

Lipid formulation: successful stories and prospective future

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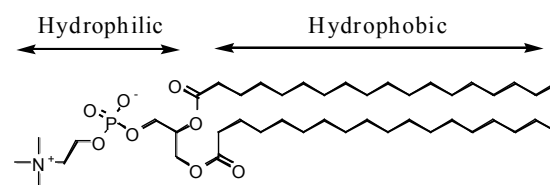


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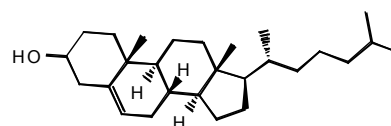
Introduction

It is estimated that 40 to 50 percent of new chemical entities (NCEs) are sparingly soluble compounds that require specialized formulations⁽¹⁾. To date, for parenteral dosage, a wide range of lipid/liposome technology options are available, which cover both the “traditional” strategies where the lipids are used as co-solvents, and “more advanced” systems where lipid particulate carriers are used to protect the active pharmaceutical ingredient (API) and improve its pharmacokinetic and pharmacodynamic performance.

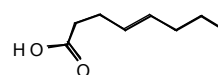
Lipids have been widely used as pharmaceutical excipients for a long time. However, the definition of lipid is usually ambiguous. One can find an excellent definition from the public website (<http://en.wikipedia.org/wiki/Lipid>): “Lipids are a class of hydrocarbon-containing organic compounds essential for the structure and function of living cells. Lipids are categorized by the fact that they are soluble in nonpolar solvents (such as ether and chloroform) and are relatively insoluble in water.” Thus, the term lipid really is a catch-all phrase for a wide variety of hydrocarbon-based molecules, which can be natural or synthetic, aliphatic or aromatic, acyclic or cyclic, straight or branched, and saturated or unsaturated. Typically, lipid molecules are predominantly hydrophobic, but also have a hydrophilic functional portion, such as hydroxyl, amine, phosphate ester, and carboxyl groups. This makes lipids a group of amphipathic molecules that possess both hydrophobic and hydrophilic portions to self-assemble into various colloid structures in physiological conditions.



1,2-Distearoyl-sn-Glycero-3-Phosphocholine (DSPC)



Cholesterol (Chol)



Octanoic Acid

Figure 1. Representative structures of various lipids.

In Figure 1, three typical lipids are used to illustrate the structural diversity of lipids. The lipids such as cholesterol (Chol) and octanoic acid, do not necessarily include two acyl chains, although diacyl chain phosphate lipids, e.g. 1,2-Distearoyl- sn- Glycerol-3-Phosphocholine (DSPC), are typically found as major components of biological membranes. From the lipid structures (Figure 1), we can understand that there is no strict boundary of the definition between lipids and detergents. For example, Chol is widely distributed in the mammalian cell membrane; but its structural analogue, cholate acid, is a natural detergent found in the human GI tract for dissolving and absorbing lipids from diet.

Lipid excipients also have a good safety record because most of the lipids used in drug formulations are natural structures found in the human body. Synthetic lipids have also been shown to be safe *in vivo* such as polyethylene glycol (PEG)-conjugated lipid.

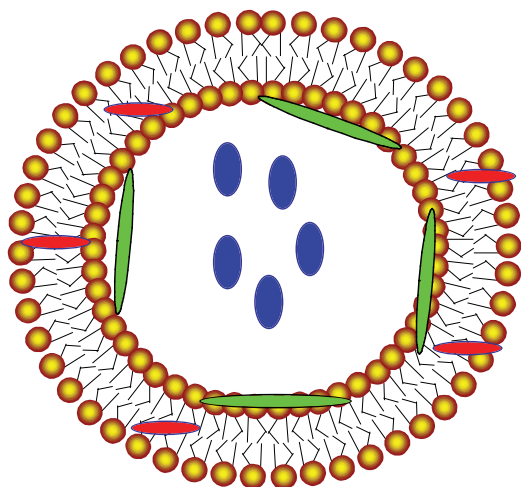


Figure 2. Typical modes for loading drugs in liposome. Highly hydrophobic drugs (red) can intercalate into the lipid bilayer; drugs (blue) with medium hydrophobicity can be encapsulated in the interior space of liposomes; and highly charged molecules (green) such as DNA and siRNA can be condensed into liposomes through the electrostatic interaction with the charged lipid headgroups on the liposome surface.

Liposomes are orderly assembled spheres composed of lipid bilayer in which the lipid hydrophilic headgroups are positioned towards aqueous environment and the hydrophobic portions are packed parallel as continuous membrane by hydrophobic interaction. Drugs can be loaded into the interior space of the liposome, be embedded in the lipid bilayer, be condensed by the lipid charges, or be mixed with lipid excipients into mixed micelles (Figure 2). In the following parts, we will discuss several commercialized formulations in which the lipids are key excipients.

DaunoXome® (liposomal daunorubicin injection)

DaunoXome is an anti-cancer drug of Gilead Sciences. DaunoXome is a special formulation of daunorubicin (in liposomes) used to treat Kaposi's sarcoma, a skin cancer. The API, daunorubicin, is a cytotoxic anthracycline antibiotic and an analogue of doxorubicin; both have similar therapeutic application as anti-cancer drugs. The mechanism of action is thought to be related to their ability to bind DNA and inhibit nucleic acid synthesis in cancer cells. Severe Kaposi's sarcoma is usually found in patients infected with the Human Immunodeficiency Virus (HIV) because the patients lose their immune response against carcinogenic mutation in cells. Kaposi's sarcoma can quickly metastasize into other organs such as lung and intestine. The inactive ingredients in DaunoXome include DSPC, Chol, sucrose, glycine, citric acid, and calcium chloride⁽²⁾. The product is intended to be administered only by intravenous infusion and was approved by FDA in 1996.

In DaunoXome, daunorubicin is encapsulated in the liposome composed of DSPC and cholesterol. DSPC has high phase transition temperature (T_m), 55 °C, and good compatibility with Chol. Normally, lipid bilayer has two thermodynamic phases: gel phase or liquid-crystal phase. At temperature $< T_m$, the lipid membrane is in the gel phase which is relatively rigid and tight because the lipid molecules have lower energy of random motion and the hydrocarbon chains are fully extended and closely packed; at temperature $> T_m$, the lipid membrane becomes disordered because the hydrocarbon chains are randomly oriented and fluid, which may decrease the liposome stability. The *in vivo* pharmacokinetics of DaunoXome is different from the conventional daunorubicin. Studies indicate that DaunoXome produces a 36-fold increase in mean area under the plasma curve compared to conventional drug (375.3 versus 10.33 $\mu\text{g}\cdot\text{hr}/\text{ml}$)⁽³⁾. A major advantage of DaunoXome is that it substantially reduces the cardio-toxicity including heart failure which is the major side effect found in the conventional daunorubicin therapy.

DOXIL® (doxorubicin HCl liposome injection)

Doxil (as Caelyx in Europe) is a liquid formulation of doxorubicin encapsulated in PEG-coated liposomes. Similar to DaunoXome, Doxil is also administered via intravenous injection for curing the HIV-related Kaposi's sarcoma; more recently for ovarian cancer.

Doxil is a landmark development in the history of liposome research. It is currently a product of ALZA, Inc., a subsidiary company of Johnson & Johnson. It was initially developed by Liposome Technology, Inc. (later Sequus Pharmaceuticals, Inc.), a company in San Francisco Bay Area. The product is approved in 1995 by FDA, which is even earlier than DaunoXome. Afterwards, the company was bought by ALZA in 1999, and subsequently becomes a product of Johnson & Johnson when

ALZA was bought by the pharmaceutical giant in 2001. Doxil is also called STEALTH® liposomes because it substantially extends the half-life of doxorubicin in vivo. Doxil has a terminal half-life of approximately 55 hours in humans.

The effect of extending half-life is attributed to the protection of liposome by the surfacial PEG molecules. PEG is a linear, inert, and hydrophilic polymer with wide application in pharmaceutical formulations. In biotechnology field, PEG was initially used to enlarge the size of proteins to reduce the renal excretion and protect the protein drugs from the recognition by protease and immune systems ⁽⁴⁾. Normally, PEG with molecular weight > 1000 Dalton is nontoxic and well tolerated in vivo. The PEG polymers on the liposome surface can reduce the liposome uptake by reticuloendothelial system (RES) which are the major cell types to clear the liposome from bloodstream. RES are part of the immune system, consisted of the phagocytic cells in reticular connective tissue, primarily monocytes and macrophages. These cells accumulate in liver, named as Kupffer cells, lymph nodes and spleen. Thus, it is not surprise that liver and spleen are two major organs to take up the liposome drugs following the intravenous injection. However, the mechanism of action regarding how the PEG polymers can decrease liposome uptake by RES is still not fully understood. A generally accepted theory is that the PEG polymers, which are in random coiled structures, can hinder the interaction of cell surface receptors of RES with liposome surface or the proteins absorbed on the liposome surface.

The Doxil liposome is composed of methoxypolyethylene glycol 2000-1,2- distearoyl-sn-glycero-3-phosphoethanolamine (MPEG-DSPE), fully hydrogenated soy phosphatidylcholine (HSPC) and Chol ⁽⁵⁾. The API, doxorubicin, is loaded into the internal space of liposome by an ammonia sulfate gradient method. In the method, the liposome is first formulated in high concentration of ammonium sulfate solution. Then the exterior ammonium sulfate is removed by filtration or dialysis. Afterwards, the preformed liposomes are mixed with doxorubicin solution. Since doxorubicin is medium hydrophobic in an uncharged form, it can diffuse across the lipid bilayer into the interior of the liposome. A spectacular effect is that the ammonium sulfate in the liposome can form salt crystal with doxorubicin, thus the high concentration of doxorubicin is stabilized in the liposome as crystal precipitation. This drug loading method is called “remote loading” in liposome field. Doxil liposome has a diameter less than 100 nm so it can be passively targeted into solid tumor sites via an effect called “Enhanced Permeation and Retention”. In addition to the stealth effect, PEG on the liposome surface can also improve the shelf stability of the liposomes in long term storage. The drug can be stable at 2-8°C for more than two years.

AmBisome® (amphotericin B liposome for injection)

AmBisome (amphotericin B liposome for injection) is a liposomal formulation of the antifungal drug amphotericin B for the treatment of confirmed infections caused by various fungal species or visceral leishmaniasis, a parasitic infection. These infections can be life threatening, particularly in patients who have depressed immune systems as a result of aggressive chemotherapy in cancer patients or HIV infection. However, antifungal drugs are usually more toxic than other types of antibiotics because both the pathogens and human cells are eukaryotic cells and have higher similarity. Thus amphotericin B can cause more severe side-effects to the patients than normal antibacterial drugs which are targeted to the prokaryotic bacteria. When liposome is used in the formulation, AmBisome remains effective in patients who have failed in prior conventional amphotericin therapy or in those who have developed intolerance to the drug ⁽⁶⁾.

AmBisome was originally approved in Europe in 1990, where it was marketed by Gilead and its NeXstar subsidiaries. In 1997, AmBisome was cleared for use in the United States, where Gilead co-markets the drug with Astellas. To date, AmBisome is available in more than 44 countries worldwide.

Systemic fungal infections used to be rare; however it is becoming increasingly serious in public population. It is estimated that there are approximately 100,000 cases of severe fungal infections in the United States each year. Some of the most common blood-borne fungal infections treated by AmBisome include cryptococcosis, aspergillosis and candidiasis. Patients most at risk for systemic fungal infections include cancer patients, bone marrow transplant and chemotherapy patients, organ transplant patients, HIV-infected patients and elderly patients ⁽⁶⁾.

AmBisome is a lyophilized product for intravenous infusion. The liposome formulation is composed of HSPC, Chol, distearoylphosphatidylglycerol (DSPG), α -tocopherol, together with sucrose, and disodium succinate hexahydrate as buffer ⁽⁶⁾. Upon reconstitution with WFI, the API can intercalate into the liposomal membrane. It should be noted that the API is highly hydrophobic and is not encapsulated in the interior aqueous space of liposome. The liposome formulation of amphotericin B uses the unique pharmacokinetic property of the liposome that it is mainly taken up by RES cells in liver and spleen, which are also major cells taking up the fungi and parasite. In severe conditions, the RES cells may not be able to destroy all the pathogen that they take up. With the help of liposomes, amphotericin B is accumulated into RES at higher concentration than the conventional therapy and, as a result, helps the RES cells to kill the eukaryotic pathogens.

Visudyne® (verteporfin for injection)

Visudyne is a light activated drug used in photodynamic therapy. The finished drug product is a lyophilized dark green cake. The API, verteporfin, a 1:1 mixture of two regioisomers (I and II), are electron-abundant hybrid ring molecules with high hydrophobicity (7). The Visudyne dry powder contains three lipids: egg phosphatidylglycerol (eggPG), dimyristoyl phosphatidylcholine (DMPC), and ascorbyl palmitate; and other two inactive ingredients: lactose and butylated hydroxytoluene (7).

Visudyne was co-developed by Novartis AG and QLT PhotoTherapeutics, Inc. The latter is a Canada based company. FDA approved Visudyne therapy in 2000 as the first drug treatment for a certain form of "wet" age-related macular degeneration (AMD). In the following year, it was approved by FDA for the treatment of pathological myopia (a form of nearsightedness) and presumed ocular histoplasmosis (a fungal infection of the eye). The drug was also granted the marketing authorization from the European Commission (EMEA) for the same indications. Visudyne therapy is a form of photodynamic therapy, bringing together a light-activated drug (Visudyne) and the use of a non-thermal ("cold") laser. The drug is administered intravenously after reconstituted with WFI. Following injection, the drug is taken up by the abnormal blood vessels in the eye. A "non-thermal" laser, shined in the patient's eye, activates the drug in a targeted area of the eye, producing a reaction that destroys abnormal, leaky blood vessels around the disease site.

Visudyne is expected to achieve billion dollar sales in long term because of its large population of patients. Medical experts estimate that 25 to 30 million people worldwide are affected by AMD. In addition, 200,000 people in North America and more than 500,000 people worldwide develop wet AMD each year (7). AMD is the leading cause of blindness in population over age 50. It is caused by a growth of abnormal blood vessels under the central part of the retina or macula and occurs as either dry or wet AMD. In the wet form, the vessels leak fluid and blood that lead to the development of scar tissue which destroys the central retina. Visudyne selectively targets the abnormal blood vessels on the retina, assisted by the "cold" laser, thus resulting in a reduction in their growth to stop the leakage associated with AMD, without affecting normal/healthy blood vessels (7).

Visudyne is generally well tolerated and has an excellent safety profile. In its formulation, the lipids excipients are key components to solubilize the API because the API is highly hydrophobic. The API is expected to intercalate into the lipid hydrophobic portions upon the drug is reconstituted in WFI. Without the lipids, it would be impractical to disperse the API into the bloodstream following the intravenous injection and subsequently deliver the drug into the eye via the circulation route.

RNA interference: Tremendous potential for gene therapy

Liposome and cationic lipids are widely used as gene delivery vectors in gene therapy. It was discovered in the late of 1980s that the cationic lipids can condense negatively charged DNA into small particles or nanoparticles to deliver DNA into cells. This topic has been widely reviewed in other publications (8). Since FDA and other regulatory agencies tend to be conservative on the safety issue of viral gene vectors, especially the cancerogenic risk of viral gene mutation and integration, lipid formulations gained more attention in recent years in the gene therapy field.

RNA interference (RNAi) is a natural cellular mechanism by which RNA is recognized as "foreign" due to its existence in a double-stranded form. Therefore cells quickly degrade the double-stranded RNA, along with the single-stranded RNA having the same sequence, to turn off the genes which can transcript into the single-stranded RNA (mRNA). RNAi is triggered by short interfering RNA (siRNA), usually ~21 nucleotides. Two scientists who uncovered the field just won the Nobel Prize of this year. The synthetic siRNA can stimulate the cellular machinery to cut up other single-stranded RNA having the same sequence as the siRNA. With appropriate design of the sequence, siRNA can be extremely specific to break up the mRNA associated with a disease-causing gene, which can be a cancer or viral gene.

siRNA only functions once it gets into cytosol. For academic research, siRNA are mainly tested in vitro, which is a simplified environment different from in vivo condition. For drug development, the chemically synthesized siRNAs must be able to travel in the bloodstream, escape from the RNase digestion, more importantly, overcome the cell membrane barrier and translocate itself into cells. So it is not surprise that drug delivery becomes the most difficult obstacle for the success of siRNA therapy. Many DNA delivery techniques are transformed to deliver siRNA in vivo. siRNA therapy is recognized as one of the new direction of gene therapy.

Sirna Therapeutics, with R&D headquarter at San Francisco, is developing human therapeutics based on siRNA. The company has partnerships with Allergan for the continued clinical development of Sirna-027 for AMD and other ocular opportunities; with GlaxoSmithKline for the development of siRNA for respiratory disease; and Targeted Genetics for the development of siRNA for Huntington's Disease (Sirna company website: <http://www.sirna.com>).

Sirna's delivery team is developing formulations and modalities, perhaps including lipid nanoparticles, to efficiently deliver siRNAs in vivo at low doses. One of their advantage is the company has established GMP production facility in Colorado. This can speed

up the process development in formulation designs to ensure cost effective manufacturing in the future.

Alnylam Pharmaceuticals, another siRNA company in Boston, was founded in 2002. The company was founded by a group of scientists who are among the first to describe the use of siRNA for silencing genes in mammalian cells, as described in the company website (<http://www.alnylam.com/>). In November 2004, Nature published a report by Alnylam scientists on the design and synthesis of siRNA with "drug-like" properties that achieve gene silencing in vivo of an endogenous, clinically-relevant gene. In their design, siRNA is conjugated to Chol to achieve a modest permeation across the cell membrane by the drug conjugate itself. In March 2006, the company published another article on Nature which demonstrates, in primates (monkey), that a systemically delivered RNAi therapeutic can potently silence an endogenous disease-causing gene in a clinically relevant manner. Alnylam and collaborators showed silencing of the gene for apolipoprotein B (apoB), a protein involved in cholesterol metabolism, with clinically significant efficacy as demonstrated by reductions in levels of cholesterol and low-density lipoproteins (LDL). In this formulation, siRNA is encapsulated into PEG-liposomal nanoparticle using proprietary technology. In April 2006, Alnylam presented Phase I clinical data with its lead product candidate, ALN-RSV01. The drug was found to be safe and well tolerated when administered intranasally in two Phase I clinical studies. ALN-RSV01 is being evaluated for the treatment of respiratory syncytial virus (RSV) infection and is the first RNAi therapeutic in human clinical development for an infectious disease. All the above mentioned information can be found in the company website. Alnylam has established partnerships with big pharma including Merck and Novartis, a medical technology company, Medtronic, and biotech company such as Biogen Idec, to drive further development of its product pipeline capabilities and clinical trials.

Prospective

There are several proven benefits found in the lipid/liposome formulation. It can overcome the low solubility of highly hydrophobic compounds in parenteral formulation. It can achieve an extended treatment effect, a targeting accumulation into the site of disease, or a simplified dosing regimen for physicians and patients by altering the pharmacokinetic behavior of the API. It also can play a role in reducing the harmful side-effects of certain drugs on healthy tissues, thereby offering the potential for an improved quality of life for the patients.

Obviously the development and manufacturing of liposome formulation, especially the "wet" formulation, is more expensive and sophisticated than normal drug formulations. Therefore, before we decide to get hands on the liposome formulation we should assess the drug property and its business prospective. A successful liposome formulation should come up with an API

which has large market capacity (presumably with projected annual sales >200 million dollar) to compensate the risk of investment. One may think 200 million dollar is not high for a NCE. However, companies may not like to use liposomes in the initial formulation of NCE because it complicates the development, rather they would use liposomes in the reformulation when the NCE passes the patent protection. So we can see that liposome formulation was widely used in the reformulation of "old" molecules such as doxorubicin and amphotericin B. In this situation, the liposome formulation must stand out with super improvement to compete with cheaper conventional drug therapies. The drug in liposome formulation also needs to be much potent, such as doxorubicin, because the loading capacity of liposome is usually limited. One estimates that most compounds can be formulated in liposome carriers at a drug/lipid ratio of 0.01 (wt/wt) (1). A less potent drug requires more drug loading into the formulation, thus increases the cost of lipids and the volume for injection.

A potentially huge opportunity for liposome formulation is on new biologicals such as DNA and siRNA because they are usually unstable in vivo and need to be carried by special delivery systems. siRNA therapy could become a new field other than protein therapy to provide blockbuster drugs if two obstacles can be cleared: one is the drug delivery technology and the other is the concern regarding the potential interference with normal cell growth and its long-term safety.

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