Available online on 15 Jun, 2021 at https://ijdra.com/index.php/journal

**International Journal of Drug Regulatory Affairs**

Published by Diva Enterprises Pvt. Ltd., New Delhi
Associated with Delhi Pharmaceutical Sciences & Research University


**Review Article**

Open Access

# Liposome Characterization, Applications and Regulatory landscape in US

**Amit Kumar*, Madhu Gupta, Simran Braya**

*Delhi Pharmaceutical Sciences and Research university, Mehrauli - Badarpur Rd, Sector 3, Pushp Vihar, New Delhi-110017, India*

## Abstract

Liposomes are lipid based drug carrier whose therapeutic performance depends on their structure. Liposomes offer several advantages over the conventional drug like target drug delivery, reduced toxicity, and extended pharmacokinetics. Characterization and Identification of critical attribute of liposomal formulation and suitable strategies for control during product development is important for quality of the liposomal drug product. This paper discusses the current status of the liposomal drug product and strategy used in regulating liposome product. Despite of lack of regulatory guidelines many liposome formulations get approved which shows the potential of liposome drug products.

**Keywords:** Liposomes, Phospholipid, Nanotechnology, Lamellarity, Liposomal Delivery, ANDA, FDA, CTD

**Article Info:** Received 28 May. 2021; Review Completed 14 Jun. 2021; Accepted 15 Jun. 2021

**Cite this article as:**
Kumar A, Gupta M, Braya S. Liposome Characterization, Applications and Regulatory landscape in US. Int J Drug Reg Affairs [Internet]. 2021 Jun 15 [cited 2021 Jun 15]; 9(2):81-89. Available from: http://ijdra.com/index.php/journal/article/view/474

**DOI:** 10.22270/ijdra.v9i2.474
*Corresponding author

## 1. Introduction

Liposomes are most popular nano-carrier used for drug delivery liposome were in 1964, in Cambridge at the Babraham Institute by the British hematologist Dr. Alec D Bangham. (1,2) Liposome is made of two greek words 'lipos' and 'soma' which means fat and body. Liposomes are basically small vesicles composed of multiple bilayer formed by using amphipathic molecules like phospholipids and separated by an aqueous compartment. In liposomal drug products, drug is encapsulated in the liposomes. Hydrophilic drug is loaded in the aqueous section and lipophilic drug is loaded in the lipid section of the liposome. (3) Liposomes are a promising drug delivery mechanism. Liposome properties vary greatly depending on lipid composition, surface charge on the liposome, duration, and the method used for formation. Furthermore the fluidity and rigidity of the liposome is dependent on the component of bilayer used in the formation. For example natural unsaturated phospholipids species forms less stable and more permeable bilayers whereas saturated phospholipids species forms impermeable and rigid bilayers. Liposomes are widely used as nano carrier for a different types of molecules in pharmaceutical and cosmetic industry. Also agriculture and nuraceuticals industries are studying liposome to encapsulate antimicrobial, antioxidants, flavoring agents which are unstable and to protect their function. (4,5)

Liposome offers various advantages like biodegradability, low toxicity, biocompatibility and can trap hydrophilic and hydrophobic drug because of this many studies are being conducted on the liposome to have better understanding on functioning of liposomes. Doxil was the first nanoscale liposomal drug product which was approved by usfda in the year 1995 for the cure of aids related Kaposi's sarcoma and ovarian cancer. It was the very first nanomedicine which got regulatory approval after that many liposomal drug got approval. (6) Due to developments in the liposomal technology currently many liposomal are present in market and also under clinical trials.

## 2. Classification of Liposomes

Liposomes can be classified on the basis of their **Structure** (7):

**Table 1.** Classification of liposome

| Vesicle type | Abbreviation | Size |
|---|---|---|
| Multilamellar Large vesicles | MLV | 0.1-6µm |
| Small unilamellar vesicles | SUV | 0.02-0.05 µm |
| Large unilamellar vesicles | LUV | >0.06 |

| | | |
|---|---|---|
| Oligolamellar vesicles | OLV | 0.1-1 µm |
| Unilamellar vesicles | UV | - wide range |
| Gaint unilamellar vesicles | GUV | >1 µm |
| Medium Unilamellar vesicles | MUV | |
| Multivesicular vesicle | MV | (>1 µm |

**3. Composition and application**

**Liposome classified into five categories for their application and composition**

i. Conventional liposomes

ii. pH sensitive liposomes

iii. Cationic liposomes

iv. Immune liposomes

v. Long circulating liposomes

**Advantages of liposome**

- Higher efficacy and increased therapeutic index of drug.
- Increased stability via encapsulation
- Liposomes are biocompatible, non-toxic, biodegradable and non- immunogenic.
- Encapsulation reduces the toxicity. (amphotericin B)
- Exposure to sensitive tissue is minimal.
- Site avoidance effect
- Active targeting by using site specific ligand.

**Disadvantages of liposome**

- Short half-life
- Phospholipid can undergoes reaction like oxidation and hydrolysis
- Leakage of the drug from liposome.
- High cost of production
- Fewer stable

**4. Characterization of Liposomes**

Characterization of liposomes is very essential for the assessment of the quality and to validate the batch-to-batch reproducibility of liposome sample. Some of the most chief parameter for characterization is stated below:

***Size and particle distribution***

Determination of distribution size is critical parameter because it provide information about liposome's quality as well as variation in batch, size distribution is also very crucial for the parental liposomal drug products. Various techniques by which size can be measured are dynamic light scattering, electron microscopy, laser light scattering etc. (8, 9)

***Lamellarity***

Number of bilayers which are present in the liposome is lamellarity and it affects the drug release kinetics, encapsulation efficacy and also the implementation of the liposomal product. It can be measured by Small angle X-ray scattering, 31P-NMR, Freeze-fracture electron microscopy. (10)

***Lipid composition and concentration***

Components which were used for the manufacturing of liposome must be analyze prior to and after the preparation. Individual component can be measured by using a chemical reacton following a spectrophotometric measurement. Barlett assay or Stewart assay is used to find out Phospholipid concentration, Cholesterol oxidase assay is used to find out Cholesterol concentration following with HPLC. (11)

***Surface charge***

Zeta potential is described as the overall charge on the liposome suspension, depending on the charge the liposome can be positively, negatively charged or neutral. Zeta potential can determine the stability of the liposome. Liposome is stable when all the particle remains suspended not aggregated. Surface charge can be measured zeta potential measurement, electrophoresis. (12, 13)

***Encapsulation efficiency***

Encapsulation efficiency is basically the quantity of drug which can be loaded into the carrier liposome. Formula used for measuring the encapsulation efficiency is % Encapsulation = drug entrapped in liposome/total drug added *100. Drug content can be found using Mini column centrifugation, ion-exchange Chromatography. (14)

***In vitro drug release***

In vitro drug release can be performed using dialysis tube diffusion technique. In this method, a weighed amount of newly formed liposome is reconstituted in release medium and taken inside a dialysis chamber. Then the whole system is placed on the top of magnetic stirrer and maintained at 37*c. sampling is done by withdrawing 1ml from the released medium along with the addition of 1ml of fresh buffer simultaneously. Samples are measured spectrophotometrically. (15, 16)

**a. Physical Characterization**

**Table 2.** Physical Characterization parameters of liposomes

| Charcterization parameters | Analytical methods |
|---|---|
| Size & Surface morphology | TEM, Freeze- fracture EM |
| Size distribution | PCR , Zetasizer , DLS, TEM, gel permeation, exclusion |
| Surface charge | Zeta potential measurement |

| | |
|---|---|
| Lamellarity | NMR, Freeze fracture EM |
| % Entrapment Efficiency | Minicolumn centrifugation, ion exachange, gel exclusion |
| Drug release | Diffusion |

**b. Chemical Characterization**

**Table 3.** Chemical characterization parameters of liposomes

| **Charcterization parameters** | **Analytical methods** |
|---|---|
| **Phospholipid** | Barlett/Stewart assay, HPLC |
| **Cholesterol** | Cholesterol oxidase assay,HPLC |
| **Drug** | Method as in individual monograph |

**c. Biological Characterization**

**Table 4.** Biological characterization parameters of liposomes

| **Charcterization parameters** | **Analytical methods** |
|---|---|
| Sterility | Aerobic or anaerobic cultures |
| Pyrogenicity | LAL test |
| Animal toxicity | Monitoring survival rates, Histopathology |

**5. Application of liposome**

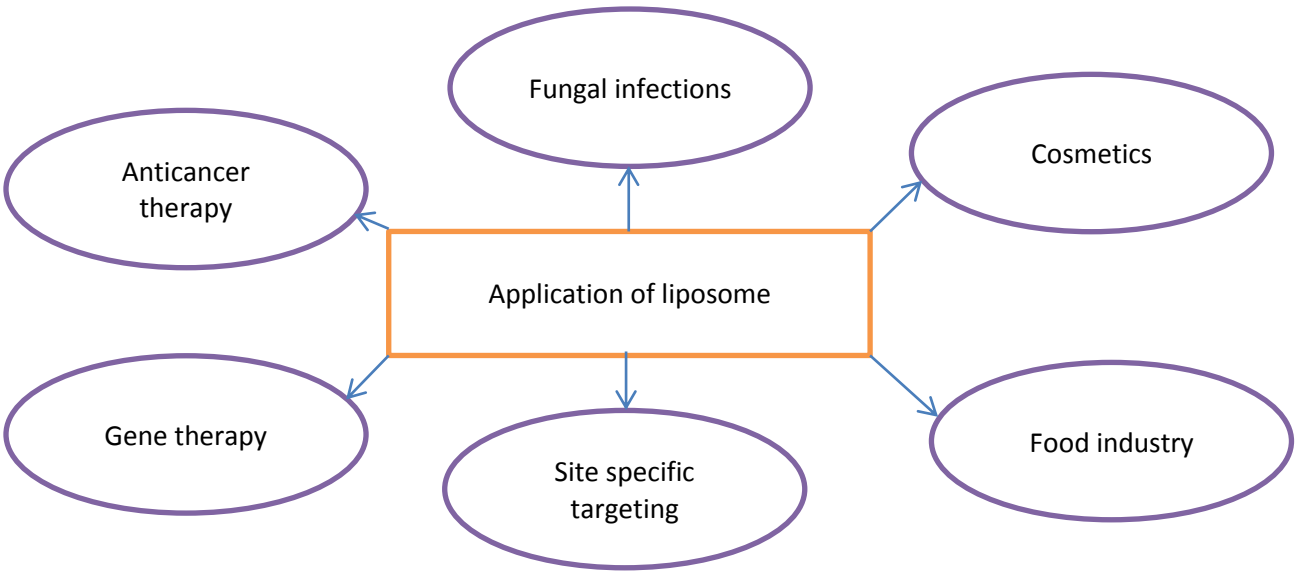


**Figure 1.** Representing the applications of liposomes

***Liposomes anticancer therapy***

The side effect of anticancer agents are a major issue in cancer chemotherapy, that is why liposomal formulations are used because of liposome increases the therapeutic activity of the drug by delivering larger quantity of drug in to tumors site and reducing impact to normal tissues. A number of liposome drug products are being used as anticancer agents because they were found least toxic and more effective in comparison to free drug. FDA approved first nano-scale drug which was doxil a liposomal formulation doxorubicin used for management of AIDS and ovarian cancer. In 2017 FDA approved vyxeos which is a cytarabine fixed combination with daunorubicin in liposomal formulation, for acute myeloid leukemia (AML) treatment. Study of vyxeos showed overall improvement in the survival rate. (17, 18)

***Liposomes for Fungal Infections***

Liposomal formulation is also used for fungal infections because many antifungal agents like amphotericin B have shown some serious toxic effect and less stability. Encapsulation of these antifungal agents showed improved result by increasing their stability activity and protection from environment. Amphotericin B in liposomal formulation (Ambisome) showed improved result in comparison to free drug. (19)

***Liposome site specific targeting***

Drug delivery to a particular site is achieved by liposomal drug delivery. Encapsulation of drug in the liposome nano carrier can deliver higher quantity of drug to a particular site which is very helpful in chemotherapy, many anticancer drug are available in liposomal formulation because of the target delivery system and which cause minimal exposure of drug to

normal tissues. By encapsulation both passive as well as active targeting can be done. (20, 21)

***Liposomes in cosmetics***

Liposomes have application in cosmetics also because by encapsulation they increases the active ingredient's stability, enhanced dermal bioavailability, skin targeting, protection from sun and sweat of skin cells and also increase skin hydration. Products like hair shampoos, lotions, moisturizers, creams etc., are already present in the liposomal form. (22)

***Liposomes in food industry***

Liposomes have great potentials in the food, beverage, and nutraceutical industries. Increased bioavailability and enriched flavors are results due to encapsulation of additives, antimicrobials and preservatives. Liposomes because of their non-toxic, biodegradable and ability to carry hydrophobic and hydrophilic product are suitable for the food industry. (23)

***Liposome in gene therapy***

Liposomes serves as an excellent gene delivery tools because of its remarkable and unique characteristics in DNA delivery. (i) low immunogenic response, (ii) ease of loading DNA in larger quantity (iii) increased DNA stability in body by using liposomes and (iv) DNA blood circulation is improved because of minimal clearance. liposomal gene therapy could benefit a various diseases like, immune system deficiencies , HIV ,neurological disorders , cystic fibrosis, hemophilia and sickle cell anemia. (24)

***Need of regulations***

- The biggest issues for regulation of liposomes is that the regulatory bodies uses safety data based upon the bulk materials which don't display same PK and PD behavior as liposomes. (25) Often scale up and manufacturing process is hit or miss for liposomes stability. Hence, stringent protocols and assurances are required for approval. (26)
- Regulatory guidelines are needed to measure liposome toxicity which is used as nanocarrier. (27)
- Genotoxicity and mutagenicity may occur because most nanomedicines directly interfere with genetic material. (28)
- There is data indicates that nanomedicines when administered intravenously particles accumulate in renal system, liver, CNS system. (29, 30)
- Also the impact of nanomedicines on the environment should be considered while regulating them. (31)
- Environmental assessment for a new nanotechnology product is required by the USFDA unless relaxation is provided.

## 6. Currently approved clinical liposomal drug therapies

**Table 5.** Some of the liposomes approved for clinical use

| Disease indication | Liposomal product | Drug (API) | Company |
|---|---|---|---|
| **Cancer therapy** | Doxil® | Doxorubicin | Janssen Johnson&Johnson |
| | Mepact® | Mifamurtide | Takeda Pharmaceutical |
| | Myocet® | Doxorubicin | Elan Corporation USA |
| | Vyxeos® | Daunorubicin: cytarabine | Jazz Pharmaceuticals |
| **Fungal diseases** | Abelcet® | Amphotericin B | Enzon |
| | Ambisome® | Amphotericin B | Astellas Pharma |
| | Nyotran® | Nystatin | Aronex Pharmaceuticals |
| **Viral vaccines** | Epaxal® | Hepatitis A vaccine | Crucell, Berna Biotech |
| | Inflexal®V | Influenza vaccine | Crucell, Berna Biotech |
| **Analgesics** | DepoDur | Morphine sulfate | SkyPharma Inc. |
| | Exparel | Bupivacaine | Pacira Pharmaceuticals, Inc. |
| **Photodynamic therapy** | Visudyne | Verteporfin | Novartis |
| | Visudin | Verteporphyrin | Novartis |

## 7. Liposomal Delivery: Future Challenges

There are three major issues that we encounter with liposomal delivery systems: absorption by the reticulo-endothelial system, large-scale processing, and phospholipid stability, all of which pose a barrier to their commercial growth.

***Uptake by Reticulo-endothelial system***

Liposomes after the systemic administration are recognized as foreign particles by the mononuclear phagocyte system (MPS) cells, mostly in the spleen and liver by fixed Kupffer cells, and are thus endocytosed. This fate is ideal for delivering drugs to these cells, but it precludes other applications, such as site-specific drug delivery through ligands expressed on the liposome surface that bind to overexpressed receptors on diseased cells. As a result, researchers began looking for liposomes that could evade MPS uptake, and a few lipid compositions that extended blood circulation time of liposomes were discovered.

***Large scale production***

Liposome preparation entails a number of steps, including the evaporation of a solvent system under reduced pressure, the preparation of a thin lipid film, and sonication. These measures, especially the preparation of thin film, are complicated to carry out on a large scale. As a result, scaling up liposome production from the laboratory to large-scale production is difficult. Chemical solvents such as chloroform, methanol, and others are not recommended in such high concentrations

to solubilize and combine lipids, according to regulatory standards.

***Stability***

While liposomes have the advantage of improving the stability of unstable drugs, the phosphdolipids used in their manufacture are highly susceptible to oxidation and/or hydrolysis. As a result, lipid-based products cannot be processed for a longer period of time. However, the goods are also available in lyophilized form, which have to be rearranged before use. Liposomes are not only physically unstable, but they are also chemically unstable. Furthermore, it has been found that electrostatic liposome stabilization is insufficient in the presence of disintegration substances such as enzymes and proteins encountered in in-vivo applications. (32)

## 8. Regulatory Landscape in US

In the United States Food and Drug Administration, (FDA) is in charge of evaluating, authorising, and overseeing medicinal products such as pharmaceuticals and medical devices. The FDA regulates a broad variety of products, including foods, cosmetics, medications, instruments, veterinary products, and tobacco products, all of which can include nanotechnology or nanomaterials. (33)

**When determining whether a product controlled by the FDA uses nanotechnology, the FDA would ask:**

- Whether an end product or material is designed to have as a minimum one outside dimension, in addition to an inner or surface structure, with inside the range of nanosacle (approximately 1 nm to 100 nm).
- When an end product or substance is designed exhibit any physical, chemical or biological effect because of its size or dimension. (34)

***The 505(b)(2) regulatory pathway***

The liposome drug products can 505(b)(2) pathway to get approved, because most of the time liposome contain an already licensed drug. This type of submission provide benefit to the applicants because FDA will allow referral to the dossier of the API which is already authorized by the FDA in other dosage forms, reducing the number of clinical trials needed and making liposome product production much less expensive than creating a new chemical entity (NCE).

***FDA Nanotechnology Task Force***

Nanotechnology task force was formed by FDA in august 2006, with a goal of determining approaches of regulatory authority that would promote the production of safe and effective FDA-regulated nanotechnology or nanomaterials products.

Rapid advances in fields such as engineering, life sciences and medicine have growth in the knowledge of nanotechnology and its existing and future applications in FDA-regulated products. Since 1970, More than 600 applications (investigational new drug (IND), new drug application (NDA), and follow up product application for drug products which include application of nanomaterials have been received by the FDA's Center for Drug Evaluation and Research (CDER), with half of them submitted in the last couple of years. (35)

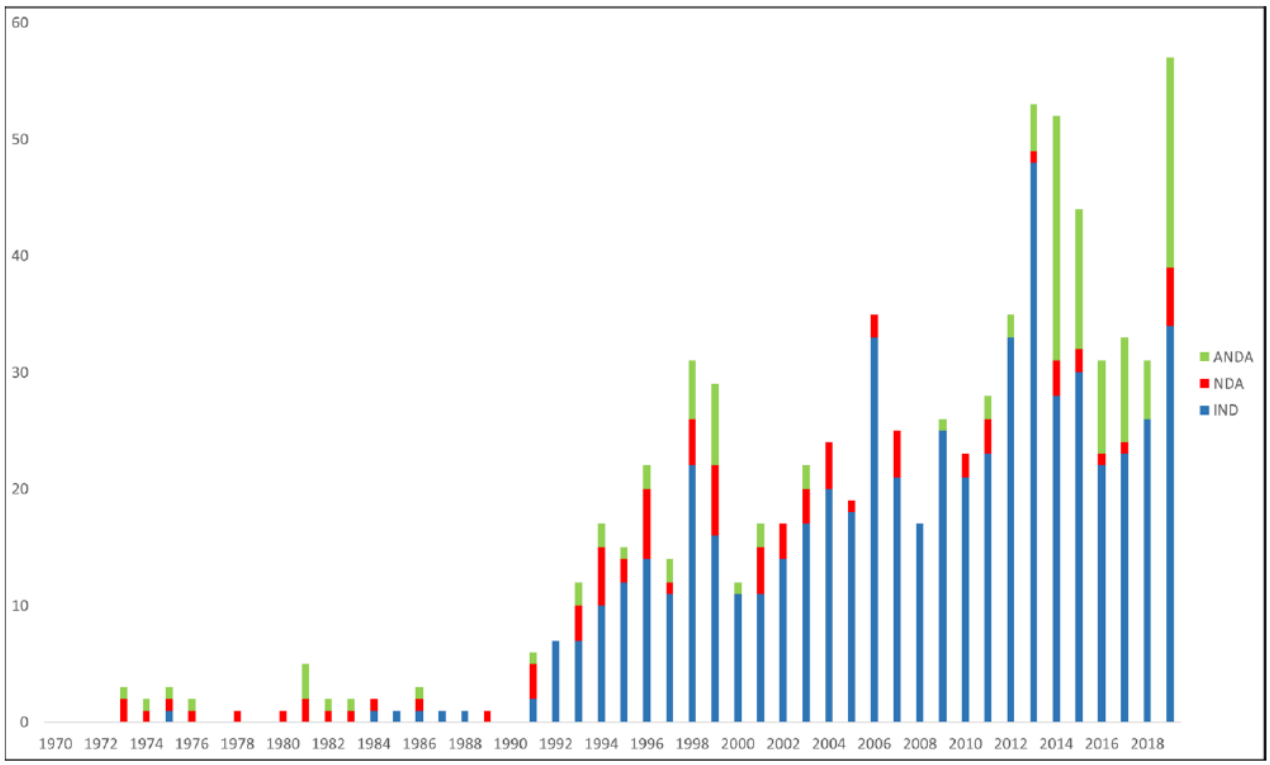


**Figure 2.** Human drug product submissions to FDA containing nanomaterials between 1970-2019.

In April 2018, FDA released its final guidance for industry on liposome drug products. It completes the October 2015 updated draught guidance for industry Liposome Drug Products, Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation.

**9. Data to be submitted by an applicant**

The US Food and Drug Administration issued guidelines on what details should be included in a new drug application or follow up application for a liposomal drug product that will be evaluated by the Center for Drug Evaluation and Research (CDER). (36)

***Chemistry, Manufacturing, and Controls***

**a. Description and Composition**

In the application applicant should include information about the liposome drug product's composition like drug substance, lipids, nonlipid components. An expression for amount of lipid and drug substance which is incorporated in the formulation should be expressed. Since any alterations in the manufacturing, including the composition of the lipids, can have a huge impact on the toxicology and pharmacological properties and quality of a liposome product, the ranges must be defined based on product development studies.

**b. Physicochemical Properties**

The structure and integrity of liposome are critical physicochemical properties that indicate the liposome drug formulation's ability to contain and maintain the drug products within the proper liposome structure. These characteristics would be used to describe a liposome formulation in general. Changes in the consistency of the liposome product, including drug leakage from the liposomes, can be caused by variations in these properties. Some of the physiochemical properties are size distribution, mean size of particles of liposomes, osmolality, zeta-potential and physical stability

**c. Critical Quality Attributes**

The parameters whose variation has an impact on critical quality attribute are determined by critical quality attribute (CQA). As a result, it must be regulated or managed in order to ensure the optimal drug product

**d. Description of Manufacturing Process and Process Controls**

A comprehensive s flow diagram of process and a summary of operations with ranges for process parameters and process controls are recommended by the FDA. Pharmaceutical production research can back up these ranges. The process and mechanism of liposomal drug loading and the purification of free (unincorporated) drug substance from the liposome formulation, should be thoroughly explained. Until commercial delivery, the production process should be tested to show accuracy and reproducibility.

**e. Control of Lipid Components**

The consistency of lipid components, especially changed lipids, can affect the quality and efficiency of a liposome medicinal product. A novel lipid component, defined as any lipid component not mentioned in the Database of inactive ingredients (IID) or a component that exceeds the amount provided in the database for the intended administration route, should have the same degree of information as a drug product. This information needs to be included in the application or in a Type IV Drug Master File (DMF)

**f. Drug Product Specification**

Applicant should provide a drug product specification that takes into account particular characteristics for the product. Some of the specification which should be includes in the product specification are physiochemical parameters, free and contained drug in liposome, lipids content used, in-vitro release of drug from the liposome, detail about the degradation product.

**g. Stability**

The microbiological, physical, and chemical stability of the liposome formulation and the integrity of the liposomes in the drug product should all be considered in stability studies. (37)

**Table 6.** Stability testing of new liposomal drug formulations:

| Study | **Stored general conditions** | **stored in a refrigerator** | **stored in a freezer** | **Duration of study** |
|---|---|---|---|---|
| **Long term** | 30°C ± 2°C /75% RH ± 5% RH | 5°C ± 3° C | - 20°C ± 5°C | 6 months or 12 months |
| **Accelerated** | 40°C ± 2°C /75% RH ± 5% RH | 25°C ± 2° C/60% RH ± 5%RH | - | 6 months |

***Post-approval Changes in Manufacturing***

The FDA claims that "Liposome drug products are complex and responsive formulations, with less stable responses to CMC changes than more traditional formulations. As a result, adjustments to the formulation, container closure, manufacturing location, or manufacturing process (including significant equipment and size changes) will almost always necessitate a supplement to the prior approval." To discuss concerns about the type of information to produce or the necessary reporting process for a post-approval update, FDA recommends contacting the appropriate review division associated with the application. (38)

***Human Pharmacokinetics: Bioavailability and Bioequivalence***

The Guidance states that calculating the quantity of drug in plasma over time is unlikely to be adequate to show bioequivalence because of the products' complexity and varying release profiles relative to other liposomal products and non-liposomal liposomal products. The drug's bioavailability at the site of action may need to be determined.

A Pre-ANDA meeting is recommended for ANDA applicants prior to submission to decide an appropriate method for determining drug bioavailability at the site of action. A Sponsor should check with the relevant CDER Review Division for NDA applications.

For an ANDA submission, product-specific bioequivalence guidances should be taken into account when submitting an ANDA. Just four of such guidance documents for liposomes have been published by the FDA so far. Draft guidelines for bupivacaine, daunorubicin citrate, amphotericin B and doxorubicin are among them. Clinical and in vitro trials to show bioequivalence are recommended in the guidance documents. (39)

***Comparison with the non-liposome drug product***

The FDA advises a Sponsor to compare its proposed innovator liposomal product to an approved non-liposomal product that includes the same active ingredient and is administered via the same route. Absorption, delivery, metabolism, and excretion (ADME) experiments should be used to compare the two drugs. A mass balance research with radio-labeled drug material may be required to characterize differences in distribution between liposomal and non-liposomal drug products. In the relevant patient population, a single-dose PK analysis comparing liposomal and non-liposomal products should also be performed.

***Biopharmaceutics***

According to the Guidance, studies should be conducted to determine whether the release characteristics of the items are identical. It should be attempted to perform IV/IVC (in vitro/in vivo correlation) studies. Even if a full connection cannot be formed, information about such relationships can be useful. To assess the potential for drug 'dose-dumping' in vivo, liposome association with blood proteins should be investigated.

***Labeling***

Labeling requirements for liposome formulation are available on the website of CDER. The liposomal product's nonproprietary name should include term that distinguishes the product form as a liposome or a pegylated liposome.

Ex

[DRUG] Liposome Type X [DOSAGE FORM]

[DRUG] Pegylated Liposome Type X [DOSAGE FORM]

When FDA approves innovator's liposome drug products it will be designated as type A and other follow up for the same drug and dosage form will be designated as type B, C, D…….Z. label should also include a caution note regarding the different behavior pattern of liposome drug product. Proper reconstitution instructions and an adequate usage time must be given for a reconstituted product. For the liposomal drug product's robustness, proper storage and reconstitution conditions should also be given. (40)

**10. Submission**

For getting market authorization for the liposomal drug product submission is made using common technical document (CTD) format. CTD format dossier is used in many regulated and semi regulated market like Us, Japan, Europe, India etc. it consists of five modules (41):

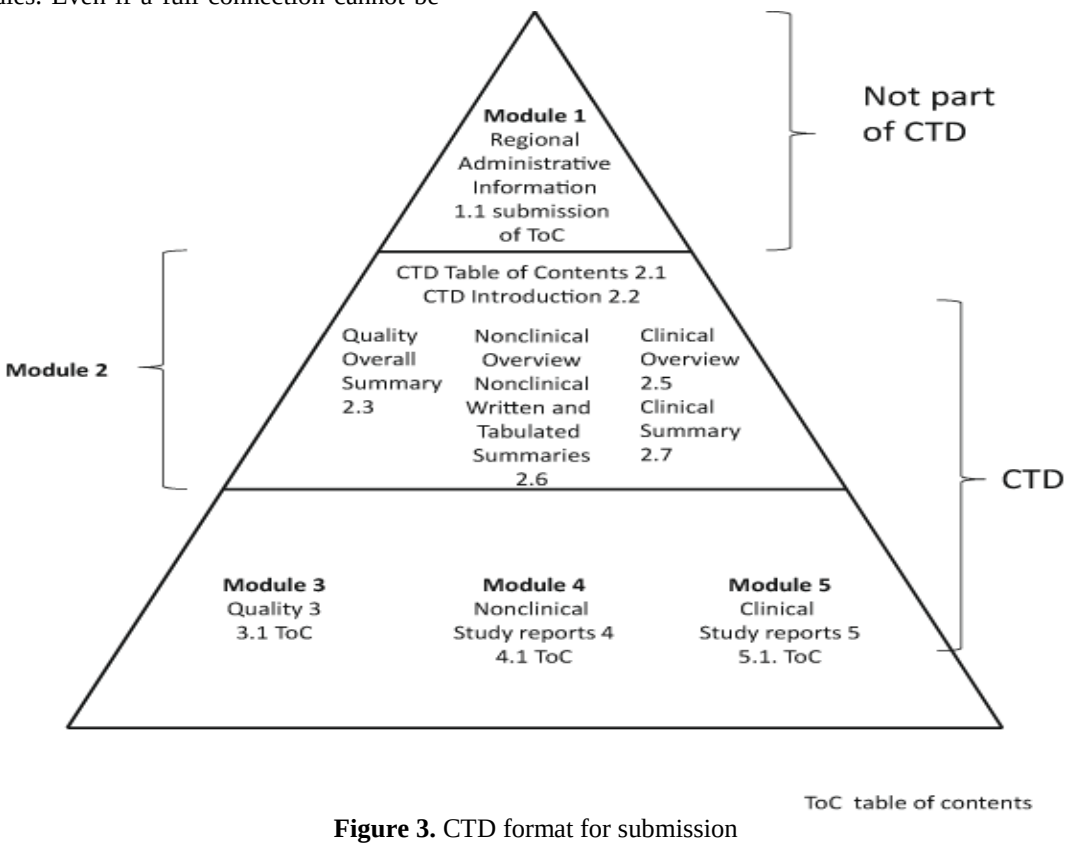


**Figure 3.** CTD format for submission

- Module 1 - Administrative Information
- Module 2 – Summaries
- Module 3 – Quality
- Module 4 - Non-Clinical
- Module 5 – Clinical

## 11. Conclusion

With the introduction of the PEGylated liposomal formulation Doxil® in 1995, liposomes were successfully introduced to the market. After their introduction, these delivery mechanisms have been investigated for diverse types of diseases ranging from tumors care to pain relief. Regulation over pharmacokinetics and pharmacodynamics properties, increased bioavailability, and low toxicity are the main advantages of liposomes. Regulatory guidance, on the other hand, has not kept pace with the rapid growth of new liposome-based drug delivery systems, and regulatory demands are, for the most part, out of date.

## Acknowledgements

We would like to express our sincere gratitude to IJDRA Journal for publishing our work.

***Financial Disclosure statement:*** The authors received no specific funding for this work.

## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## References

1. Bangham AD. Liposomes in Biological Systems. Chichester: John Wiley and Sons; 1980.
2. Deamer DW. From "banghasomes" to liposomes: a memoir of Alec Bangham; 1921-2010.
3. Johnston MJ, Semple SC, Klimuk SK, Ansell S, Maurer N, Cullis PR. Characterization of the drug retention and pharmacokinetic properties of liposomal nanoparticles containing dihydrosphingomyelin. Biochim Biophys Acta. 2007 May; 1768(5):1121-7. doi: 10.1016/j.bbamem.2007.01.019. Epub 2007 Feb 1. PMID: 17321495.
4. Mu L., Sprando R. L. Application of nanotechnology in cosmetics. Pharm. Res. 2010; 27: 1746−1749.
5. Betz G. Aeppli, A. Menshutina N. Leuenberger H. In vivo comparison of various liposome formulations for cosmetic application. Int. J. Pharm. 2005; 296:44−54
6. Y. Barenholz. Doxil®—the first FDA-approved nano-drug: from an idea to a product, in: D. Peer (Ed.), Handbook of Harnessing Biomaterials in Nanomedicine: Preparation, Toxicity, and Applications. Singapore: Pan Stanford Publishing; 2012.p. 335-398
7. Amarnath S, Sharma US. Liposomes in drug delivery: progress and limitations. Int J Pharm. 1997;154:123-140
8. Berger N.; Sachse A.; Bender J.; Schubert R.; Brandl, M. Filter extrusion of liposomes using different devices: comparison of liposome size, encapsulation efficiency, and process characteristics. Int. J. Pharm. 2001; 223:55-68.
9. Provder T. Challenges in particle size distribution measurement past, present and for the 21st century. Prog. Org. Coat. 1997; 32:143-153
10. .Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: state of the art. J. Colloid Sci. Biotechnol. 2012;1:147- 168.
11. Edwards K.A, Baeumner A.J. Analysis of liposomes. Talanta. 2006; 68:1432-1441.
12. Hunter R.J, Midmore B.R, Zhang H. Zeta Potential of Highly Charged Thin Double-Layer Systems. J. Colloid Interface Sci. 2001; 237:147-149.
13. Schlieper P, Medda P.K, Kaufmann R. Drug-induced zeta potential changes in liposomes studied by laser Doppler spectroscopy. Biochim. Biophys. Acta. 1981; 644:273-283.
14. Wang T, Deng Y, Geng Y, Gao Z, Zou J, Wang Z. Preparation of submicron unilamellar liposomes by freeze-drying double emulsions. Biochim. Biophys. Acta. 2006; 1758:222-231.
15. Gillet A, Lecomte F, Hubert P, Ducat E, Evrard B, Piel G. Skin penetration behaviour of liposomes as a function of their composition. Eur. J. Pharm. Biopharm. 2011; 79:43-53.
16. Elsayed M.M, Abdallah O.Y, Naggar V.F, Khalafallah N.M. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. Int. J. Pharm. 2006; 322:60-66.
17. Nagayasu A, Uchiyama K, Kiwada H. The size of liposomes: a factor which affects their targeting efficiency to tumors and therapeutic activity of liposomal antitumor drugs. Adv. Drug Delivery Rev. 1999; 40:75-87.
18. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends PharmacolSci. 2009; 30:592-599.
19. Dupont B. Overview of the lipid formulations of the amphotericin B. J AntimicrobChemother. 2002; 49:31-36.
20. Fendler JH, Romero A. 1977. Liposomes as drug carriers. Life Sci. 20:1109-1120.
21. Heneweer C, Gendy SE, Peñate-Medina O. Liposomes and inorganic nanoparticles for drug delivery and cancer imaging. TherDeliv. 2012; 3:645-656.
22. Soni V, Chandel S, Jain P, Asati S. Role of liposomal drug-delivery system in cosmetics. 2016; 93-120.
23. Mirafzali Z, Courtney S, Thompson K.T. Application of Liposomes in the Food Industry. 2014; 139-150.
24. Balazs DA, Godbey W. Liposomes for use in gene delivery. J Drug Deliv. 2011; 2011:326497. doi: 10.1155/2011/326497
25. Bawa R, Audette G.F; Reece B. Handbook of Clinical Nanomedicine: Law, Business, Regulation, Safety, and Risk, Pan Stamford Series on Nanomedicine Florida, USA: CRC Press; Taylor and Francis. Boca Raton. 2016; vol. 2.
26. Kelly B, 2010 Nanomedicines: regulatory challenges and risks ahead [Internet]. COV; 2010 Oct [cited 2021 Mar 05]. Available from: https://www.cov.com/-/media/files/corporate/publications/2010/10/nanomedicines regulatory.pdf
27. Madoa M, Tabib J.K, Nicholls F, Linga W, Wilhelm C, Debargee O, Gazeauc F, and Clement O. Contrast Media Mol. Imaging; 2013.
28. Zhang X.Q, Xu X, Bertrand N, Pridgen E, Swami A, Farokhzad O.C. Adv. Drug Delivery Rev. 2012;64.
29. Manke A, Wang L, Rojanasakul Y. BioMed Res. Int. 2013; 942916.
30. Kermanizadeh A, Balharry D, Wallin H, Loft s, Møller P, Crit. Rev. Toxicol. 2015.
31. Mahapatra I, Clark J.R.A, Dobson P.J, Owen R, Lynch I, Lead J.R. Environ. Sci. Nano. 2018;5.
32. Sengar G, Tripathy P. Pharmaceutical regulatory agencies and organizations around the world: Scope and challenges in drug development [Internet]. Pharmatutor; 2021 [cited 2021 Mar 07]. Available from: https://www.pharmatutor.org/articles/pharmaceuticalregulatory-agencies-and-organizations-around-worldscope-challenges-in-drug-development.

33. US Food and Drug Administration (USFDA) [Internet]. US FDA; 2021 Feb 23 [cited 2021 Mar 03]. Available from: https://www.fda.gov/science-research/nanotechnology-programs-fda/nanotechnology-task-force
34. US Food and Drug Administration (USFDA) Nanotechnology—Over a Decade of Progress and Innovation; 2020 Jul.
35. US Food and Drug Administration (USFDA). Guidance for industry - Liposome drug products (Draft guidance 2191). August; 2002.
36. FDA, CDER. Liposome Drug Products Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labelling Documentation Guidance for Industry. April; 2015.
37. ICH Expert Working Group. ICH Guideline Q1A(R2) Stability Testing of New Drug Substances and Products. Int Conf Harmon. 2003.
38. US Food and Drug Administration (USFDA). Guidance for industry - Changes to an Approved NDA or ANDA. Guidance for Industry [Internet]. US FDA; 2004 Apr [cited 2021 Mar 02]. Available from:
39. Formal Meetings between FDA and ANDA Applicants of Complex Products under GDUFA. Guidance for Industry [Internet]. US FDA; 2020 Nov [cited 2021 Mar 04]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/formal-meetings-between-fda-and-anda-applicants-complex-products-under-gdufa-guidance-industry
40. Getting Liposome Drug Products Approved: They Are Non-biological Complex Drugs [Internet]. Camargo; 2018 May 16 [cited 2021 Mar 04]. Available from: https://camargopharma.com/resources/blog/getting-liposome-drug-products-approved-they-are-non-biological-complex-drugs.
41. Electronic Common Technical Document (eCTD) [Internet]. US FDA; 2021 Jun 07 [cited 2021 Jun 09]. Available from: https://www.fda.gov/drugs/electronic-regulatory-submission-and-review/electronic-common-technical-document-ectd.