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White Paper

Successful drug development with synthetic lipids: critical aspects and strategies

Shiksha Mantri, Adela Kasselkus, Diamanto Pommerehne

The role of lipids in drug development

Currently gaining enhanced momentum due to their vital role in the development of vaccines against COVID-19, lipid-based formulations and lipid nanoparticles have shown a lot of promise in drug development and delivery particularly because of their ability to:

- Enhance active pharmaceutical ingredient (API) stability by protecting the API from immune response, proteases and other factors (Yingchoncharoen, 2016)
- Boost the solubility and bioavailability of drugs with poor water solubility (Yingchoncharoen, 2016)
- Passively target inflamed or tumor tissues due to their leaky vasculature, known as the enhanced retention and permeation effect (Danhier, 2010)
- Improve the toxicity profile of the entrapped API; targeted drug delivery could improve the toxicity profile of the API further as the APIs are delivered directly to the site of action (Yingchoncharoen, 2016)
- Ability to deliver difficult APIs such as RNA, which are prone to instability, nuclease-mediated lysis, strong immune responses, and inability to reach the site of action.

In 1995, the U.S. Food and Drug Administration (FDA) approved the first liposomal drug, Doxil®, which encapsulated the cytotoxic drug doxorubicin. Since then liposome drug development continues to trend upward due to advances in liposome and lipid nanoparticle production technologies.

Figure 1 shows the marketed liposomal formulations in six therapeutic categories, with the most liposomal drugs indicated for the treatment of cancer (Bulbake, 2017). Today, there are 18 liposomal drugs on the market and hundreds of liposomal drugs in clinical trials for a wider range of ailments. There is active work in developing generics of off-patent liposomal drugs and the U.S. Food and Drug Administration (FDA) has issued product-specific guidances for several generic versions of liposomal products.

The latest trend in lipid-based drug delivery research and drug development is in the field of nucleic acid delivery, for APIs such as short RNAs for gene silencing or activation (siRNA, miRNA, saRNA) and long RNA (mRNA) for applications in cancer therapy, enzyme replacement therapy, vaccines, and more. In 2018, the first liposomal drug for gene therapy, Onpattro®, encapsulating siRNA, was approved by the FDA for the treatment of hereditary transthyretin amyloidosis (hATTR). Lipid based RNA formulations are also finding their use as vaccines for infectious diseases. The first vaccine to enter clinical trials for COVID-19 was a mRNA vaccine, where the mRNA of a viral antigen was encapsulated in a lipid nanoparticle. More gene therapy drugs using lipids are expected to be approved in the next few years.

MilliporeSigma

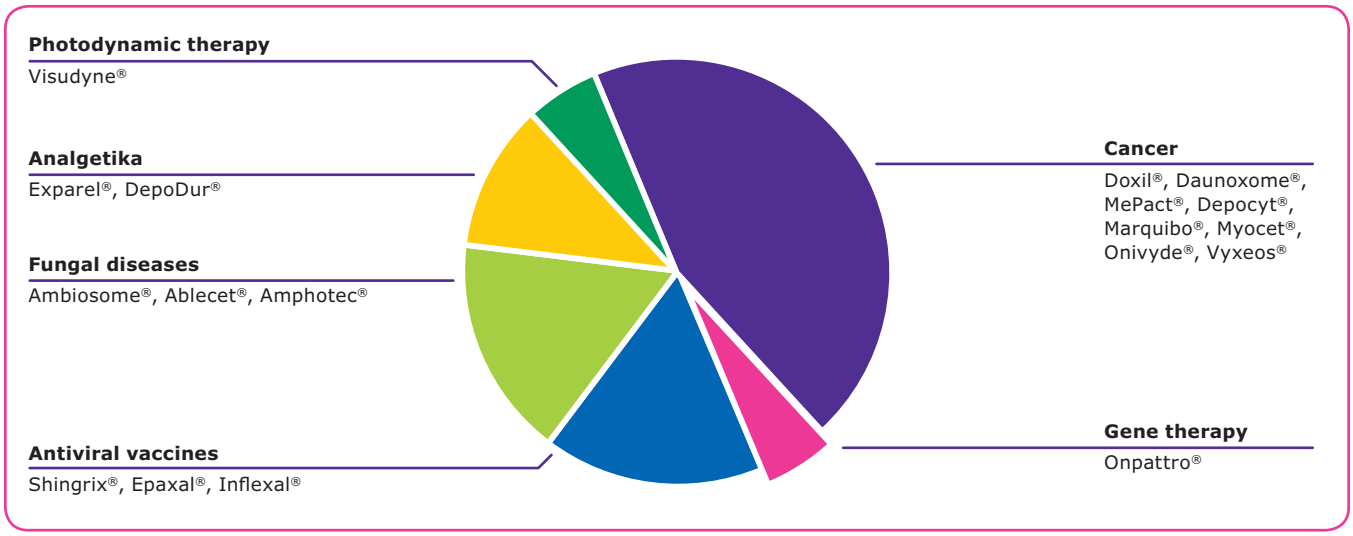

Figure 1. Lipid-based drugs on the market today (reproduced with changes from Bulbake, 2017).

Methods of liposome manufacturing

There are numerous methods for liposome manufacturing (Wagner and Vorauer-Uhl, 2011). The challenge for liposome drug producers lies in ensuring liposome manufacturing in a scalable, robust and efficient process. The manufacturing process shown in Figure 2a typically involves dissolution of lipid molecules in a solvent, a drying step and a hydration step under agitation followed by energy input such as sonification or extrusion to downsize the vesicles to unilamellar vesicles of a homogeneous distribution. Depending on whether the API is hydrophobic or hydrophilic, the API is added either with the organic solvent during the initial dissolution of the lipid or with the aqueous solution during the hydration step. In most cases, purification is the final step.

Another manufacturing method of note is the ethanol injection method, where the lipids are dissolved in ethanol and rapidly mixed with an aqueous medium containing the API (Figure 2b).

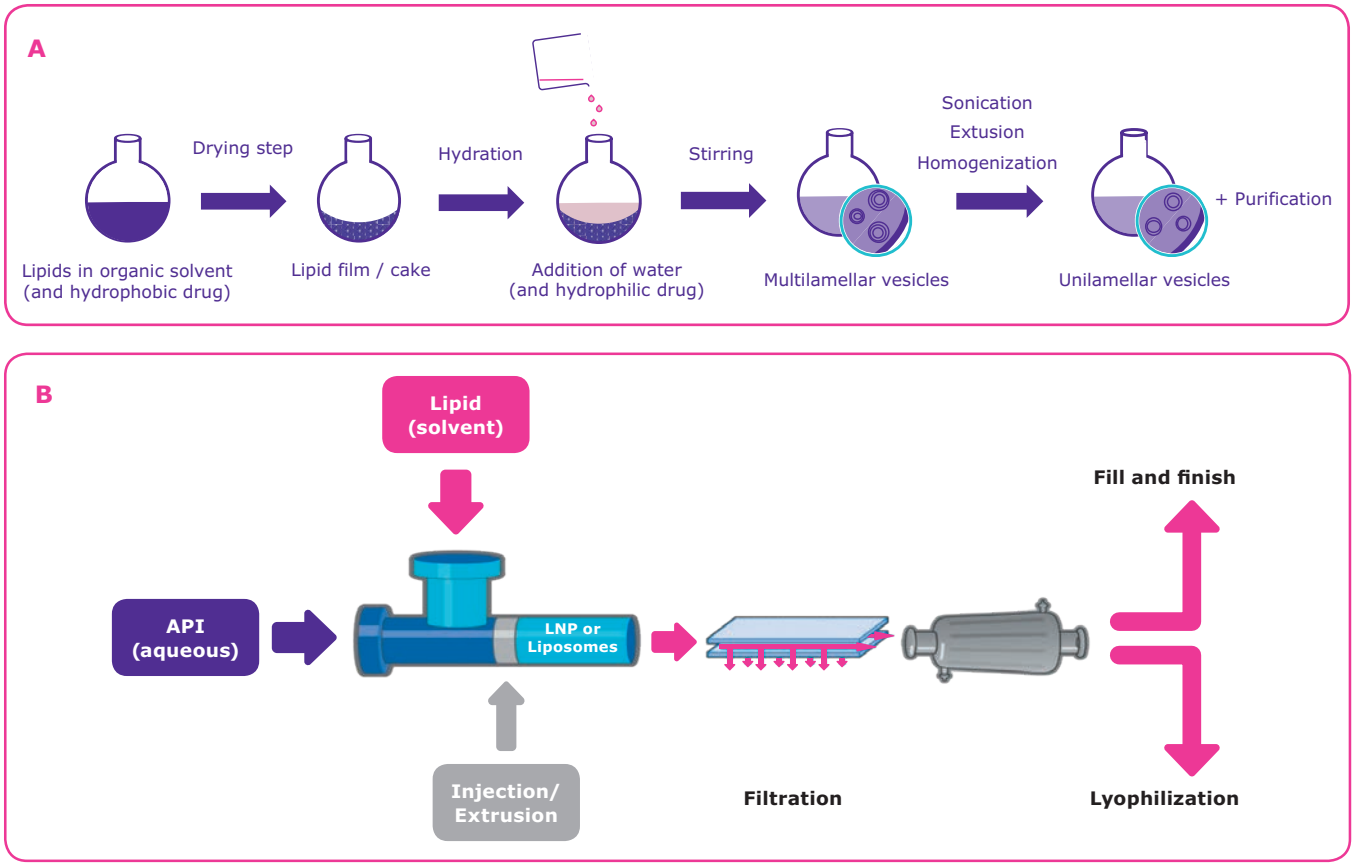

Figure 2. Schematic overview of the liposome manufacturing process. A) Rehydration method B) Ethanol injection method for manufacturing liposomes (Charcosset et al., 2015).

The manufacturing method is often based on the final application. For example, the ethanol injection method is suitable for the production of small unilamellar liposomes and stable nucleic acid lipid particles (SNALPs) which are used in intravenous applications. However, this technology is not suitable to create large liposomes as multilamellar liposomes (MLVs) and multivesicular vesicles (MVVs), which are used for vaccines administered by subcutaneous injection or intramuscular injection. In this case, the rehydration method is used.

Critical aspects to consider while choosing lipids

Lipid type, source and quality have a direct impact on the impurity profile and properties such as the particle characteristics, stability and release profile of the final formulation. In consequence, to achieve reproducible results with the final formulation, consistent quality of lipids are required, which is dependent on the quality of the raw materials used to synthesize the lipids, and good material characteristics of the lipid itself.

Choice of lipids: Synthetic vs. naturally derived
There are some advantages of using synthetic lipids over tissue derived lipids: unlike synthetic lipids that are chemically synthesized in the lab and consist of a single lipid of known quality, tissue-derived lipids are usually mixtures of lipids that are egg-derived or bovine-derived. Besides the risk of batch-to-batch variability, there is also a risk of viral or protein contamination in tissue-derived lipids.

Lipid purity
Lipid purity is critical because it influences the lipid's stability, the bilayer structure in formulation, the formulation stability and release profile. Lipid purity can be optimized by the quality of the starting materials, and modifying the manufacturing and purification techniques.

Lipid purity starts with high and consistent quality raw materials that offer following attributes:

- Low level of byproducts
- Defined stereochemistry (D/L) and isomeric purity (cis/trans)
- Low bioburden and endotoxin levels
- Plant derived raw materials with bovine spongiform encephalopathy (BSE)/transmissible spongiform encephalopathy (TSE) and non-genetically modified organism (GMO) certificates
- Use of Class II and III solvents; Class I solvents should be avoided based on guidelines from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) in ICH Q3C.

The final good manufacturing practice (GMP) process needs to be scalable and reproducible regarding yield and product quality. Manufacturing costs are driven by product concentration, yield and reaction/work-up time. Reaction conditions that could lead to isomerization should be avoided. Scalability should be considered from the very beginning to ensure economy of scale with increasing batch size. The manufacturing process should aim to reduce the number of chemical synthesis steps and clearly define the GMP steps.

The purification process steps must be scalable as well. If possible, crystallization or liquid/liquid extraction methods should be used. Similarly, chromatography should be avoided because the process is complex, expensive and not scalable. Filtration over silica gel is a good alternative.

Lipids:

DOPC	1,2-Dioleoyl-sn-glycero-3-phosphocholine
DOPE	1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine
R/S-DOTAP Cl	R/ S-1,2-dioleoyl-3-trimethylammonium-propane (chloride salt)
GDO	Dioleoyl-rac-glycerol

Other terms used:

RNA	Ribonucleic acid
siRNA	Short interfering RNA
saRNA	Short activating RNA
miRNA	microRNA
mRNA	Messenger RNA
HPLC	High-performance liquid chromatography
DSC	Differential scanning calorimetry
XRD	X-ray diffraction

Consistent quality
Synthetic lipids need to have consistent quality in every step of the drug development process. This means avoiding variability in the formulation development process as well as avoiding bridging toxicity studies, which also saves time and reduces costs. Working with a specialized life science supplier that offers a consistently high product quality is one strategy for achieving consistent quality.

Good material characteristics
The material's characteristics – solubility, crystallinity, stability and flowability – play an important role in the drug product GMP manufacturing process. Lipids are waxy by nature, which can result in slow dissolution rates and lead to challenges when handling large amounts.

Optimizing the lipid's surface characteristics for a fast and complete dissolution is a prerequisite for a reproducible liposome manufacturing process. The available processes to enhance a material's surface are:

- Cryo-milling
- Spray drying
- Crystallization
- Lyophilization

These processes provide solubility improvements, higher purity, enhanced stability and easier handling characteristics, all of which will enable an easier formulation process and is relevant to GMP requirements. The use of spray drying and lyophilization processes result in a material of very high surface area and good handling characteristics. Homogeneity in lipid mixtures can be ensured with spray drying and lyophilization.

Crystallization is one of the most commonly used methods for surface enhancement. MilliporeSigma holds patents to produce certain crystalline lipid compounds such as DOPC and DOTAP Cl. Both DOPC and DOTAP Cl, are typically available in the market as amorphous material. The amorphous versions are difficult to weigh precisely due to their lumpiness and have poor dissolution characteristics.

In contrast, crystalline DOPC and DOTAP Cl offer several benefits over amorphous DOPC including:

- Enhanced stability: confirmed by stability studies of more than seven years at 25 °C/60% rH
- Fast dissolution rate
- Free flowing powder, allowing for easy weighing and portioning

Crystalline forms of DOPC, and the pure enantiomers (R, and S) and racemic mixture DOTAP Cl, are available from MilliporeSigma.

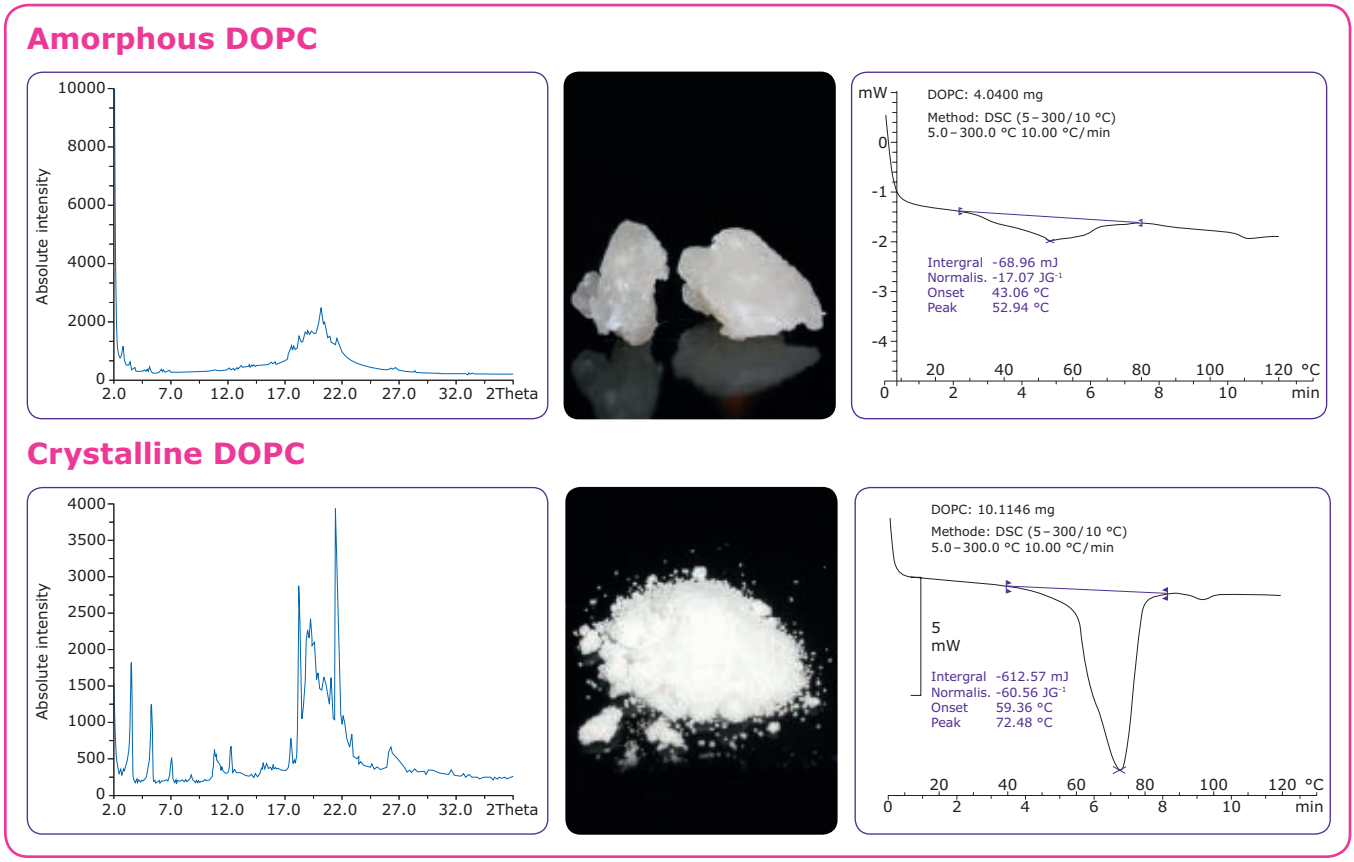

Figure 3. Comparison of amorphous and crystalline DOPC: XRD (left), optical appearance (middle), DSC (right).

Figure 4. Wax-like, amorphous DOPE in comparison to free flowing powder DOPE.

Another example of how process improvements can dramatically change the physical properties of lipids is DOPE. Typically, DOPE is available as lumps, gel or foam format in its amorphous form, and has limited dissolution even after lyophilization. MilliporeSigma has improved the manufacturing process of DOPE such that the final compound is a free-flowing powder, which enables fast and complete dissolution. Powder DOPE also offers easier handling compared to conventional material of wax-like consistency.

Troubleshooting lipid purity

Figure 5 illustrates many impurities found in a commercially available sample of Dioleoyl-rac-glycerol (GDO), which had an impact on the final formulation (Figure 5a). For a defined and controlled behavior of the final formulation, we have produced a highly pure and defined mixture of GDO isomers (1,3-GDO and 1,2-GDO). This was achieved by chemically synthesizing 1,3-GDO and 1,2-GDO with high purity as seen in the HPLC trace in Figure 5b. The new lipid product can be delivered with consistent quality allowing reproducible formulation results.

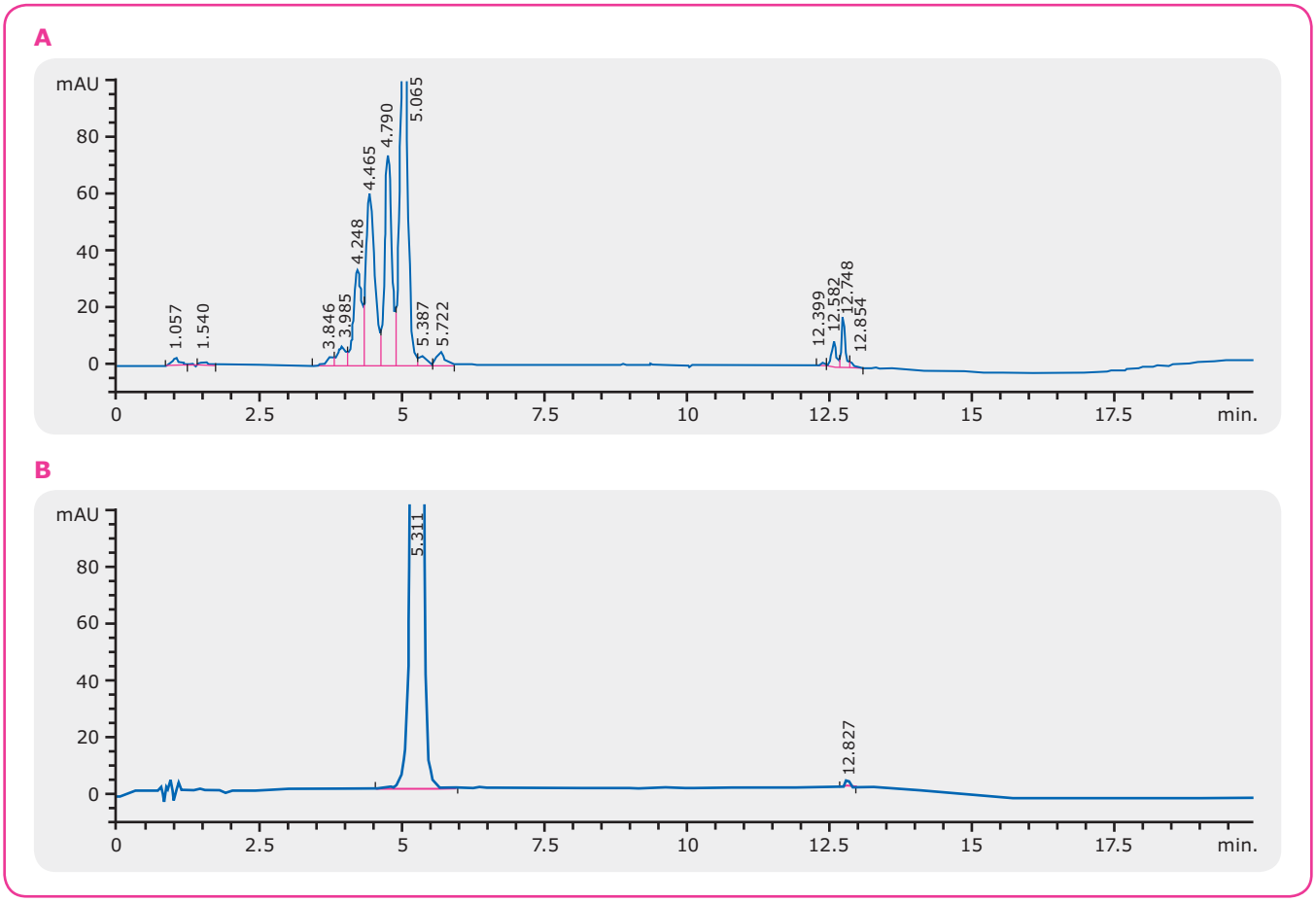

Figure 5. Comparison of (A) commercially available sample of dioleoyl-rac-glycerol GDO with many impurities and (B) high purity 1,3-GDO & 1,2-GDO from MilliporeSigma.

New lipid technologies

Thanks to exciting advances in the field of new lipid structures, it is possible to change the properties of the formulation completely. For example, the gene therapy field has seen several generations of cationic and ionizable lipids with novel headgroups, linkers containing hydrolysable ester bonds and disulfide bridges, and lipidoids.

An unmet need in lipid-based delivery is targetability, to the site of action. Peptides or mAbs could be potential targeting agents when effectively displayed on the surface. However, conjugating peptides directly to lipids is not straightforward as the functional groups of the side chains of the constituent amino acids can also react. The side reactions lead to unwanted by-products that are difficult to purify, and lead to low process yields, which results in a final, expensive product that is not scalable and unsuitable for GMP production.

A solution to this challenge is the in-solution lipidation of the peptide of interest. MilliporeSigma utilizes a solid phase synthesis process which can be automated. It starts with a patented lipidated amino acid attached to a resin and subsequent amino acids are added one by one. Fast work-up can be accomplished by simply washing the resin. This solid phase synthesis results in a lower priced product, and in a scalable process that is suitable for GMP production.

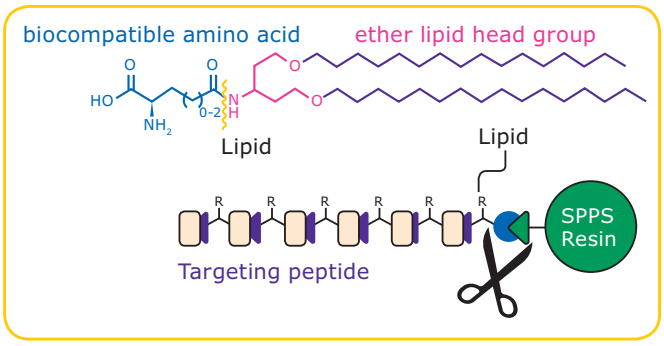

Figure 7. Customized lipid from solid phase synthesis.

Product development of lipids

Product development should ideally follow the drug development stages as outlined in Figure 6. The initial step of developing a new lipid is finding a cost-effective synthetic route, performing feasibility studies, and doing lab scale manufacturing experiments. This should be done during the early preclinical stages of drug development. During the late preclinical stage and Phase I clinical trials, which occur over a period of several years, process optimization should be the focus. This includes identifying the key raw materials and developing all the analytical methods required, specific to the lipid. During the clinical trials, the lipid chemical synthesis should be scaled up accordingly, which includes implementation of analytical methods, and establishing in-process controls. Stability studies should also be ongoing at this stage. During late-stage clinical trials, critical parameters for the process need to be defined, process validation of the lipids should be planned, pre-qualified raw material suppliers should be fixed, and rigorous risk analysis and intermediates testing should be done. As drug development is a long and costly process, choosing the wrong material will lead to negative financial implications and delays.

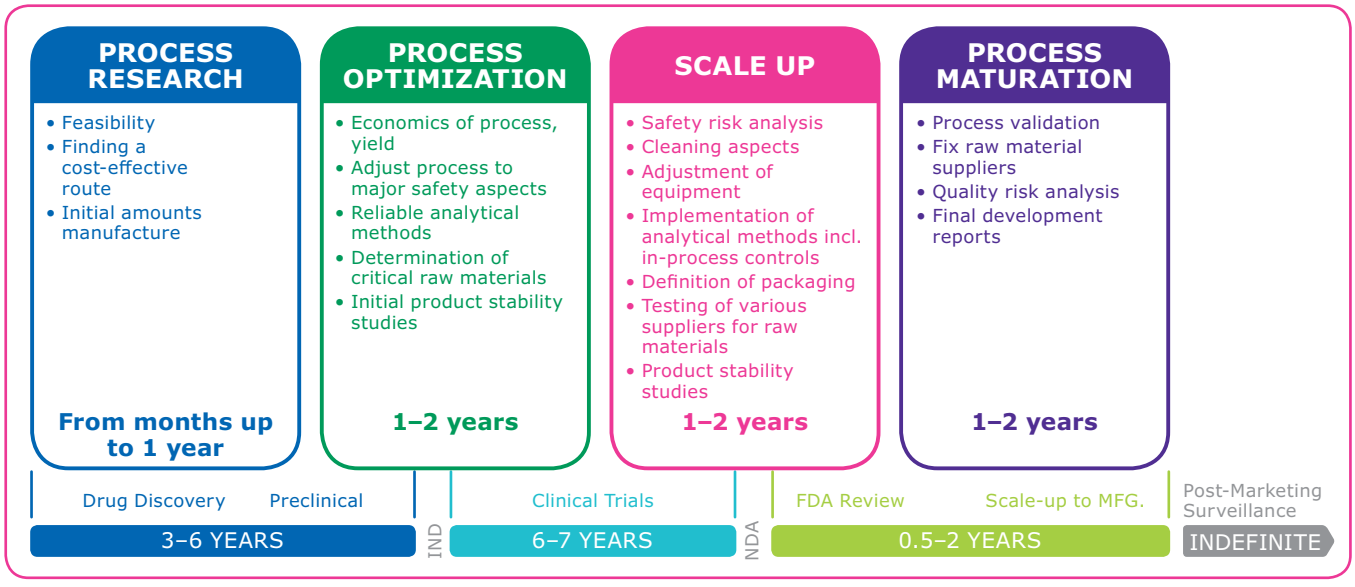

Figure 6. Overall pharmaceutical product development process timelines and the corresponding process development behind lipid-based formulations.

Regulatory aspects for lipid-based drug formulations

There is no clear path to regulatory approval of liposome drug products due to the absence of global harmonized regulatory requirements for lipid excipients. Since the purity and quality of the lipid components can affect the quality of the lipid-based formulation, detailed information on chemistry, manufacturing and controls is requested by regulatory authorities. Some of the recent guidelines address various regulatory aspects of liposome drug products that are relevant to this discussion.

Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation

The FDA finalized its guidance on liposomes in April 2018. The guidance document addresses what information should be submitted to FDA by the drug sponsor in new drug applications (NDAs) or abbreviated new drug applications (ANDAs) (FDA, 2018). The guidance refers to several guidelines established by the ICH, including ICH Q11: Development and Manufacture of Drug Substances. The FDA liposome guidance notes the quality and performance of a liposome drug product can be impacted by the quality of lipid components, and recommends the level of detail in the submission should be comparable to that for a drug substance.

Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product

The European Medicines Agency (EMA) published a revised refection paper in March 2013 on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product. The reflection paper highlights how the quality of the lipid materials is essential for the quality of the drug product, and how characterization and specification of the lipid is vital (EMA, 2013). The level of information to provided with the submission depends on the complexity of the excipients.

Guideline for the Development of Liposome Drug Products

Japan's Ministry of Health, Labour and Welfare (MHLW) Japan published guidelines for the Development of Liposome Drug Products in March 2016. The MHLW guidelines note the quality of liposome components should be evaluated and controlled to a greater extent than general excipients because they have a significant impact on the drug product (MHLW, 2016).

ICH Guidelines

In order to provide the high level of detail required by the different global authorities, guidelines have been established that should be taken into consideration. For instance, in addition to ICH Q11, several ICH guidelines are applicable including:

- ICH Q7 (API GMP): Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients Q7
- ICH Q1A(R2): Stability Testing of New Drug Substances and Products
- ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology
- ICH Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances

IPEC Guidelines

Apart from the regulatory authorities, it is worthwhile to consider the guidelines set by the International Pharmaceutical Excipients Council (IPEC) Federation. The IPEC is a global industrial organization that aims to promote the best use of pharmaceutical excipients and harmonize standards by developing guidelines for various aspects of excipients, e.g.:

- IPEC PQG Excipient Good Manufacturing Practices Guide
- IPEC Excipient Good Distribution Practices Guide
- IPEC Excipient Qualification Guide
- IPEC Excipient Stability Guide
- IPEC Excipient Composition Guide
- IPEC-Americas Excipient Significant Change Guide
- IPEC-Americas Excipient Master File Guide

The IPEC Federation is made up of the five-existing regional IPECs: IPEC-Americas, IPEC China, IPEC Europe, IPEC Japan and IPEC India. The IPEC guidelines have been used globally by many companies and regulatory authorities to develop appropriate standards for excipient control.

Regulatory Submission

The submission of the excipient information to regulatory authorities presents another challenge. In some countries such as the United States, Canada, Japan and China, excipient information can be submitted in the form of a drug master file (DMF) or dossier by the excipient manufacturer. In Europe, this regulatory procedure does not exist for excipients. The excipient information must be submitted by the finished drug product manufacturer as part of its application.

Due to the challenging regulatory environments, it is recommended that the drug manufacturer works closely with a supplier that provides regulatory expertise and counsel through all phases of clinical development and commercialization, covering all aspects of quality assurance and documentation.

Conclusion

For successful drug development with synthetic lipids, the GMP manufacturing process needs to be scalable and reproducible in quality and yield. This is a prerequisite for a consistent quality in the final product. The quality of lipids used has a major impact on the performance of the liposomal formulation. Lipids are available in different formats, physical states, and purities from different suppliers, and it is essential to choose the right lipids with the best characteristics depending on the application. When working with any novel lipids, feasibility studies to find the most optimal synthesis route and purification steps, that can be scaled up for GMP production are key.

While the regulatory process for liposome drug products is complicated with many different guidelines from different global authorities, they all agree lipid quality is critical.

To avoid high costs and surprises later in the drug development process, it is important to plan the product development beforehand and work with the same quality of excipients throughout drug development. This highlights the importance of working with the right supplier that offers consistent high-quality products, understands all steps of the drug development process and the regulatory environment and provides a high-level of customer support.

With over 25 years of experience, MilliporeSigma specializes in synthesizing top-quality lipids, following current GMP (cGMP) processes that adhere to ICH Q7. For more information on our portfolio, visit our webpage: www.EMDMillipore.com/lipids

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