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ANTIBIOTIC LIPOSOMAL FORMULATIONS FOR IMPROVING RESPIRATORY HEALTH IN CYSTIC FIBROSIS: A REVIEW ON TRENDS IN LIPOSOMAL RESEARCH

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ABSTRACT

Cystic fibrosis results in defective mucociliary clearance together with persistent pulmonary infections mostly due to Pseudomonas aeruginosa. The effectiveness of conventional antibiotics remains limited because they clear rapidly from the body and bacteria develop growing resistance patterns. Liposomal antibiotics enable better pulmonary tissue retention along with more precise drug distribution and fewer required medication doses. Using Arikayce, LipoBiEDT-TOB™ and Pulmaquin™ has shown potential for treating cystic fibrosis. Better therapeutic outcomes will be achieved through future developments in PEGylated along with stimuli-responsive and gene-integrated liposomal systems.

Keywords: Cystic fibrosis, liposomes, antibiotic resistance, pulmonary drug delivery, *Pseudomonas aeruginosa*, Arikayce, PEGylation, Nano medicine.

1. INTRODUCTION

One of the common life-threatening autosomal recessive genetic disorders, with the highest prevalence in Europe, North America, and Australia is Cystic fibrosis (CF). Lung disease accounts for nearly 95% of early deaths in CF patients, along with the development of habitual infection [1,2].

Cystic fibrosis

In fact, the initial periodic stains cause chronic infections even though the lungs are not inflamed or infected at birth. CFTR is structurally distinct from other ATP-binding cassette proteins [3]. It results from a mutation in the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR), a transmembrane channel that conducts chloride and regulates anion transport and mucociliary clearance in the airways. When CFTR is not functioning properly, mucus is retained, which increases the risk of chronic infection and lung-damaging local airway inflammation [4,5].

2. EPIDEMIOLOGY

The healthcare pathogen Pseudomonas aeruginosa primarily attacks sick patients who are immunocompromised or who suffer from continuous lung diseases [6]. Healthcare-associated infections involving Pseudomonas aeruginosa generally occur as ventilator-associated pneumonia and surgical site infections and urinary tract infections and bacteremia and affect 7.1%–7.3% of infected patients. According to the Cystic Fibrosis Foundation Patient Registry Pseudomonas aeruginosa detects in 49.6% of cystic fibrosis patients and the infection rate rises among older patients reaching 74.1% in those aged 26 and above. The bacterium develops biofilms which help it stay longer and be more resistant in clinical environments and transmission occurs through reservoirs of water and medical tools [7].

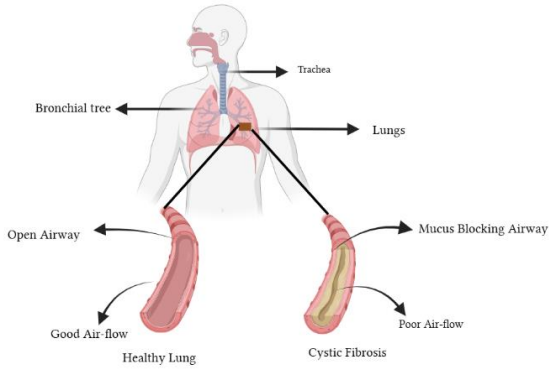

Fig 1. Schematic representation of Cystic Fibrosis

3. PATHOGENESIS

Difficulties with the imperfect CF gene cause CFTR protein malfunction to develop later into discriminated sodium and chloride conductance on epithelial cell plasma membranes. Consequently, this results in airway surface fluid depletion and abnormal glandular secretions [8,9]. The symptoms of the disease result from mucus thickening which causes obstruction in small airways [10]. Infectious sputum stagnation in the lungs develops from epithelial ciliary defects which both remove

bacteria from the airways and decrease sodium chloride ion intake [11,12]. The subsequent medical condition happens after bronchiectasis when bronchi become enlarged and weak which results in large airway obstruction [13]. Recurrent bacterial infections and inflammation occur due to airway blockage resulting in reduced lung function and respiratory failure that becomes a significant cause of death from CF [14].

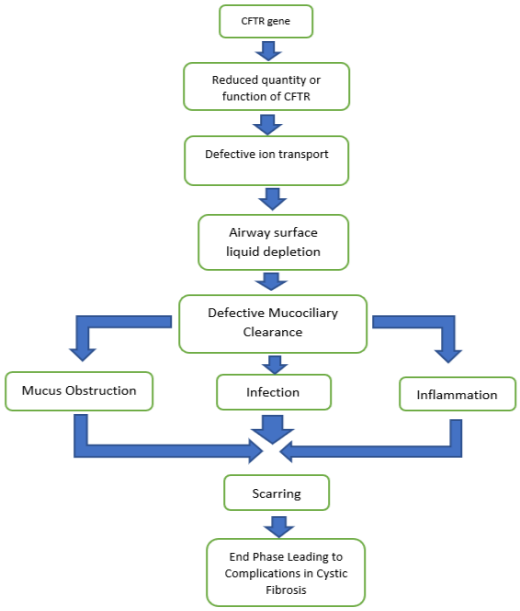

Fig.2 Pathogenesis of Cystic Fibrosis

4. MECHANISM OF ANTIBIOTICS

4.1 Inhibition of DNA Replication: Antibiotics inhibit DNA replication by blocking essential bacterial enzymes namely DNA gyrase and topoisomerase IV which drive this vital cellular process. Bacterial survival depends on these enzymes because they help address the topological issues that occur when DNA unwinds. The antibiotics stop the essential enzyme functions leading to impaired bacterial cell reproduction and genetic stability and thus resulting in bacterial death [15].

4.2 Prevention of Protein Synthesis: The mechanism of action called prevention of protein synthesis targets bacterial ribosomes as another critical method. The inhibition of translation by protein synthesis blocking agents prevents bacterial ribosomes from creating essential proteins needed for bacterial growth and operation. The molecular blockade hinders essential protein generation processes which make it impossible for bacteria to survive and multiply [16,17].

4.3 Inhibition of Cell Wall Synthesis: Several antibiotics affect the bacterial cell wall through specific inhibition mechanisms which disrupt the essential cell wall structure responsible for cellular shape and stability. Through their action on peptidoglycan synthesis antibiotics make the bacterial cell wall unstable which results in cell death by lysis. Such antibiotics work best against bacteria in growth phase because these pathogens depend on robust cell wall synthesis to remain structurally intact [18].

4.4 Disruption of Membrane Function: Some antibiotic compounds damage bacterial cell membranes to disrupt their essential structural integrity. The antibiotics create cellular membrane dysfunction which leads to vital substance leakage and eventually kills the cells. Different types of antibiotics function through this mechanism which demonstrates the fundamental importance of membrane stability for bacterial survival [19,20].

5. MECHANISM OF ACTION OF LIPOSOMAL ANTIBIOTICS

5.1 Disruption of cell wall and cell membrane: The evolution of microorganisms has included cell membrane development as their physical protection against antibiotic treatment. Bacteria have two natural membrane components that contribute to their negative surface charge. The outer membranes of Gram-negative bacteria contain lipopolysaccharides and Gram-positive bacteria possess teichoic acids which incorporate phosphate or carboxyl functionalities [21,22]. Although these antimicrobials can usually penetrate bacterial cell membranes easily their movement is restricted due to the highly polar membrane properties thus resulting in diminished antibacterial action. Electrostatic adsorption between the cell wall and NPs results in membrane depolarization while lowering membrane permeability and disrupting membrane fluidity to cause cell death.

5.2 Generation of reactive oxygen species (ROS): The biological system produces ROS during its ongoing oxidative metabolic process. Cells undergo growth cycles with signaling processes while needing survival functions and

death sequences and these processes get influenced by the activity of ROS [23].

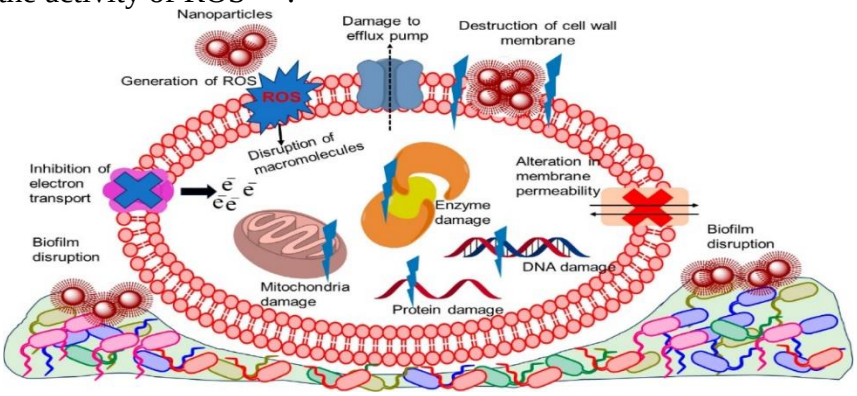

Fig 3. Mechanism of action of Liposomal antibiotics. Courtesy: ACS Omega 2023, 8, 39, 35442-35451

Drugs	Marketed Product	Lipid composition Encapsulation	Target
Amikacin	Arikayce	1,2-Dipalmitoyl-sn-glycero-3-phosphocholine+cholesterol liposome	MAC lung disease CF, NCFB Pseudomonas aeruginosa
Tobramycin	LipoBiEDT-TOB ™	Liposomal bismuth-ethanedithiol-loaded tobramycin	Pseudomonas aeruginosa Burkholderia cepacian
Ciprofloxacin	Lipoquin™	Hydrogenated soy phosphatidylcholine +cholesterol liposome	CF, NCFB Pseudomonas aeruginosa
	Pulmaquin™	Hydrogenated soy phosphatidylcholine +cholesterol liposome	CF, NCFB Pseudomonas aeruginosa MAC
Amphotericin B	Ambisome™	Liposomes	Prophylaxis/treatment for IPA and pulmonary IFI in patients with high-risk hematologic malignancies or LTR

Table no. 1 FDA-Approved Drug Products Used for Treating Pulmonary Infections[25]

5.3 Damage of intracellular components: Bacteria need cellular homeostasis together with functioning intracellular signaling pathways to survive and fulfill their biological functions. Blocking cellular pathways leads to cell death when NPs are developed to perform this function. Protein synthesis alterations together with DNA damage and gene expression modifications represent several disturbances reported in the literature [24].

6. RATIONALE FOR USING LIPOSOMAL ANTIBIOTICS

Patients find it difficult to administer inhaled antibiotics and antifungals because these medications require multiple daily doses due to their short half-life [25,26]. Antibiotic delivery through liposomes enables a controlled drug release and enables macrophage drug uptake through phagocytosis because of which liposomal antibiotic formulations provide better treatment for intracellular infections including NTM. Liposomes act to minimize drug-associated irritation or the lung signaling that happens with aerosol-based deposition. The presence of drug in the lung that results from inhaled liposomes allows infrequent doses because the formulation persists in the respiratory tissues [27].

7.LIPOSOMES

The liposomal structure consists of spherical lipid vesicles measuring between 50–500 nm in diameter which form from emulsifying natural or

synthetic lipids in aqueous solutions to create one or more bilayers [28,29,30].

7.1 Advantages of liposomes

- Increases efficacy and therapeutic index of the drug
- Increases stability via encapsulation
- Liposomes serve as non-toxic vesicles which exhibit biocompatibility and flexibility as they break down completely with no immunogenicity during both systemic and non-systemic deliveries.
- Liposomes function as protective barriers which prevent susceptible tissues from coming into contact with dangerous drugs [31,32,33].

7.2 Disadvantages of Liposomes

- The production of liposomes utilizes traditional methods that contain toxic organic solvents that simultaneously threaten stability.
- The physical chemical and biological instability of liposomes reduces their effective shelf life along with their operational performance.
- The creation of uniform liposomes together with their complex preparation requirements results in high energy costs and time-consuming methods [34,35].

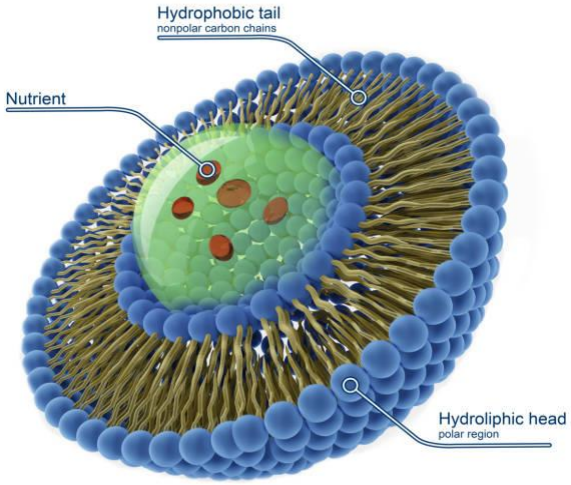

Fig 4. A schematic representation of liposomal drug delivery

8. FORMULATION COMPONENTS OF THE LIPOSOMES

Liposomes exist as systems which possess multiple different structural components that both build their framework and make them functional.

1. **Phospholipids:** The main structural components of liposomes originate from phospholipids because these molecules create the essential lipid bilayer. Commonly used phospholipids include:
- The liposome formulation industry utilizes natural phospholipid Soybean Phosphatidylcholine as

its main ingredient.

- Egg Phosphatidylcholine: Another natural source of phospholipids.
- Synthetic Phospholipids: Such as dioleoyl phosphatidylcholine (DOPC) or distearoyl phosphatidyl choline (DSPC). [36,37]

2. **Cholesterol:** Liposomes contain cholesterol as a component which improves membrane stability through regulation of fluidity and reinforces the lipid bilayer structure. The presence of liposomes in biological fluids remains stable through structural protection from cholesterol and analogous substances.
3. **Drugs:** Liposomes have the ability to incorporate therapeutic agents of different types including drugs as follows:

- **Hydrophilic drugs:** The water-soluble drugs are commonly placed within the aqueous section of liposome particles.
- Hydrophobic drugs: these drugs find their placement inside the bilayer layer structure of the liposome (38).

4. **Stabilizers and Additives:** Special stabilizers and additives are included with liposomes to enhance their stability as well as their operational performance through added measures.

- **Polymers:** Stealth liposomes with extended circulation time are created by using polymers specifically polyethylene glycol (PEG).
- **Membrane proteins:** These act as enhancers for targeting functions and biodistribution through their incorporation into the liposomal system.

5. **Buffers:** The incorporation of buffering agents defends the pH stability of liposomal formulations when drugs are encapsulated in order to guarantee their stability and effectiveness [39,40].

6. **Ionic agents:** The inclusion of ionic agents functions to alter liposome charge properties which affects their antiallergic and tissue cell-connecting interactions. [41].

9. CLASSIFICATION OF LIPOSOMES

Liposomes can be classified based on:

9.1 Based on Structure

- **Unilamellar liposomes:** Liposomes with a Single Bilayer Structure Are Called Unilamellar Liposomes since They Encompass a Liquid Core in One Membrane. These vesicles function well for drug encapsulation together with drug release processes. SUVs and LUVs belong to the subcategories of unilamellar vesicles. The size of SUVs lies between 20-100 nanometers while the dimension of LUVs spans above 100 nanometers.
- **Multilamellar liposomes:** The drug loading capacity becomes higher when lipid bilayers form into multiple concentric layers in multilamellar liposomes. These vesicles may encapsulate bigger amounts of aqueous solution. The lipid layers enable drug release to happen over time through a sustained mechanism. Multilamellar liposomes have dimensions which surpass those of unilamellar liposomes.
- **Multivesicular vesicles (MVVs):** A special lipid structure known as Multivesicular Vesicles contains small vesicles placed inside a larger vesicular compartment. The controlled drug release function results from this structural format. The stability together with the bioavailability of drugs inside the microencapsulation increases when using MVVs. The delivery system benefits from these components for specific targeting applications [42,43].

9.2 Based on Functionality

- **Conventional Liposomes:** General drug delivery operations require conventional liposomes which represent basic liposomal structures. The vesicles contain drugs which belong to both hydrophilic and hydrophobic categories. Their circulation period remains short since the immune system rapidly removes these vesicles. Basic liposomal formulations use the traditional liposomes as the fundamental building block.

- **Stealth Liposomes:** The Stealth Liposomes incorporate polyethylene glycol (PEG) on their surfaces to achieve immune system evasion and an extended circulation duration in the bloodstream. The addition of PEG produces a coating that obstructs the opsonization process thus leading to improved stability during circulation and better biodistribution. Such targeted delivery systems enable drug administration while decreasing the occurrence of adverse effects on the patient. Liposomes exist as a prevalent therapeutic agent for cancer treatment along with its other various applications.

- **Charged Liposomes:** The presence of PEG coating and membrane surface charges in charged Liposomes leads to either positive or negative charges that boost biological membrane binding abilities. Such charges boost both drug uptake by cells and increase delivery efficiency. The classification system divides liposomal structures into two groups: cationic and anionic liposomes that bear positively or negatively charged surfaces. The science utilizes these vesicles frequently for gene delivery as well as vaccine formulations [44].

9.3 Based on Composition

- **Phospholipid-Based Liposomes:** Liposomes made with phospholipids constitute the main building block of their lipid bilayer structure. The common types of phospholipids used in pharmaceuticals are phosphatidylcholine together with phosphatidylethanolamine. Providing biocompatibility together with biodegradability are characteristics of these liposomes. Liposomes find their place in different drug delivery applications because of their extensive utilization.
- **Cholesterol-Containing Liposomes:** The addition of cholesterol in liposomes serves to adjust membrane fluidity and stability level. Drugs can achieve better encapsulation because cholesterol strengthens the lipid bilayer structure. These drug carriaging systems have

enhanced resistance against normal alterations that occur naturally in biological spaces. Such liposomes find frequent usage in stable formulation development.

- **Polymer-Modified Liposomes:** Drug delivery efficiency and circulation time of liposomes improve when they contain polymers or membrane proteins as modifications. These enhancements allow the liposomes to better distribute throughout the body structure. Medical professionals design these vesicles for particular therapeutic fields and targeted drug delivery applications. The addition of polymers to liposomes enhances drug pharmacokinetic behavior of intravelopated drugs. [45]

9.4 Based on the Targeting Mechanism

- **Passive Targeted Liposomes**: Liposomes implementing passive targeting benefit from enhanced permeability and retention (EPR) effect that allows accumulation within tumor tissues. The drug delivery relies upon tumors presenting leaky blood vessels. For specific cancers this technique shows effective results although it lacks specificity. Conventionally applied liposomal preparations use passive targeting as an established technique.
- **Active Targeted Liposomes:** These incorporate surface-facing ligands which act as receptors that connect to target cells. Targeted delivery systems achieve better therapeutic benefits together with decreased adverse effects through their specific targeting methodology. Active targeting brings the most benefit to cancer treatments together with targeted therapies for local-area diseases. Liposomes enhance drug delivery precision because of their design [46].
- **Stimuli-Responsive Liposomes**: The design of Stimuli-Responsive Liposomes enables them to release their cargo when stimulated by particular external signals including pH variations and temperature or light fluctuations. The controlled drug release happens precisely where the treatment needs to take effect. The use of liposomes that respond to stimuli makes treatments more effective while decreasing the side effects that spread throughout the body. Scientists focus on developing this field as a new research approach for drug delivery systems. [47]

10. METHODS OF PREPARATION

1. Thin Film Hydration Method:

This method involves dissolving lipids in organic solvent then removing the solvents through vacuum pressure to form a thin film which gets hydrated by an aqueous buffer. The aqueous buffer solution fills the hydrated film above the Tm point of the lipids while containing drugs that dissolve in water. The method of hydration controls how well drugs are encapsulated and slower hydration speeds lead to better encapsulation [48,49].

2. Reverse-Phase Evaporation Method:

These produces water-in-oil emulsions through mixing lipids in solution with an aqueous phase while using organic solvents. The removal of organic solvent from the mixture results in liposome formation. This procedure functions as a substitute to thin-film hydration which delivers superior control of liposome dimensions [50].

3. Detergent Removal Method:

This uses lipids that are mixed with a surfactant with high critical micelle concentration (CMC) while remaining in an organic solvent solution. A lipid film gets mixed micelles through the addition of aqueous solutions containing drug molecules which later get purified through dialysis and alternative methods to remove the surfactant. The method of surfactant removal leads to the potential loss of hydrophilic drugs [51].

4. Dehydration-Rehydration Method:

Represents an organic solvent-free procedure that combines lipids with drug molecules in an aqueous solution and applies sonication for production. The method incorporates dehydration into making a multilayered film followed by hydration step to form large vesicles. The simplicity of this method creates large variations in the sizes of produced liposomes during the fabrication process [52].

5. Heating Method:

Involves saturating lipids with aqueous solution during heating periods lasting at least one hour above phospholipid Tm temperatures until reaching 100°C when cholesterol is added. σαν stabilizing agent between 3-5% of glycerin or propylene glycol is added to the formulation to maintain stability and resist coagulation.

6. pH Jumping Method:

Exposing a combination of phosphatidic acid and phosphatidylcholine in aqueous solution to a rapid pH increase by four times allows breaking multilamellar vesicles (MLVs) into small unilamellar vesicles (SUVs) using the solvent-free pH Jumping method. The proportion of phosphatidic acid against phosphatidylcholine controls how much SUVs and LUVs will form in the production process. [53]

7. Supercritical Fluidic Method

Supercritical Fluidic Method functions through dissolving lipids with supercritical carbon dioxide (CO_2) instead of organic solvents. The pressure decreases causes liposome formation when aqueous phase flows continuously through a supercritical lipid solution system. The encapsulation efficiencies reach 5-fold greater levels through this method but production costs remain high and output yields remain low [54,55,56,57].

8. Microfluidic Channel Method

A microfluidic channel system applies ethanol or isopropanol to dissolve lipids before injecting them into an aqueous solution contained inside channels. The process of continual mixture between organic and aqueous solutions produces liposomes that get stabilized through surfactant addition to prevent coagulation. The described approach enables scientists to generate uniform liposomes between each experimental run [58,59,60].

9. Solvent Injection Method:

Rapid injection of lipids and hydrophobic active agents in solution form using a solvent creates the basis for this method. The approach needs an aqueous solution concentration that exceeds the organic substance by at least ten to twenty times before vacuum evaporates the organic solvent. Properties of liposomal formulations have higher polydispersity indexes (PDI) when created through this approach [61]. (61)

10. Freeze-Thaw Cycles:

This represent a fundamental technique which aids in both improving the encapsulation efficiency and enhancing liposome lamellarity during the liposome preparation process. The liposome stability alongside its characteristics improves due to the freeze-thaw cycle process [62].

11. EVALUATION OF LIPOSOMES

Physico-chemical evaluation studies for liposomes

11.1 Physical evaluation: Particle size:

a) **Particle size**: DLS (Dynamic light scattering) is used to determine the size of liposomes. By injecting the samples in liquid form, graphs highlight the size of the particles in suspension. [63]

b) **Zeta potential:** The surface electrical charge of liposomes needs evaluation because it plays crucial role in maintaining stability. The Zeta potential depends on the electric charge. The combination of phospholipid "head" constituents with medium pH levels determines whether liposomes acquire negative or positive or non-charged surface properties. Surface electric charge density together with its nature affecting both liposomal stability and their biodistribution kinetics while regulating target cell liposome uptake [64,65]. (64,65)

c) **Polydispersity index:** The PDI serves as a critical computation element to determine the dispersal pattern of nanocarrier particles and measure their general sample uniformity. A PDI value below 0.3 defines the ideal condition for lipid-based drug delivery systems since it shows optimal population distribution and resulting stability and efficacy. High heterogeneity measurement values above 0.7 tend to decrease formulation efficacy and make pharmaceutical creations less acceptable to patients. Overall success and safe delivery of medical chemicals demand precise PDI control for optimal outcomes in treatment [66].

11.2 Determination of Encapsulation Efficiency

Liposomal antibiotic encapsulation efficiencies (EE) is calculated through an experimental method that measures antibiotic content within liposomes against initial solution amounts using the following equation (1) [67,68].

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Concentration of encapsulated drug}}{\text{Initial drug concentration}} X\ 100$$

11.3 Microscopic Methods for Analyzing the Morphology of Liposomes

By using microscopic methods researchers can see both the external form along with the internal structure of liposomes. The microscopic methods include both optical microscopy and electron microscopy. The use of optical microscopy allows manipulation using visible light but delivers limited results about liposomal structure [69].

11.4 In vitro release studies

The test evaluates drug release using the Franz diffusion cell system under in vitro conditions. One ml (100 mg/ml) of solution is placed into the beaker holding dissolution medium in phosphate buffer saline with a pH value of 7.4. A dissolution medium at 37 ± 0.5°C is placed inside the beaker. The setup operates under a magnetic stirrer set to 50 rpm during testing. Direct testing for drug release through the Franz diffusion cell requires buffers prepared to match intestinal environmental characteristics and cerebrospinal fluid conditions (pH 7.4). The solution volume includes 5 ml of dissolution medium that is removed throughout time intervals to maintain sink conditions. UV spectroscopy measures the filtered samples at a particular wavelength. The experimental runs are performed three times with mean values and standard deviations shown for each measurement.

11.5 Physicochemical Stability

The stability level of liposomes determines their clinical usefulness and effectiveness potential. Assessments of liposome stability commonly include physical evaluations at various time intervals (days, weeks or months) to evaluate drug loss and size measurements of nanoparticles. Physical degradation of lipid membranes together with particulate aggregation remain as undesirable changes in liposome formulations throughout their usage period [73,74].

12. CHALLENGES

The diverse genetic makeup together with different patient characteristics in cystic fibrosis (CF) treatment hinders clinicians from predicting exact drug treatment responses in specific patients. Cystic fibrosis originates from a single gene mutation yet multiple genetic variants exist among the various causal elements in the disorder. Treatment options for CF require immediate improvement because patient genetic differences present multiple obstacles to effective medication selection. Research focuses on developing safe alternatives to drug treatments which react to CFTR modulators because people with gene variations responding to these treatments have seen tremendous benefits from their use.

13. CONCLUSION

Liposomal antibiotics show great potential to advance respiratory infection treatment in cystic fibrosis (CF). The potential therapeutic advancement in CF treatment comes from liposomes because they enhance delivery of medications while decreasing toxicity and inhibiting biofilm resistance. The clinical adoption of respiratory treatments for cystic fibrosis faces obstacles from drug leakage incidents and stability system problems as well as issues with industrial-sized manufacturing processes. The limitations of liposomal research exist although the ongoing scientific advancements in the field push forward innovation in CF treatment.

14. FUTURE PERSPECTIVES

Studies should aim at improving liposomal formulations by extending their residence time within the lungs and shortening dosing intervals while building methods to enhance patient drug compliance. The research into PEGylated and functionalized liposomes presents potential opportunities to extend drug presence in the lungs thus advancing CF therapy pathways. Current research demonstrates that liposomes containing functional foods and plant extract exhibit strong potential to decrease inflammation associated with cystic fibrosis in the lungs. Future medical breakthroughs need to incorporate gene therapy inside liposomal systems for targeting the primary genetic defects that cause CF. The ongoing rise of success using liposomal drug delivery systems indicates they can transform CF treatment by minimizing usage of standard antibiotics while enhancing sustained patient results.

15. REFERENCES

1. Elborn JS. Cystic fibrosis. Lancet 2016; 388:2519–2531
2. Parihar A, Prajapati BG, Paliwal H, Shukla M, Khunt D, Bahadure SD, Dyawanapelly S, Junnuthula V. Advanced pulmonary drug delivery formulations for the treatment of cystic fibrosis. Drug Discovery Today. 2023 Jul 31:103729.
3. Rey MM, Bonk MP, Hadjiliadis D. Cystic fibrosis: Emerging understanding and therapies. Annu Rev Med [Internet]. 2019;70(1):197–210.
4. Roberts JM, Dai DLY, Hollander Z, Ng RT, Tebbutt SJ, Wilcox PG, et al. Multiple reaction monitoring mass spectrometry to identify novel plasma protein biomarkers of treatment response in cystic fibrosis pulmonary exacerbations. J Cyst Fibros [Internet]. 2018;17(3):333–40.
5. Turcios NL. Cystic fibrosis lung disease: An overview. Respir Care [Internet]. 2020;65(2):233–51.
6. Reynolds D, Kollef M. The Epidemiology and Pathogenesis and Treatment of Pseudomonas aeruginosa Infections: An Update. Drugs. 2021 Dec;81(18):2117-2131. doi: 10.1007/s40265-021-01635-6. Epub 2021 Nov 7. PMID: 34743315; PMCID: PMC8572145.
7. Filbrun AG, Lahiri T, Ren CL. Introduction and epidemiology of cystic fibrosis. In: Handbook of Cystic Fibrosis. Cham: Springer International Publishing; 2016. p. 1–4.
8. Ratjen FA. Cystic fibrosis: pathogenesis and future treatment strategies. Respir Care. 2009 May;54(5):595-605. doi: 10.4187/aarc0427. PMID: 19393104.
9. Velard F, Delion M, Tabary O, Guillaume C, Sermet-Gaudelus I, Pimpec-Barthes FL, et al. WS06.5 New insights into the pathogenesis and potential therapies of cystic fibrosis (CF) bone disease. J Cyst Fibros [Internet]. 2016;15:S10.
10. Parkins MD, Floto RA. Emerging bacterial pathogens and changing concepts of bacterial pathogenesis in cystic fibrosis. J Cyst Fibros [Internet]. 2015;14(3):293–304. Available from: http://dx.doi.org/10.1016/j.jcf.2015.03.012
11. Peters S. Cystic fibrosis: a review of pathophysiology and current treatment recommendations. S D Med. 2014 Apr;67(4):148-51, 153. PMID: 24791377.
12. Ramsey BW. Management of pulmonary disease in patients with cystic fibrosis. N Engl J Med. 1996 Jul 18;335(3):179-88. doi:10.1056/NEJM199607183350307. Erratum in: N Engl J Med 1996 Oct 10;335(15):1167. PMID: 8657217.
13. Haworth CS, Bilton D, Elborn JS. Long-term macrolide maintenance therapy in non-CF bronchiectasis: evidence and questions. Respir Med. 2014 Oct;108(10):1397-408. doi: 10.1016/j.rmed.2014.09.005. Epub 2014 Sep 16. PMID: 25301290.
14. Fiel SB. Clinical management of pulmonary disease in cystic fibrosis. Lancet. 1993 Apr 24;341(8852):1070-4. doi: 10.1016/0140-6736(93)92423-q. PMID: 8096969.
15. Halawa EM, Fadel M, Al-Rabia MW, Behairy A, Nouh NA, Abdo M, Olga R, Fericean L, Atwa AM, El-Nablaway M, Abdeen A. Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. Frontiers in Pharmacology. 2024 Jan 12; 14:1305294.
16. Gupta A, Mumtaz S, Li C-H, Hussain I, Rotello VM. Combatting antibiotic-resistant bacteria using nanomaterials. Chem Soc Rev [Internet]. 2019;48(2):415–27.
17. Metz M, Shlaes DM. Eight more ways to deal with antibiotic resistance. Antimicrob Agents Chemother [Internet]. 2014;58(8):4253–6.
18. Lim SB, Banerjee A, Önyüksel H. Improvement of drug safety by the use of lipid-based nanocarriers. J Control Release [Internet]. 2012;163(1):34–45.
19. Hosnedlova B, Kabanov D, Kepinska M, B Narayanan VH, Parikesit AA, Fernandez C, et al. Effect of Biosynthesized Silver Nanoparticles on Bacterial Biofilm Changes in S. aureus and E. coli. Nanomaterials (Basel) [Internet]. 2022;12(13):2183.
20. Wang C-H, Hsieh Y-H, Powers ZM, Kao C-Y. Defeating antibiotic-resistant bacteria: Exploring alternative therapies for a post-antibiotic era. Int J Mol Sci [Internet]. 2020;21(3):1061.
21. Ghosh R, De M. Liposome-Based Antibacterial Delivery: An Emergent Approach to Combat Bacterial Infections. ACS omega. 2023 Sep 22;8(39):35442-51.
22. Khatib, I., Khanal, D., Ruan, J., Cipolla, D., Dayton, F., Blanchard, J. D., & Chan, H.-K. (2019). Ciprofloxacin nanocrystals liposomal powders for controlled drug release via inhalation. International Journal of Pharmaceutics, 566, 641–651.
23. Blanken D, Foschepoth D, Serrão AC, Danelon C. Genetically controlled membrane synthesis in liposomes. Nat Commun [Internet]. 2020;11(1):4317.
24. Subramani T, Ganapathyswamy H. An overview of liposomal nano-encapsulation techniques and its applications in food and nutraceutical. J Food Sci Technol [Internet]. 2020;57(10):3545–55.
25. Bassetti M, Vena A, Russo A, Peghin M. Inhaled liposomal antimicrobial delivery in lung infections. Drugs. 2020 Sep;80(13):1309-18.
26. Cipolla D, Blanchard J, Gonda I. Development of liposomal ciprofloxacin to treat lung infections. Pharmaceutics. 2016 Mar 1;8(1):6.
27. Lee M-K. Liposomes for enhanced bioavailability of water-insoluble drugs: In vivo evidence and recent approaches. Pharmaceutics [Internet]. 2020;12(3):264.
28. T. Sun, Y.S. Zhang, B. Pang, D.C. Hyun, M. Yang, Y. Xia, Engineered nanoparticles for drug delivery in cancer therapy, Angew. Chem. 53 (2014) 12320–12364.
29. S. Jha, P.K. Sharma, R. Malviya, Liposomal drug delivery system for cancer therapy: advancement and

patents, Recent Pat. Drug Deliv. Formulation 10 (2016) 177–183.
30. Delma KL, Lechanteur A, Evrard B, Semdé R, Piel G. Sterilization methods of liposomes: Drawbacks of conventional methods and perspectives. Int J Pharm [Internet]. 2021;597(120271):120271.
31. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013 Feb 22;8(1):102. doi: 10.1186/1556-276X-8-102. PMID: 23432972; PMCID: PMC3599573.
32. Carugo D, Bottaro E, Owen J, Stride E, Nastruzzi C. Liposome production by microfluidics: potential and limiting factors. Sci Rep [Internet]. 2016;6:25876.
33. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: An updated review. Pharmaceutics [Internet]. 2017;9(2):12.
34. Ahmed KS, Hussein SA, Ali AH, Korma SA, Lipeng Q, Jinghua C. Liposome: composition, characterisation, preparation, and recent innovation in clinical applications. J Drug Target. 2019 Aug;27(7):742-761. doi: 10.1080/1061186X.2018.1527337. Epub 2018 Oct 15. PMID: 30239255.
35. Gouda A, Sakr OS, Nasr M, Sammour O. Ethanol injection technique for liposomes formulation: An insight into development, influencing factors, challenges and applications. J Drug Deliv Sci Technol [Internet]. 2021;61(102174):102174.
36. van der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. Biochim Biophys Acta [Internet]. 2017;1859(9 Pt B):1558–72.
37. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, et al. A review on phospholipids and their main applications in drug delivery systems. Asian J Pharm Sci [Internet]. 2015;10(2):81–98.
38. Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: Structure, composition, types, and clinical applications. Heliyon. 2022 May 1;8(5).
39. Pavelić Z, Skalko-Basnet N, Jalsenjak I. Characterisation and in vitro evaluation of bioadhesive liposome gels for local therapy of vaginitis. Int J Pharm [Internet]. 2005;301(1–2):140–8.
40. Monteiro N, Martins A, Reis RL, Neves NM. Liposomes in tissue engineering and regenerative medicine. J R Soc Interface [Internet]. 2014;11(101):20140459.
41. Wu X, Dai X, Liao Y, Sheng M, Shi X. Investigation on drug entrapment location in liposomes and transfersomes based on molecular dynamics simulation. J Mol Model. 2021;27(4):111.
42. Maja L, Željko K, Mateja P. Sustainable technologies for liposome preparation. J Supercrit Fluids [Internet]. 2020;165(104984):104984.
43. Andra VVSNL, Pammi SVN, Bhatraju LVKP, Ruddaraju LK. A comprehensive review on novel liposomal methodologies, commercial formulations, clinical trials and patents. Bionanoscience [Internet]. 2022;12(1):274–91.
44. Nikam NR, Patil PR, Vakhariya RR, Magdum CS. Liposomes: A novel drug delivery system: An overview. Asian J Pharm Res [Internet]. 2020;10(1):23.
45. Dhillon A, Singh R, Senwar KR. An extensive review on novel liposomes : Classification, methodology, characterization, current formulations. Int J Drug Deliv Technol [Internet]. 2024;14(03):1842–52.
46. Sheoran R, Khokra SL, Chawla V, Dureja H. Recent patents, formulation techniques, classification and characterization of liposomes. Recent Pat Nanotechnol [Internet]. 2019;13(1):17–27.
47. Burdaev NI, Nikolaeva LL, Kosenko VV, Shprakh ZS, Bunyatyan ND. Liposomes as drug carriers: Classification, preparation methods, and medicinal use. Bull Sci Cent Expert Eval Med Prod [Internet]. 2023;13(2–1):316–32.
48. Tagrida M, Prodpran T, Zhang B, Aluko RE, Benjakul S. Liposomes loaded with betel leaf (Piper betle L.) ethanolic extract prepared by thin film hydration and ethanol injection methods: Characteristics and antioxidant activities. J Food Biochem [Internet]. 2021;45(12):e14012.
49. Al-Amin MD, Bellato F, Mastrotto F, Garofalo M, Malfanti A, Salmaso S, et al. Dexamethasone loaded liposomes by thin-film hydration and microfluidic procedures: Formulation challenges. Int J Mol Sci [Internet]. 2020;21(5):1611.
50. Liu G, Hou S, Tong P, Li J. Liposomes: Preparation, characteristics, and application strategies in analytical chemistry. Crit Rev Anal Chem [Internet]. 2022;52(2):392–412.
51. Lombardo D, Kiselev MA. Methods of liposomes preparation: Formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. Pharmaceutics [Internet]. 2022;14(3):543.
52. Has C, Sunthar P. A comprehensive review on recent preparation techniques of liposomes. J Liposome Res [Internet]. 2020;30(4):336–65.
53. Shah S, Dhawan V, Holm R, Nagarsenker MS, Perrie Y. Liposomes: Advancements and innovation in the manufacturing process. Adv Drug Deliv Rev [Internet]. 2020;154–155:102–22.
54. Bigazzi W, Penoy N, Evrard B, Piel G. Supercritical fluid methods: An alternative to conventional methods to prepare liposomes. Chem Eng J [Internet]. 2020;383(123106):123106.
55. Trucillo P, Martino M, Reverchon E. Supercritical assisted production of lutein-loaded liposomes and modelling of drug release. Processes (Basel) [Internet]. 2021;9(7):1162.
56. Trucillo P, Cardea S, Baldino L, Reverchon E. Production of liposomes loaded alginate aerogels using two supercritical CO2 assisted techniques. J CO2 Util [Internet]. 2020;39(101161):101161.

57. Trucillo P, Campardelli R, Reverchon E. Liposomes: From Bangham to supercritical fluids. Processes (Basel) [Internet]. 2020;8(9):1022.
58. Rebollo R, Oyoun F, Corvis Y, El-Hammadi MM, Saubamea B, Andrieux K, et al. Microfluidic manufacturing of liposomes: Development and optimization by design of experiment and machine learning. ACS Appl Mater Interfaces [Internet]. 2022;14(35):39736–45.
59. Buttitta G, Bonacorsi S, Barbarito C, Moliterno M, Pompei S, Saito G, et al. Scalable microfluidic method for tunable liposomal production by a design of experiment approach. Int J Pharm [Internet]. 2024;662(124460):124460.
60. Zhang G, Sun J. Lipid in chips: A brief review of liposomes formation by microfluidics. Int J Nanomedicine [Internet]. 2021;16:7391–416.
61. Gouda A, Sakr OS, Nasr M, Sammour O. Ethanol injection technique for liposomes formulation: An insight into development, influencing factors, challenges and applications. J Drug Deliv Sci Technol [Internet]. 2021;61(102174):102174.
62. Bernal-Chávez SA, Romero-Montero A, Hernández-Parra H, Peña-Corona SI, Del Prado-Audelo ML, Alcalá-Alcalá S, et al. Enhancing chemical and physical stability of pharmaceuticals using freeze-thaw method: challenges and opportunities for process optimization through quality by design approach. J Biol Eng [Internet]. 2023;17(1):35.
63. Miere F, Fritea L, Cavalu S, Vicaș SI. Formulation, Characterization, and Advantages of Using Liposomes in Multiple Therapies. Pharmacophore. 2020;11(3-2020):1-2.
64. Németh Z, Csóka I, Semnani Jazani R, Sipos B, Haspel H, Kozma G, et al. Quality by design-driven zeta potential optimisation study of liposomes with charge imparting membrane additives. Pharmaceutics [Internet]. 2022;14(9):1798.
65. Chowdhury N, Chaudhry S, Hall N, Olverson G, Zhang Q-J, Mandal T, et al. Targeted delivery of doxorubicin liposomes for Her-2+ breast cancer treatment. AAPS PharmSciTech [Internet]. 2020;21(6):202.
66. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics [Internet]. 2018;10(2):57.
67. Mugabe C, Azghani AO, Omri A. Preparation and characterization of dehydration-rehydration vesicles loaded with aminoglycoside and macrolide antibiotics. Int J Pharm [Internet]. 2006;307(2):244–50.
68. Gbian DL, Omri A. The impact of an efflux pump inhibitor on the activity of free and liposomal antibiotics against Pseudomonas aeruginosa. Pharmaceutics [Internet]. 2021;13(4):577.
69. Bibi S, Kaur R, Henriksen-Lacey M, McNeil SE, Wilkhu J, Lattmann E, et al. Microscopy imaging of liposomes: from coverslips to environmental SEM. Int J Pharm [Internet]. 2011;417(1–2):138–50.
70. Nallamothu R, Wood GC, Pattillo CB, Scott RC, Kiani MF, Moore BM, et al. A tumor vasculature targeted liposome delivery system for combretastatin A4: design, characterization, and in vitro evaluation. AAPS PharmSciTech [Internet]. 2006;7(2):E32.
71. Dhas N, Preetha HS, Dubey A, Ravi G, Govindan I, Rama A, et al. Factorial design-based fabrication of biopolymer-functionalized Asiatic acid-embedded liposomes: in-vitro characterization and evaluation. J Appl Pharm Sci [Internet]. 2022; Available from:
72. Jindal S, Awasthi R, Singare D, T. Kulkarni G. Preparation and in vitro evaluation of Tacrolimus loaded liposomal vesicles by two methods: A comparative study. J Res Pharm [Internet]. 2021;25(1):1–1. Available from:
73. Briuglia M-L, Rotella C, McFarlane A, Lamprou DA. Influence of cholesterol on liposome stability and on in vitro drug release. Drug Deliv Transl Res [Internet]. 2015;5(3):231–42.
74. Large DE, Abdelmessih RG, Fink EA, Auguste DT. Liposome composition in drug delivery design, synthesis, characterization, and clinical application. Adv Drug Deliv Rev [Internet]. 2021;176(113851):113851.