Research Article

Preparing Liposome Mixtures with Nippon Fine Chemical's High-purity Phospholipids and Presome® Series

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Abstract

A wide range of liposome and lipid nanoparticle drug products has been approved in the three decades since the approval of AmBisome® in the United States as the world's first liposome drug product. Many of these products are formulated with phospholipids or cholesterol produced by Nippon Fine Chemical (NFC) or with Presome®, which is produced using a proprietary manufacturing process of NFC. This article introduces NFC-produced high-purity phospholipids and cholesterol and discusses the advantages and uses of Presome.

Keywords: Phospholipids, Presome®, liposome, lipid nanoparticle, drug delivery system, formulation technology

Introduction

The phospholipids used to make liposome and lipid nanoparticle (LNP) drug products are a key component of biological membranes and have high biocompatibility, making them relatively well suited for drug product applications. In these formulations, a drug is contained in the liposomes, which act as nano-scale capsules that serve as drug delivery systems (DDS). Active research has sought to leverage the properties of the phospholipids used to make liposome drug products that achieve better therapeutic efficacy, a more favorable safety profile and lower burden on patients than conventional drugs in order to improve patient quality of life. This research has led to many approved liposome drug products that benefit patients in clinical practice. The recent approval of COVID-19 vaccines manufactured by Pfizer and Moderna has highlighted again the promise of LNP drug products containing mRNA active ingredients. The development history and benefits of these products are detailed in many publications.

Pharmaceutical companies worldwide are finding new effects for approved drugs with established safety and pharmacokinetic profiles by modifying the dosage form or route of administration. Their robust drug repositioning efforts represent a means for the discovery of drugs for treating diseases different from those for which the original drugs are indicated. Since drug substances that have already undergone clinical testing have been evaluated for safety and toxicity, the basic research and nonclinical testing steps can be skipped or simplified, which helps to significantly lower drug development costs. In the domain of new drug development, intense effort is being poured into satisfying unmet medical needs associated with the treatment of refractory orphan diseases with no established effective treatments. Within these efforts toward drug repositioning and satisfying unmet medical needs, liposome formulation research and the use of LNP platforms for existing drugs are accelerating in pharmaceutical companies.

The materials that are commonly used in liposome and LNP drug products are high-purity, pharmaceutical-grade phospholipids and cholesterol manufactured and marketed by NIPPON FINE CHEMICAL CO., LTD. Drugs ranging from small-molecule compounds to macromolecules can be encapsulated in liposomes, which are containers with a lipid bilayer that forms readily in water. Liposomes therefore make ideal drug capsules. LNPs have the ability to protect small interfering RNAs (siRNAs) from degradation by nucleases and other enzymes following administration into the body. Additionally, LNPs facilitate siRNA uptake into the cytoplasm when siRNAs (which are larger and more negatively charged than small-molecule drugs) are encapsulated in these vessels that readily permeate the hydrophobic cell membrane.

Table 1. Approved nanomedicine products

Product name (Year and region of marketing)	Drug	Developer and manufacturer	Lipid components	Indications
AmBisome® ('90, US)	Amphotericin B	Gilead	HSPC/DSPG/Cholesterol	Mycoses
Doxil®/Caelyx® ('95, US/'96, EU)	Doxorubicin	ALZA	HSPC/Cholesterol/MPEG-DSPE	Ovarian cancer, etc.
DaunoXome® ('95, US)	Daunorubicin	Gilead	DSPC/Cholesterol	Kaposi's sarcoma
Visudyne® ('00, US)	Verteporfin	QLT	DMPC/EPG	Age-related macular degeneration
EXPAREL® ('12, US)	Bupivacaine	Pacira	DEPC/DPPG/Cholesterol/Tricaprylin	Relief of postoperative pain
ONIVYDE® ('15, US)	Irinotecan	Merrimack	DSPC/Cholesterol/MPEG-DSPE	Pancreatic cancer
VYXEOS® ('17, US/'18, EU)	Daunorubicin and Cytarabine	Celator/ Jazz Pharma	DSPC/Cholesterol/DSPG	Acute myeloid leukemia
ARIKAYCE® ('18, US)	Amikacin	INSMED	DPPC/Cholesterol	Pseudomonas aeruginosa infections
ONPATTRO® ('18, US/'18, EU)	siRNA	Alnylam	DSPC/Cholesterol/DMG-PEG/DLin-MC3-DMA	hATTR amyloidosis
Comirnaty® ('20, Worldwide)	mRNA	Pfizer/ BioNtech	DSPC/Cholesterol/PEG lipids/cationic lipids	SARS-CoV-2 vaccine
mRNA-1273 ('20, Worldwide)	mRNA	Moderna	DSPC/Cholesterol/PEG lipids/cationic lipids	SARS-CoV-2 vaccine

Industrial Application of High-purity Phospholipids in Nanomedicine Products

Issued in April 2018 by the United States Food and Drug Administration, "Liposome Drug Products, Guidance for Industry" recommends that the purity of esterified fatty acids in phosphatidylcholine be controlled. For example, the fatty acids constituting hydrogenated soybean phosphatidylcholine (HSPC), which is used in the manufacture of various drug products, contain stearic acid and palmitic acid bonded at a molar ratio of about 8:1. As hydrogenated soybean phosphatidylcholine is a naturally derived phospholipid, this fatty acid ratio often varies depending on the soybean source, country of origin, purification method, and even across batches. This variability also occurs in egg phosphatidylcholine, which is derived from chicken eggs. NFC has data showing that the feed and rearing conditions of the laying chickens affect the fatty acid purity of egg phosphatidylcholine. Variability in the fatty acid ratio across batches means, chemically, that a mixture of multiple compounds is present, and variation in fatty acid purity means that the ratios of these multiple compounds in the mixture change. Such variation in the proportion of fatty acids, which provide nanoscale drugs with hydrophobicity, could affect the performance of liposomes made from them.

As stated in the FDA's "Liposome Drug Products, Guidance for Industry," controlling the purity of fatty acids is difficult because control must extend back to soybeans or chickens. To circumvent this difficulty, test data must be acquired in the liposome or LNP design stage to cover such changes in fatty acid purity. Furthermore, the purity of raw material fatty acids must be checked on a by-batch basis even after approval so that the drug product can be manufactured without out-of-specification materials. The issuance of the Guidance prompted a gradual reduction in the use of naturally derived lecithin in formulation research except in generic drug development, and its replacement with lecithin with a distinct fatty acid profile, i.e., high-purity phospholipids. High-purity phospholipids are typically produced using fatty acids purified to a level of at least 99%. High-purity phospholipids are an excellent ingredient for achieving unwavering liposome performance. NFC already had a lineup of high-purity phospholipids with fatty acid purity in the early 1990s and now commercially produces at least several tons annually. These phospholipids are found in Gilead's AmBisome® and other drug products.

A range of production techniques for consistently manufacturing phospholipids at a 100 kg scale is required if high-purity phospholipids are to be used as a pharmaceutical raw material. Fatty acids of a purity with at least 99.0% are needed to produce high-purity phospholipids, but are rarely available for direct purchase in sufficiently large quantities. NFC often must perform purification in house. Boosting purity, however, is generally difficult. Advanced technological capabilities are needed to remove trace impurities with different numbers of carbons, such as removing stearic acid present in trace amounts in palmitic acid.

High-purity Cholesterol

Cholesterol, by strengthening interactions between lipid molecules in unsaturated lipid membranes, decreases membrane permeability and fluidity, which confers a anti-fluidizing effect. Adding cholesterol to a saturated lipid membrane, however, eliminates phase transitions so that the membrane has fluidity even at the temperature of the gel phase. In this sense, cholesterol has a condensing effect. The presence of cholesterol eliminates dramatic changes in fluidity at phase transitions, resulting in decreased fluidity at temperatures exceeding the phase transition point and increased fluidity below the phase transition point.

This property is pronounced when at least 30 mol% cholesterol is added relative to phospholipid. Manufacturers therefore often use cholesterol with high-purity phospholipids in liposome and LNP drug products. Cholesterol is often produced by purifying naturally derived materials, which frequently contain related substances as impurities including lathosterol, desmosterol, and dihydrocholesterol. The structural similarity of these impurities to cholesterol makes achieving high purity difficult through repeated purification alone.

We use proprietary technology to manufacture and sell Cholesterol HP, an injection-grade high-purity cholesterol product. Our cholesterol products have a purity exceeding 99.0% and are used in many marketed liposome drug products.

Research and Development of Liposome Drug Products

When suspended in an aqueous solution, phospholipids form a lipid bilayer that in turn forms enclosed vesicles containing an aqueous phase. These enclosed vesicles, which are liposomes, are capable of containing a wide range of active substances. The Bangham's method is used to prepare liposome batches

of several milliliters needed in the research stage. Several other methods, most of which are easily accomplished, are described in the literature. Bangham's method works well in the early stages of research but is almost always insufficient once formulation development proceeds to a stage where a pilot scale of 1 or 10 L is required. At these scales, liposome preparation methods different from those used to produce small, lab-scale batches must be used. Changing preparation methods, however, can result in poor reproducibility of product performance and other problems. Regardless of which method is selected, achieving a uniform mixture and hydration of the raw material phospholipids and cholesterol at the molecular level are the most important requirements for proper liposome preparation. Without sufficient mixing, hydrophobic cholesterol aggregates precipitate out without being incorporated into the liposome membrane. Even when the raw materials are uniformly mixed at the molecular level, the presence in the mixture of clumps, solids, and similar shapes that are not readily hydrated may require more robust hydration techniques, which could in turn require new equipment investment. Although many techniques that use organic solvents to produce liposomes are available, these techniques burden the manufacturer with the new tasks of establishing methods for removing residual organic solvent from the drug product and controlling the level of removal.

Presome is a Lipid Blend that Simplifies Liposome Preparation

Presome® is a lipid blend made using proprietary technology developed by NFC that greatly simplifies the complicated process of liposome production. Presome can be added to an aqueous solution free of organic solvents and gently mixed to prepare a liquid mixture of multi-lamellar liposomes measuring about 1 μm in diameter. These liposomes can later be resized to suit the purpose (**Figure 1**). Although Presome is a mixture of powdered lipids, it is not a liquid mixture of lipids with the solvent removed but rather a mixture of lipids specially processed at the molecular level for liposome preparation. The result is a product that simplifies liposome production, enables large-scale liposome production, and allows the preparation of highly concentrated liposome liquid mixtures. Some of the features of Presome are listed below.

- Many of the complex procedures involved in preparing liposome mixtures can be eliminated.
- Large-scale, high-concentration liposome mixtures (up to 20 kg per batch) can be prepared.
- Liposome mixtures can be prepared without organic solvents.
- Presome can be ordered with custom lipid consistencies.
- Presome containing certain lipid-soluble substances can be ordered.
- NFC's high-purity phospholipids, high-purity cholesterol, and functional phospholipids can be used as lipid raw materials.
- Consistent quality control and GMP control are possible.

Presome contains raw materials suited to sustainable development goals. On the market for over 20 years, Presome has an established reputation and is used in marketed products and investigational drugs. Presome, along with our high-purity phospholipids and cholesterol, is available as a reagent for you to purchase and try through Tokyo Chemical Industry Co., Ltd.

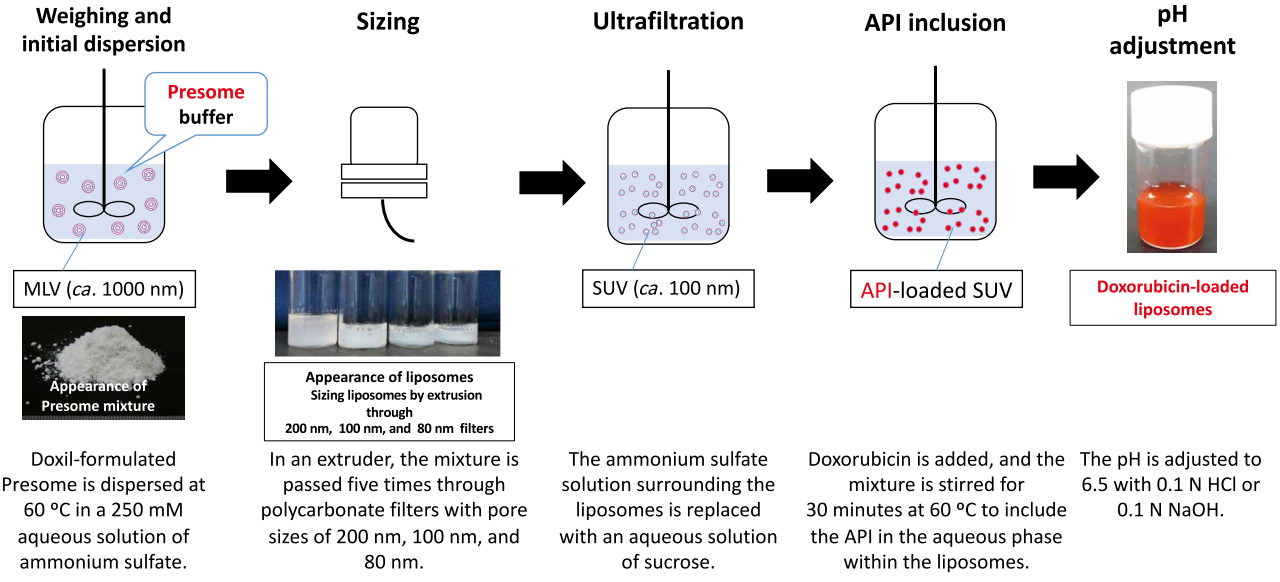

Figure 1. Preparation of doxorubicin-loaded liposomes with Presome techniques

Using Presome to Prepare Sample Batches of Commercially Available Liposome Products

To demonstrate the usefulness of Presome, we prepared three commercially available liposome drug products using pre-formulated Presome, using the methods described in the relevant journal articles (1,2,3). The active pharmaceutical ingredient (API) encapsulation rates and physical properties of the resulting liposomes are shown in Table 2.

Table 2. API encapsulation rates and physical properties of liposomes prepared with Presome

Formulation name	References	API	API encapsulation rate1	Lipid concentration	Drug concentration	Particle size (PDI)
Doxil®	(1)	Doxorubicin	100%	HSPC/Cholesterol/MPEG2000-DSPE= 9.6/3.2/3.2 (mg/mL)	2.0 mg/mL	101 nm (0.048)
ONIVYDE®	(2)	Irinotecan	99%	Not determined.	2.3 mg/mL	101 nm (0.048)
Pulmaquin®	(3)	Ciprofloxacin	98%	HSPC/Cholesterol = 62.4/26.9 (mg/mL)	43.7 mg/mL	103 nm (0.038)

1) Yield from liposomes relative to the amount of the API initially added.

Remote loading was used to include each API into liposomes as described above. Each API was efficiently encapsulated, and the liposomes contained the expected concentrations of lipids. These trials highlight the potential of Presome for preparing products equivalent to commercially available drug products. They also suggest that Vyxeos® could be prepared with Presome.

Conclusion

Now that laboratories across the globe have been actively researching and developing liposome drug products for over three decades, LNP coronavirus vaccines have recently emerged as a miracle of modern medicine. Pharmaceutical companies are rethinking their DDS technologies to reposition their existing drug lineup so as to satisfy unmet medical needs for therapeutic and prophylactic coronavirus drugs. Their efforts are apparent in intensifying research into nanomedicine products including liposomes and LNPs. As this research proceeds, NFC is continuing to produce more high-purity phospholipids and cholesterol every year, and nanomedicine stands to make more strides forward. As the manufacturer of these raw materials, NFC is committed to producing products of stable quality to satisfy the R&D institutes, pharmaceutical companies, medical institutions, doctors, and patients we serve, and enable our clients to manufacture products with excellent performance reproducibility. After all, our mission is to add new value to the field of nanomedicine.

Nippon Fine Chemical commemorated its 100th anniversary in February 2018. Established in 1918 as Nippon Camphor Co., Ltd., the company overcame a major challenge when the camphor monopoly system was abolished after the war, switching to fatty acid and other oil-related products business. In 1971, the company name was changed to the current "Nippon Fine Chemical Co., Ltd.", and since its fresh start as a fine chemical manufacturer, the company has expanded its business portfolio as a pioneer in the chemicals sector. Going forward, we want to be indispensable to all people, a company that continues to innovate and grows sustainably.

References

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Related Products

Cholesterol (>99%) (stabilized with α-Tocopherol)	1g	5g	C3624
DOP-DEDA		50mg	D5882
Presome® ACD-1		100mg	P2807
Doxorubicin Hydrochloride	25mg	100mg	D4193
Daunorubicin Hydrochloride	20mg	100mg	D4532
Bupivacaine Hydrochloride	5g	25g	B3925
Irinotecan Hydrochloride Trihydrate		100mg	I0714
Cytarabine	1g	5g	C2035
Amikacin Sulfate	5g	25g	A2281
Ciprofloxacin	5g	25g	C2510

* These related products are for research purposes only.

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