such as non-toxic, biodegradable lipid components, non-immunogenic, lowers systemic toxicity, targeted delivery, and improved pharmacokinetic properties. However, development, manufacturing and characterization of this liposomal formulation is burdensome due to complexity involves with the composition and manufacturing process[49,52–54]. Furthermore, analytical method development and characterization of the complex lipid based drug delivery system is one major challenge the current pharmaceutical companies are facing. *In-vitro* bioequivalence testing and evaluation of new methods for dissolution and other physical and chemical attributes needs to be considered while developing the lipid based formulations[55,56].

5. Conclusion

Lipid based drug delivery systems have been successfully translated into clinical application. The delivery of therapeutics by lipid based systems can alter the biodistribution and therapeutic index profile of active substances. Extensive research is being carried out using lipid based drug delivery systems in diverse areas including the delivery of anti-cancer, anti-fungal, anti-inflammatory drugs and therapeutic genes. Several lipid-based drug products successfully translated into the clinic and other formulations are in different phases of clinical investigation. In summary, the lipid-based drug delivery systems currently in clinical trials may provide benefits to the diversified patient population for various therapeutic applications.

Compliance with ethical standards

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Authors contribution

All authors contributed to data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure of conflict of interest

All authors declare that there is no conflict of interests regarding publication of this paper.

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