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A comprehensive review on Liposomes: As a novel drug delivery system

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Abstract

Liposomes, as versatile lipid-based nanoparticles, have emerged as promising drug delivery systems in recent years. This comprehensive review aims to provide an in-depth analysis of the advancements, challenges, and potential applications of liposomes in drug delivery. The review covers various aspects of liposome-based drug delivery, including their structure, formulation methods, advantages, limitations, and recent breakthroughs in the field. Furthermore, we discuss the diverse range of drugs and therapeutic agents that can be encapsulated within liposomes, as well as their clinical applications in targeting specific diseases.

Keywords: Liposome; Controlled Release; Targeted Drug Delivery; Novel Delivery

1. Introduction

The Greak words 'Lipos' which means fat and 'Soma' that means body, was combined to form spherical concentric vesicles called liposomes. Liposomes are round sac phospholipid molecules. It encloses a water droplet especially as form artificially to carry drug into tissue membrane. Liposome is a nanoparticle (size-100nm) [1]. Liposome were first described by Bangham in 1961, it turned into an accidental discovery in which he scattered the phosphatidyl choline molecule in water, for the duration of this he located that the molecule was forming a closed bilayer shape having an aqueous segment which were entrapped by means of a lipid bilayer[2]. Liposomes are useful because they act as carriers for a variety of drugs and have potential therapeutic or other properties. Various carriers such as nanoparticles, microparticles, polysaccharides, lectins, and liposomes can be used to target drug to a specific sites. Liposomal drug delivery is gaining interest due to its contribution to various areas like drug delivery, cosmetics, and biological membrane structure [3]. A liposome is a tiny bubble (vesicle), with a membrane composed of a phospholipid bilayer. Membranes are usually made of phospholipids like phosphatidylet-hanolamine and phosphatidylcholine. Phospholipids are amphiphilic with its polar head as hydrophilic and hydrocarbon tail as hydrophobic [4].

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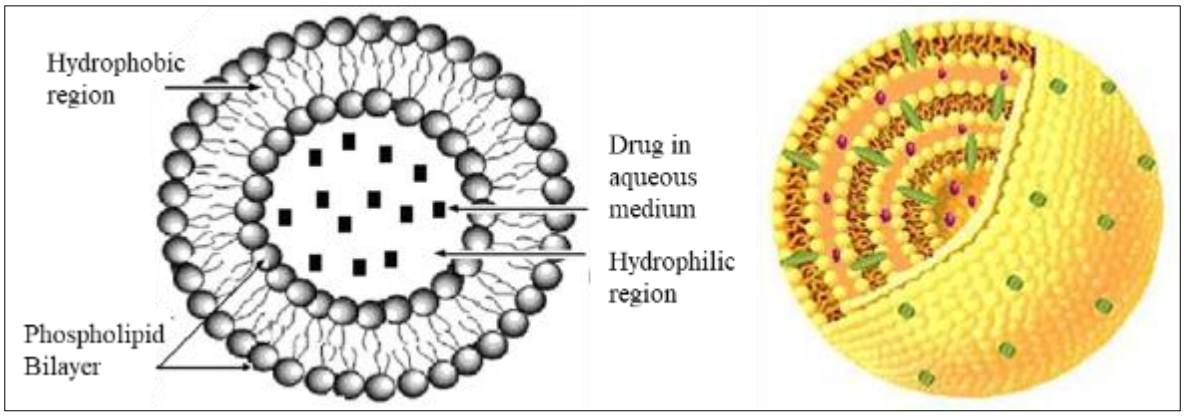


Figure 1 Basic Liposome Structure and multilamellar Liposome

2. Structure of liposomes: [5,6]

2.1. Phospholipids

- Naturally occurring phospholipids used in liposome:
  - Phosphatidylethanolamine
  - Phosphatidylcholine
  - Phsphatidylserine

- Synthetic phospholipids used in the liposomes are:
  - Dioleoyl phosphatidylcholine
  - Disteroyl phosphatidylcholine
  - Dioleoyl phosphatidylethanolamine

2.2. Cholesterol

Cholesterol may be included into phospholipids membrane in very high awareness up to 1:1 or 2:1 molar ratios of cholesterol to phospatidylcholine. Being an amphipathic molecule, cholesterol inserts into the membrane with its hydroxyl group of cholesterol orientated towards the aqueous floor and aliphatic chain aligned parallel to the acyl chains inside the center of the bilayers and additionally it growth the separation between choline head organizations and gets rid of the everyday electrostatic and hydrogen bonding interaction [6].

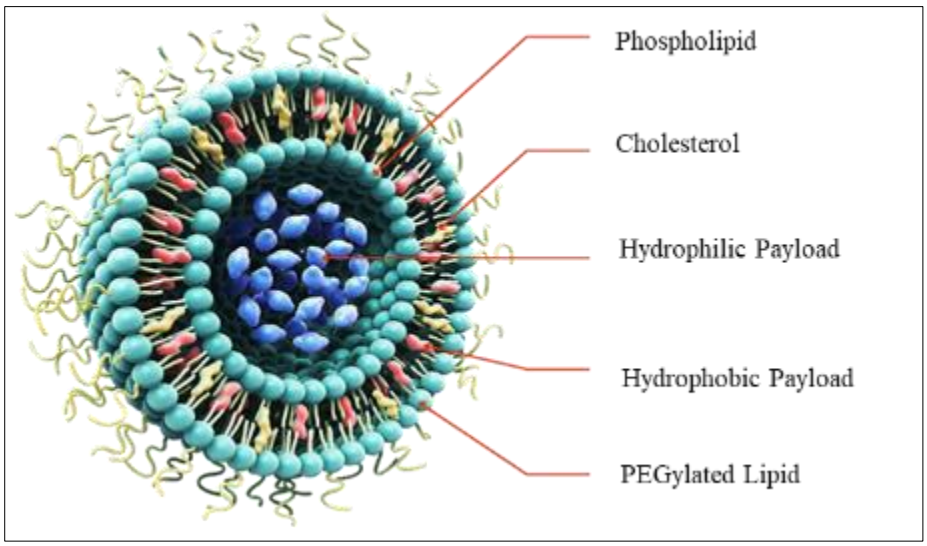


Figure 2 An illustration of liposome and its structural components

3. History of Liposomes

Liposomes were first discovered in the mid-1960s by British hematologist Dr. Alec D. Bangham [2]. He was studying the structure of cell membranes and stumbled upon the liposome while using an electron microscope [5]. Liposomes are tiny spherical structures made up of lipid bilayers, similar to the structure of cell membranes. Dr. Bangham's groundbreaking work laid the foundation for understanding and developing liposomes for various applications [8].

In the following decades, liposomes gained recognition for their potential in drug delivery. Their ability to encapsulate drugs and transport them to specific sites in the body revolutionized the field of pharmacology [9]. Liposomal drug delivery systems allowed for controlled release and reduced side effects of many medications [10].

Since then, liposomes have found applications not only in drug delivery but also in cosmetics, food technology, and gene therapy. [11] Researchers have developed various types of liposomes with different sizes, compositions, and surface modifications to optimize their performance for specific applications [11,12,13].

Today, liposomes continue to be an essential tool in the fields of medicine and biotechnology, with ongoing research aimed at improving their effectiveness and versatility in delivering therapeutic agents and other bioactive compounds [14,15,16].

3.1. Advantages of Liposomes: [1,6,17,18]

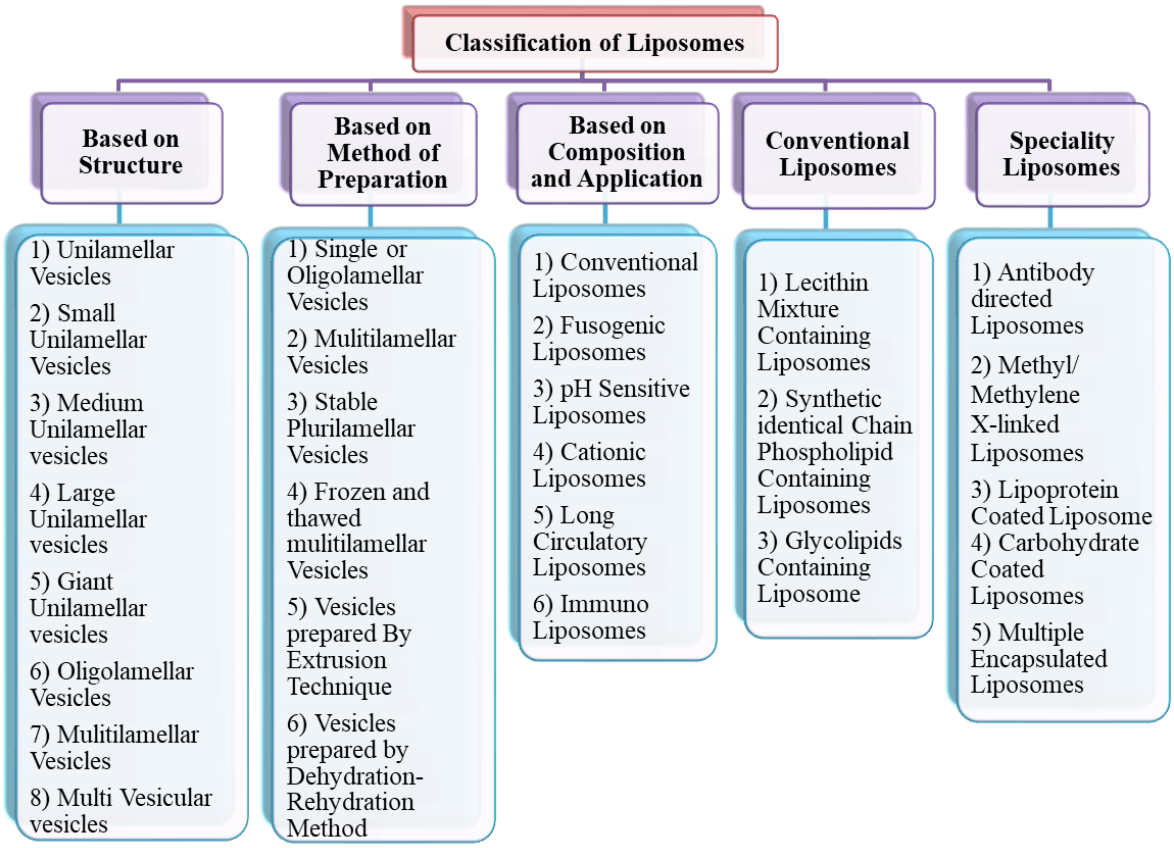
- Targeted Drug Delivery: Liposomes can encapsulate drugs and deliver them to specific target tissues or cells, allowing for targeted therapy. This minimizes the exposure of healthy tissues to the drug, reducing side effects.
- Improved Bioavailability: Liposomes can encapsulate poorly water-soluble drugs, improving their solubility and bioavailability, which can be a critical factor in drug effectiveness.
- Sustained Release: Liposomes can release drugs gradually over time, leading to sustained therapeutic effects and reducing the need for frequent dosing.
- Protection of Sensitive Compounds: Liposomes can protect sensitive drugs or bioactive compounds from degradation due to environmental factors, such as enzymes, pH changes, or oxidation.
- Versatility: They can be tailored in terms of size, composition, and surface modifications to optimize their performance for specific drugs or therapeutic applications.
- Reduced Toxicity: Liposomal drug delivery can reduce the toxicity of certain drugs by minimizing their exposure to healthy tissues while targeting diseased cells.
- Enhanced Cellular Uptake: Liposomes can improve the cellular uptake of drugs or therapeutic agents, making them more effective in treating diseases.
- Cosmetic Applications: Liposomes are used in cosmetics to enhance the penetration of active ingredients into the skin, improving their efficacy.
- Food Technology: In the food industry, liposomes are used to encapsulate and protect flavors, vitamins, and nutrients, enhancing the quality and shelf life of food products.
- Vaccine Delivery: Liposomes play a crucial role in vaccine development by improving the stability and delivery of antigens, enhancing immune responses.
- Biocompatibility: Liposomes are generally well-tolerated by the body, making them suitable for various medical and cosmetic applications.
- Research Tools: Liposomes are valuable tools in biomedical research for delivering biomolecules, dyes, or other compounds to cells for experimental purposes.
- Diagnostic Applications: Liposomes are used in diagnostic assays for drug screening, disease detection, and other diagnostic purposes.
- Immunogenicity: Liposomes can enhance the immunogenicity of vaccines, resulting in a stronger and more specific immune response.
- Customization: Researchers can customize liposomes for specific applications by modifying their properties, such as size, charge, and surface functionality.

3.2. Disadvantages of Liposomes: [1,6,17,19]

- Storage Stability: Liposomes can be prone to instability during storage, leading to aggregation, leakage of encapsulated substances, or changes in size and structure.
- Scalability: Scaling up the production of liposomes can be challenging and costly, which may limit their widespread use in large-scale pharmaceutical manufacturing.

- Uniformity: Achieving uniformity in liposome size and composition can be difficult, affecting their performance and reproducibility.
- Short Circulation Half-Life: Liposomes can be rapidly cleared from the bloodstream by the body's immune system, limiting their time window for drug delivery.
- Immunogenicity: Some liposome formulations may trigger an immune response, potentially causing adverse reactions in the body.
- Limited Drug Loading: Liposomes have a finite capacity for drug loading, which can be a limitation when trying to deliver high doses of certain drugs.
- Complexity: The development of liposomal formulations requires expertise and can be a complex process, which may limit their accessibility to researchers and manufacturers.
- Expense: Producing liposomal formulations can be costly, which may lead to higher drug prices for liposome-based therapies.
- Compatibility Issues: Some drugs may not be suitable for encapsulation in liposomes due to compatibility issues, limiting the range of drugs that can benefit from liposomal delivery.
- Clinical Translation: Despite promising results in preclinical studies, not all liposome-based therapies have successfully transitioned to clinical use, highlighting challenges in translating laboratory findings to real-world applications.
- Interaction with Biological Systems: Liposomes may interact with proteins or cells in ways that affect their stability, drug release, or performance.
- Biodegradability: Depending on their composition, liposomes may not be readily biodegradable, which can raise environmental concerns.
- Niche Applications: Liposomes may not be suitable for all drug delivery needs, and alternative delivery systems may be preferred in certain cases.
- Release Control: Achieving precise control over drug release kinetics from liposomes can be challenging, which may be critical for some therapeutic applications [20].
- Regulatory Approval: Obtaining regulatory approval for liposomal drug products can be a complex and time-consuming process, adding to the development timeline and cost [21].

4. Classification of Liposomes: [19,22]



4.1. Mechanism of formation of Liposomes: [19-25]

Liposome performs their motion by four distinct Mechanism-

- Endocytosis – This take location via phagocytic cells of reticuloendothelial system together with neutrophils.
- Adsorption – It occurs to the cellular surface through non precise electrostatic forces or by using interplay with cell surface additives.
- Fusion- It takes place by means of the insertion of liposomal bilayer into plasma membrane with continuous release of liposomal content into the cytoplasm.
- Lipid exchange- on this transfer of liposomal lipids to the cellular membrane without association of liposomal contents.

4.2. Method of preparation: [26,27,28]

4.2.1. Thin-Film Hydration Method: [29,30]

This is one of the most widely used methods for liposome preparation.

Steps:

- Dissolve lipids (phospholipids and cholesterol) in an organic solvent like chloroform or methanol to create a lipid solution.
- Evaporate the solvent under reduced pressure to form a thin lipid film on the walls of a round-bottom flask or a glass vial.
- Hydrate the lipid film by adding an aqueous solution (e.g., buffer or distilled water) and vortexing or sonicating to form multilamellar vesicles (MLVs).
- Optionally, you can reduce the size of MLVs to smaller unilamellar vesicles (SUVs) through extrusion or sonication.

4.2.2. Reverse Phase Evaporation Method: [30,31]

This method is used for preparing liposomes with high encapsulation efficiency.

Steps:

- Dissolve lipids in an organic solvent along with an aqueous solution containing the substance to be encapsulated.
- Evaporate the organic solvent under reduced pressure to form a water-in-oil emulsion.
- Remove the organic phase, leaving behind liposomes containing the encapsulated substance.

4.2.3. Extrusion Method

This method is used to prepare liposomes with uniform size and narrow size distribution.

Steps:

- Prepare liposomes using the thin-film hydration method to create MLVs.
- Pass the liposome suspension through a series of polycarbonate membrane filters with defined pore sizes using a hand-held extruder or a high-pressure extruder.
- This process results in smaller liposomes with a consistent size.

4.2.4. Sonication Method: [32,33,34]

This method is used to downsize liposomes or to prepare small unilamellar vesicles (SUVs).

Steps:

- Prepare liposomes using the thin-film hydration method or another appropriate method.
- Subject the liposome suspension to high-frequency ultrasound (sonication) to reduce the size of the liposomes.

4.2.5. Detergent Removal Method: [35,36]

This method is used for preparing liposomes encapsulating hydrophobic substances.

Steps:

- Dissolve lipids and the hydrophobic substance in a detergent solution.
- Remove the detergent using techniques like dialysis or chromatography to obtain liposomes with the encapsulated substance.

4.2.6. Freeze-Thawing Method: [37,38,39]

This method is used for preparing liposomes with enhanced stability.

Steps:

- Freeze the liposome suspension at a low temperature, typically below the lipid's phase transition temperature.
- Thaw the frozen suspension at a higher temperature, repeating the cycle multiple times.
- This process helps to entrap substances within liposomes and reduce leakage.

5. Marketed formulations of Liposomes: [23]

Product	Drug	Company
Ambisome™	Amphotericin B	Nexstar pharmaceuticals Inc., CO
Abelcet™	Amphotericin B	The Liposome Company, NJ
Amphocil™	Amphotericin B	Sequus pharmaceuticals, Inc., C.A.
Doxil™	Doxorubicin	Sequus pharmaceuticals, Inc., C.A.
DaunoXome™	Daunorubicin	Nexstar pharmaceuticals, Inc., CO
MiKasome™	Amikacin	Nexstar Pharmaceuticals, Inc., CO
DC99™	Doxorubicin	Liposome CO., NJ, USA
Epaxel™	Hepatitis A Vaccine	Swiss Serum Institute, Switzerland.
ELA-Max™	Lidocaine	Biozone Labs, CA, USA.

5.1. Evaluation of Liposomes: [1]

5.1.1. Characterization of Liposome Structure

Morphology

- Transmission Electron Microscopy (TEM): Provides high-resolution images of liposome size, shape, and lamellarity.
- Scanning Electron Microscopy (SEM): Offers surface information and morphology details.

Size and Size Distribution:

- Dynamic Light Scattering (DLS): Measures particle size, size distribution, and polydispersity.
- Nanoparticle Tracking Analysis (NTA): Tracks and sizes individual liposomes in a liquid suspension.

Zeta Potential: [40-44]

- Electrophoretic Light Scattering: Determines the surface charge of liposomes, which affects stability and colloidal behavior.

Lipid Composition Analysis

- High-Performance Liquid Chromatography (HPLC): Identifies and quantifies lipids in liposomal formulations.

5.1.2. Liposome Properties:

Encapsulation Efficiency

- UV-Visible Spectroscopy or Fluorescence Spectroscopy: Measures the concentration of encapsulated drugs or molecules.

Stability:

- Assessing changes in size, poly dispersity, and zeta potential over time under various storage conditions (e.g., temperature, pH).

Drug Release Kinetics

- *In vitro* release studies to determine the rate and extent of drug release from liposomes.

5.1.3. Biological Evaluation: [44]

In vitro Cell Studies

- Cell viability assays (MTT, Alamar Blue) to assess liposome cytotoxicity.
- Cellular uptake studies to evaluate liposome internalization and drug delivery efficiency.

In vivo Studies

- Animal models to evaluate the pharmacokinetics, biodistribution, and therapeutic efficacy of liposomal drug formulations.

5.1.4. Biocompatibility and Toxicity Assessment

- Hemolysis Assay: Measures the potential for liposomes to cause red blood cell damage.
- Immunogenicity Assessment: Investigates the immune response to liposomes.

5.1.5. Drug Release Studies

- Dialysis Method: Evaluates drug release kinetics under sink conditions by dialyzing liposomal suspensions against a release medium.
- Franz Diffusion Cell: Measures drug permeation through a membrane to mimic transdermal drug delivery.

5.1.6. Surface Modification Analysis

- Surface Characterization: Techniques like X-ray Photoelectron Spectroscopy (XPS) and Fourier-Transform Infrared (FTIR) spectroscopy to analyze modifications made to the liposome surface.

5.2. Application for Liposomes

5.2.1. Drug Delivery: [45]

- Liposomes are commonly used as drug delivery vehicles to encapsulate and deliver both hydrophobic and hydrophilic drugs.
- They can improve drug solubility, stability, and bioavailability.
- Liposomal drug formulations can target specific tissues or cells, reducing systemic side effects.

5.2.2. Vaccines: [46-49]

- Liposomes are used as adjuvants or carriers for vaccines to enhance immunogenicity.
- They can improve antigen delivery to immune cells, leading to a stronger immune response.

5.2.3. Cosmetics and Skincare: [50,51]

- Liposomes are utilized in cosmetics and skincare products for controlled release of active ingredients, such as vitamins and antioxidants.
- They can enhance the penetration of ingredients into the skin, improving their efficacy.

5.2.4. Gene Delivery: [52,53]

- Liposomes can be used to deliver genetic material, including DNA and RNA, for gene therapy applications.
- They protect and facilitate the transport of genetic cargo into target cells.

5.2.5. Diagnostics:

- Liposomes can serve as carriers for contrast agents in medical imaging, such as magnetic resonance imaging (MRI) and ultrasound.
- They enable targeted imaging of specific tissues or cells.

5.2.6. Cancer Therapy: [54,55]

- Liposomal formulations of chemotherapy drugs, like Doxil (liposomal doxorubicin), are used to treat cancer.
- They can improve drug circulation time and reduce damage to healthy tissues.

5.2.7. Food Technology

- Liposomes are applied in the food industry for encapsulating and protecting sensitive ingredients, such as vitamins, flavors, and antioxidants.
- They can improve the stability and bioavailability of these additives in food products.

5.2.8. Biotechnology

- Liposomes are used in research and biotechnology applications for drug screening and delivery to cells *in vitro.*
- They are valuable tools for studying cell membrane interactions and drug transport mechanisms.

5.2.9. Transdermal Drug Delivery: [56]

- Liposomal formulations can be applied topically to deliver drugs through the skin.
- They offer controlled release and can avoid the first-pass metabolism in the liver.

5.2.10. Personal Care Products

- Liposomes are employed in personal care products such as sunscreens and moisturizers to enhance the delivery of active ingredients.

5.2.11. Veterinary Medicine

- Liposomes are used in veterinary medicine for drug delivery to animals, similar to their applications in human medicine.

5.2.12. Environmental Remediation:

- Liposomes can be utilized for the controlled release of remediation agents in environmental cleanup efforts.

5.2.13. Intracellular Delivery: [57]

- Liposomes are valuable tools in research for delivering molecules into specific organelles within cells.

5.2.14. Nutraceuticals: [51,58]

- Liposomes are used to enhance the bioavailability of nutraceutical compounds in dietary supplements.

5.2.15. Wound Healing

- Liposomal formulations can be applied to wound dressings to promote the controlled release of wound-healing agents.

5.3. Recent Approaches: [15, 16, 37, 41, 44, 54]

Liposomes have been studied and used as drug delivery systems for several decades, and their future prospects continue to be promising. Liposomes are lipid-based vesicles that can encapsulate a wide range of drugs, including both hydrophobic and hydrophilic compounds. They offer several advantages as drug carriers, which contribute to their ongoing relevance and potential in the field of drug delivery. Here are some future prospects for liposomes as novel drug delivery systems:

- Targeted Drug Delivery: Liposomes can be designed to target specific tissues or cells, which can enhance the therapeutic efficacy of drugs while minimizing side effects. This targeted drug delivery is particularly important for treating diseases like cancer, where precision in drug delivery is crucial.[59]
- Personalized Medicine: Liposomes can be customized to encapsulate different drugs and combinations of drugs, allowing for personalized treatment regimens. This could revolutionize healthcare by tailoring drug therapies to individual patient needs.
- Co-Delivery of Therapeutics: Liposomes can carry multiple drugs simultaneously, making them suitable for co-delivery of synergistic therapeutic agents. This approach can improve treatment outcomes for various diseases and conditions.
- Controlled Release: Liposomes can be engineered to release drugs in a controlled and sustained manner, extending the therapeutic effect and reducing the frequency of dosing. This is especially valuable for chronic conditions.
- Vaccine Delivery: Liposomes have shown promise as carriers for vaccines. They can enhance the stability and immunogenicity of antigens, potentially leading to the development of more effective vaccines for infectious diseases.
- Gene Therapy: Liposomes can be used to deliver genetic material, such as small interfering RNA (siRNA) or CRISPR-Cas9 components, to target cells for gene therapy applications. This holds great potential for treating genetic disorders.
- Crossing Biological Barriers: Liposomes can be modified to overcome biological barriers, such as the blood-brain barrier, facilitating the delivery of drugs to the central nervous system for the treatment of neurological disorders.
- Combination Therapies: Liposomes can be used to combine different therapeutic modalities, such as chemotherapy and immunotherapy, for synergistic effects in cancer treatment.
- Improvements in Formulation: Ongoing research aims to enhance liposomal formulations by improving stability, reducing toxicity, and increasing drug-loading capacity, which will expand their utility.
- Regulatory Approvals: As more liposome-based drug delivery systems undergo clinical trials and gain regulatory approvals, their use in healthcare will likely become more widespread.

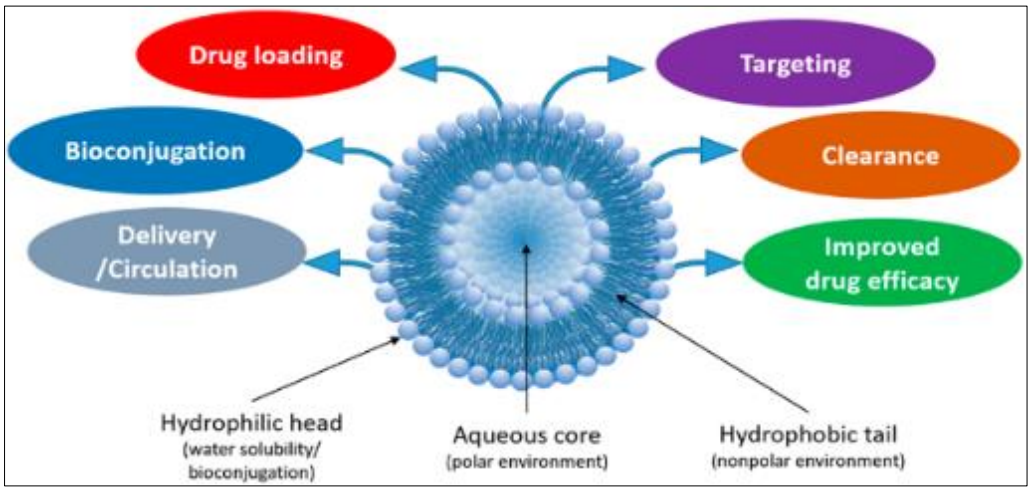


Figure 3 Structure and function of Liposomes [60]

6. Conclusion

Liposomes represent a promising and innovative drug delivery system with a wide range of applications in the field of pharmaceuticals. Over the years, extensive research has demonstrated their potential to overcome various challenges

associated with traditional drug delivery methods. Liposomes have emerged as a promising class of drug delivery systems that offer significant advantages for enhancing the therapeutic efficacy and safety of various drugs. While challenges remain, the continued innovation and refinement of liposomal technologies hold great promise for the future of drug delivery in the pharmaceutical industry. Liposomes represent an exciting and versatile approach to drug delivery, with the potential to revolutionize the pharmaceutical industry by improving drug efficacy, reducing side effects, and enabling precise targeting of therapies. Further advancements in liposomal technology are likely to expand their use in a wide range of medical applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Sawant GS, Sutar KV, Kanekar AS. Liposome: A Novel Drug Delivery System. International Journal of Research and Review. 2021; 8(4): 252-268.

[2] Mishra H, Chauhan V, Kumar K, Teotia D. A Comprehensive review on Liposomes: a novel drug delivery system. Journal of Drug Delivery and Therapeutics. 2018; 8(6): 400-404.

[3] Sharma D, Ali AAE, Trivedi LR. An Updated Review on: Liposomes as drug delivery system. PharmaTutor. 2018; 6(2):50-62.

[4] Dhandapani N, Thapa A, Goti S, Bhattara. R. Liposomes as novel drug delivery system: A comprehensive review, Nagasamy Venkatesh Dhandapani Int. J. Res. Pharm Sci. 2013; 4(2):187-193.

[5] Dr. Tiwari,Talreja S & Ms Swarnima Pandey. A review on use of novel drug delivery systems in herbal medicines, Science and Engineering Journal. 2020; 24(8):190-197.

[6] Dwivedi C, Yadav R, Tiwari S, T. Satapathy. Roy A, Role of Liposome in novel drug delivery systems. Journal of Drug Delivery & Therapeutics; 2014; 4(2):116-129.

[7] Sudhakark, Shivkanya, Fuloria, Subramaniyan V. Kathiresan V. Sathasivam. Review Ultraflexible Liposome Nanocargo as a Dermal and Transdermal Drug Delivery System.Nanomaterials. 2021; 11; 2557.

[8] A. D. Bangham. Liposome: The Babraham Connection. Chemistry and Physics of Lipids. 1993; 64:1-3.

[9] Bangham A. D, Standish M. M, Weissmann G. The action of steroids and streptolysins on the permeability of phospholipid structures to cations. Journal of Molecular Biology. 1965; 13(1): 253-259.

[10] Touitou, E. Drug delivery across the skin. Expert Opin. Biol. Ther. 2002; 2: 723–733.

[11] Abd El-Alim, S. H.; Kassem, A. A. Basha, M.; Salama, A. Comparative study of liposomes, ethosomes and transfersomes as carriers for enhancing the transdermal delivery of diflunisal. *In vitro* and in vivo evaluation. Int. J. Pharm. 2019; 563, 293–303.

[12] Ramadon, D. Mc Crudden, M. T. C. Courtenay, A. J. Donnelly, R. F. Enhancement strategies for transdermal drug delivery systems. Current trends and applications. Drug Deliv. Transl. Res.2022 12(4):758-791.

[13] Trucillo, P. Campardelli, R.Reverchon, E. Liposomes: From Bangham to Supercritical Fluidss. Processes 2020; 8: 1022. 1-15.

[14] Arora, D. Nanda, S. Quality by design driven development of resveratrol loaded ethosomal hydrogel for improved dermatological benefits via enhanced skin permeation and retention. Int. J. Pharm. 2019; 567: 118448.

[15] Weissig, V. Liposomes Came First. The Early History of Liposomology. Methods Mol. Biol. 2017; 1522: 1–15.

[16] Taglietti, M. Hawkins, C. N. Rao, J. Novel Topical Drug Delivery Systems and Their Potential Use in Acne Vulgaris. Skin Ther. Lett. 2008; 13: 2.6-8.

[17] Gomez-Hens, A. Fernandez-Romero, J. M. Analytical methods for the control of liposomal delivery systems. Trends Anal Chem, 2006; 25:167–178.

[18] Meiwan Chen Yanfang Zhou, Jingjing Huang, Ping Zhu, Xinsheng Peng, and Yitao Wang. Liposome-Based Delivery Systems in Plant Polysaccharides. Journal of Nanomaterials. 2012; 12: 1-4.

[19] Nalawade V, Patil K. International Journal of Publication and Review. 2023; 4(1):1795-1801.

[20] Tianshu Lian, Rodney J. Y. HO. Trends and Developments in Liposome Drug Delivery Systems. Jounral of pharmaceutical sciences. 2001; 90(6): 667-680.

[21] Pradhan, N. Kumar, S. Saha, et al. Liposome: Method of preparation, advantages, evaluation and its application. Journal of Applied Pharmaceutical Research. 2015; 3(3): 1-8.

[22] Gabrijelcic V, Sentjure M and Kristl J. Evaluation of liposomes as drug carriers into the skin by one di-mensional EPR imaging. Int J Pharm. 1990; 62: 75-79.

[23] Cabral E. C. M, Zollner R. L. And Santana M. H. A. Brazilian Journal of Chemical Engineering Preparation And Characterization Of Liposmes Entrapping Allergenic Protein Received: 2004, 21, (02): 137 - 146.

[24] Bangham, A.D. and R.W. Horne. Negative staining of phospholipids and their structured by surface active agents as observed in the electron microscope. J. Mol. Biol. 1964; 8: 660–668.

[25] Kaur D. Kumar S. Niosomes: present scenario and future aspects. Journal of Drug Delivery and Therapeutics, 2018; 8(5):35-43.

[26] Hong MS. Prolonged blood circulation of methotrexate by modulation of liposomal composition. Drug Delivery .2001; 8: 231–237.

[27] Jesorka A, al. Liposomes: technologies and analytical applications, Annu. Rev. Anal. Chem. 2008; 1: 801–832.

[28] Vemuri S, et al. Preparation and characterization of liposomes as therapeutic delivery systems: a review, Pharm. Acta Helv. 1995; 70: 95–111.

[29] Shailesh S, Neelam S, Sandeep K, Gupta GD. Liposomes: A review. Journal of Pharmacy Research.2009; 2(7):1163-1167.

[30] Godbole MD, Mathur VB. Selection of phospholipid and method of formulation for optimum entrapment and release of lamivudine from liposome. Journal of Drug Delivery and Therapeutics. 2018; 8(5-s):175-183.

[31] Kaur D, Kumar S. Niosomes: present scenario and future aspects. Journal of Drug Delivery and Therapeutics. 2018; 8(5) 35-43.

[32] Kirby C, Gregoriadis G. Dehydration-Rehydration Vesicles: Simple Method for High Yield Drug Entrapment in Liposomes. Biotechnology 2, 1984, 2: 979–984.

[33] Rahman A, Uahengo V, Likius D. Mini review on emerging methods of preparation of liposome and its application as Liposome drug delivery systems. Open J Pharmacol Pharmacother. 2018; 3(1): 005-021.

[34] John F. Deeken, Rebecca Slack, Phase I trial in advanced malignancies with liposome encapsulated paclitaxel (LEP).Cancer Chemother Pharmacol. 2013; 71(3): 627–633.

[35] Riaz Mohammad. Review Liposomes Preparation Method Pakistan Journal of Pharmaceutical Sciences.1996; 19(1): 65-77.

[36] Dwivedi. C, Yadav. R Tiwari. S.P, Liposome: a Powerful Approach for Vesicular Drug Delivery System, Elixir Pharmacy 81 (2015) 31461-31474.

[37] Rajan K. Verma and Sanjay Garg, “Current Status of Drug Delivery Technologies and Future Directions.” Pharmaceutical Technology" On-Line. 2001; 25 (2), 1–14.

[38] Dwivedi Chandraprakash, Verma. Review on Preparation and Characterization of Liposomes with Application. Journal of Scientific & Innovative Research. 2013; 2 (2): 1-31.

[39] Bangham AD, Standish MM, Watkin JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J, Mol, Biol. 1965; 13: 238-252.

[40] Zili Gu, Candido G. Da Silva, Koen van der Maaden, Ferry Ossendorp and Luis J. Cruz. Liposome-Based Drug Delivery Systems in Cancer Immunotherapy. Pharmaceutics, 2020; 12.1-25.

[41] B. Pradhan, N. Kumar, S. Saha, et al. Liposome: Method of preparation, advantages, evaluation and its application. Journal of Applied Pharmaceutical Research. 2015; 3(3): 1-8.

[42] Caponigro F, Cornelia P, Budillon A. Phase I study of Caelyx (doxorubicin HCL, Pegylated liposomal) in recurrent or metastatic head and neck cancer. Annals of Oncology. 2000; 11(3): 339-342.

[43] L. Kaur, P. Kaur, and MU. Khan. Liposome as a drug carrier- A review. International Journal of Research in Pharmacy and Chemistry. 2013; 3(1): 16.121-128.

[44] Ramkrishna S, Sunit K. S, Soumyaranjan S. Liposome as drug delivery system: A brief review. International Journal of Research in Pharmaceutical Science. 2014; 5(4): 309-321.

[45] S. Marripati, K. Umashankar, P. Jayachandra Reddy. A Review on Liposomes. International Journal of Research in Pharmaceutical and Nanoscience. 2014; 3(3): 159-169.

[46] Shashi K. Satinder K.Bharat P. A Complete Review on Liposome. International Research Journal of Pharmacy. 2012; 3(7): 10-16.

[47] Keller, B.C. Liposomes in Nutrition. Trends Food Sci. Technol. 2001; 12: 25–31.

[48] Rao M, Alving CR. Delivery of lipids and liposomal proteins to the cytoplasm and Golgi of antigen-presenting cells. Advance Drug Delivery Review. 2000; 41: 171-188.

[49] Lian T, Bui T, Ho RJ. Formulation of HIV-envelope protein with lipid vesicles expressing ganglioside GM1 associated to cholera toxin B enhances mucosal immune responses Vaccine .1999; 18:604-611.

[50] Bui T, Dykers, T, Hu SL, Faltynek CR, Ho RJ. Effect of MTP-PE liposomes and interleukin-7 on induction of antibody and cell-mediated immuneresponses to a recombinant HIV-envelope protein. J Acquir Immune De®c Syndr . 1994; 7: 799-806.

[51] Gluck R. Liposomal presentation of antigens for human vaccines. Pharm Biotechnol. 1995; 6: 325-345.

[52] Gabrijelcic V and Sentjure M. Influence of hydrogel on liposome stability and on liposome stability and on the transport of liposome entrapped substance in to the skin. Int J Pharm .1995; 118: 209-212.

[53] 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; 1768:1121–1127.

[54] Atrooz OM. Effects of alkylresorcinolic lipids obtained from acetonic extract of Jordanian wheat grains on liposome properties. Int J Biol Chem. 2011; 5(5): 314–321.

[55] Hatwar P. R, Dr. Bakal R. L, Dumare A. A, Dere M. D, Hage. P. D, Targeted drug delivery system for the treatment of Cancer, WJPR. 2023; 12(12): 808-816.

[56] Deulkar D. A, kubade J. A, Hatwar P. R, Dr. Bakal R. L. A review on transdermal drug delivery system, GSC Advances Research and Reviews. 2024; 18(02): 347-361.

[57] Liu W., Ye A., Singh H. Progress in Applications of Liposomes in Food Systems. In Microencapsulation and Microspheres for Food Applications; Elsevier: Amsterdam. The Netherlands. 2015; 151–170.

[58] Shashi K., Satinder K., Bharat P. A Complete Review on Liposome. International Research Journal of Pharmacy. 2012; 3(7): 10-16.

[59] Bagmar N. A, Hatwar P. R, Dr. Bakal R. L. A review on Targeted Drug Delivery System, WJPR. 2023; 12(19): 288-298.

[60] http://images.app.goo.gl/uV9HszgnHjLtcEgH9