Nano-Liposomal Delivery Systems and their Applications in Bacterial Resistance

Ruqaya Mohammed Ahmed*, Tabarek H. Mahmood, Zain Saleh Al-Tarawneh***, Qasim Khalid Alazzawi***, Ali Mahmoud Al-Samydai*****

Department of Pharmacy, Al-Ma'mon University College, Baghdad, Iraq.

****Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq.***

***** Faculty of Pharmacy, Department of pharmaceutical and pharmaceutical technology Al-Ahliyya Amman University, Amman, Jordan.***

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Corresponding Author email:
a.alsamydai@ammanu.edu.jo
Orcid: https://orcid.org/0000-0003-0093-2310

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Abstract:

Nanotechnology has been introduced in several health-related aspects, particularly in therapy. Delivery therapeutic agents using nanoliposomes have approved several improvements in their physiochemical properties, selectivity, potency, and general pharmacokinetics and pharmacodynamics. Antibiotic resistance is a major threat to the healthcare system in therapeutic and economic aspects. Developing systems of nano-sized antibacterial agents delivered by nano-liposomes shows a promising solution that would increase the effectiveness, decrease the required therapeutic doses, and save the health economy. Various types of nanoliposomes were incorporated including the cationic, anionic, and neutrally charged molecules, this technology targeted G-negative, G-positive, fungal strains, and other pathogens. Nanoliposomes could overcome the dilemma of biofilm formation and enhance selective targeting of the antibiotic agent.

Keywords: Nanoliposomes, Antibiotic resistance, Drug delivery, Antibacterial agents.

أنظمة التوصيل النانوية الدهنية وتطبيقاتها في مقاومة البكتيريا

رقية محمد احمد*، تبارك حسن محمود، زين صالح الطراونة***، قاسم خالد العزاويد***، علي محمود الصميدعي*****

قسم الصيدلة، كلية المأمون الجامعة، بغداد، العراق.

****** جامعة ابن سينا للعلوم الطبية والصيدلانية، بغداد، العراق.***

*******كلية الصيدلة، قسم الصيدلانيات و التقنية الصيدلانية، جامعة عمان الأهلية، عمان، الأردن.***

خلاصة

تم تقديم تقنية النانو في العديد من الجوانب المتعلقة بالصحة، وخاصة في مجال العلاج. أثبتت أنظمة إيصال العوامل العلاجية باستخدام النانوليبيسومات تحسينات كبيرة في خصائصها الفيزيائية والكيميائية، الانتقائية، الفعالية، والحركية الدوائية العامة. يُعتبر مقاومة المضادات الحيوية تهديدًا كبيرًا للنظام الصحي من الناحيتين العلاجية والاقتصادية. تطوير أنظمة للعوامل المضادة للبكتيريا ذات الحجم النانوي وإيصالها عن طريق النانوليبيسومات يُظهر حلاً واعدًا يمكن أن يزيد من الفعالية، يقلل من الجرعات العلاجية المطلوبة، ويوفر للاقتصاد الصحي. تم دمج أنواع مختلفة من النانوليبيسومات، بما في ذلك الجزيئات الموجبة والسالبة الشحنة والجزيئات ذات الشحنة المحايدة. استهدفت هذه التقنية السلالات البكتيرية السالبة والموجبة الجرام، الفطريات، ومسببات الأمراض الأخرى. يمكن للنانوليبيسومات التغلب على معضلة تكوين الأغشية الحيوية وتعزيز الاستهداف الانتقائي للعوامل المضادة للبكتيريا.

الكلمات المفتاحية: النانوليبوزومات، مقاومة المضادات الحيوية، توصيل الأدوية، العوامل المضادة للبكتيريا.

1. Introduction

1.1 Overview of Antibacterial Resistance

Over recent years, bacterial infections caused new diseases that have led to major threats to human health, resulting in increasing morbidity rates all over the world. Antibiotics are considered life-saving compounds but have been extensively used for improper indications or dosing. Therefore, Resistance to multiple antibiotics was merged, which increases the risk of spreading uncontrolled bacterial infections. The risk of antibiotic resistance is among the top health hazards as it has been declared by the World Health Organization (WHO), it will be ranked first for death causes by 2050 replacing cardiac diseases. [1][2].

Nanotechnology provides a promising solution to the resistance to bacterial infection. Nanoparticles, which are emerging as a new branch of medicine, are capable of eradicating pathogenic bacteria through multiple mechanisms of action, including breaking the cell membrane, forming free radicals, disrupting the bacterial cell wall, inhibiting DNA or protein synthesis enzymes, and producing photocatalytic reactive oxygen species. They may also damage cellular or viral components and disrupt biofilms. [1].

Nano-antibiotics offer several advantages over conventional antibiotics, including reduced toxicity, fewer side effects, and lower management and manufacturing costs. Additionally, nano-drugs demonstrate greater potency and higher efficacy compared to traditional medications [2].

Liposomes are spherical nanodelivery systems consisting of lipid bilayers capable of encapsulating both lipophilic and hydrophilic compounds. They are biocompatible and biodegradable, offering significant advantages for antibiotic delivery. Depending on the site of action, liposomes can deliver drugs with synergistic effects. They enhance the bioavailability of antimicrobial and antibacterial agents at targeted sites through sustained release, improve drug solubility, and protect these agents from degradation. Moreover, liposomes modify the pharmacokinetics and biodistribution of drugs, thereby reducing antibacterial toxicity and increasing therapeutic activity. [3][4][5].

1.2 Importance of Liposomes in Antibacterial Therapy

Liposomes are commonly used as carriers in pharmaceutical industries and cosmetics. On the other hand, cytotoxic agents such as doxorubicin and antifungal drugs such as amphotericin were prepared as liposomal formulations and available in the pharmaceutical market. Liposomes were known to improve drug pharmacokinetics, enhancing their solubility, consequently increasing their bioavailability, and drug circulation [9][10]. Extensive researches were performed to develop and enhance the currently available antibiotics by making modifications that produce the drug in a new form such as liposomal formulations, one of the most important properties of liposomes is that it is easy to be modified by even changing lipid types of liposome in liposomal formulation, size, charge, pH sensitivity, temperature sensitivity, and membrane fluidity.

The advantage of liposome as a carrier is that it can sustain the release of antibiotics in the circulation, which allows for maintaining the proper drug concentration for a long time, this could improve the currently used antibiotics that provide a quick and short effect making the patient need several doses per day. The vesicles improve the drug kinetics, decrease the hydrolytic activity of enzymes, first-pass effect, chemical changes and immunological deactivation. One of the problems of using liposome as a drug delivery system is that they are recognized as

foreign antigens by the body's immune system and are opsonized due to their negatively charge, the phagocytes engulf the liposomes before they reach to their target, resulting in a lower blood time and faster clearanc, so liposome design including (size,charge,PDI) should improve to decrease their recognition by phagosites and immune cells[9][11] . .

Liposomes is a spherical vesicle that composed of phospholipids, cholesterol was added to promote liposomal membrane rigidity, improve drug loading and stability, PEG (polyethylene glycol) was also added to the liposomes to increase their bloodstream circulation[12][10].

2. Fundamentals of Liposomal Technology

2.1 Structure and Composition of Liposomes

Liposomes are the most charming nano-carrier to scientists. They could be used in targeted drug delivery systems. The lipid-based nanoparticles consist of special properties, liposomes are spherical and composed of lipid vesicles (usually 50–500 nm in diameter), liposomes are composed of one or more lipid bilayers, it compromise of a hydrophilic head and lipophilic tail, they are of small particle size and composed of phospholipids (even natural lipid-like soybean phosphatidylcholine or synthetic di-alkyl or tri-alkyl lipids) that could encapsulate drugs of both hydrophobic and hydrophilic characteristics. [13][14][15].

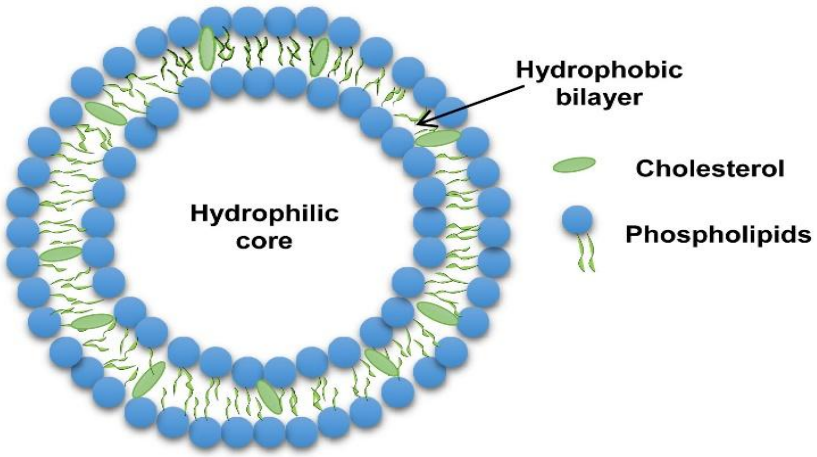

Figure1: Schematic representation of liposome[15]**.**

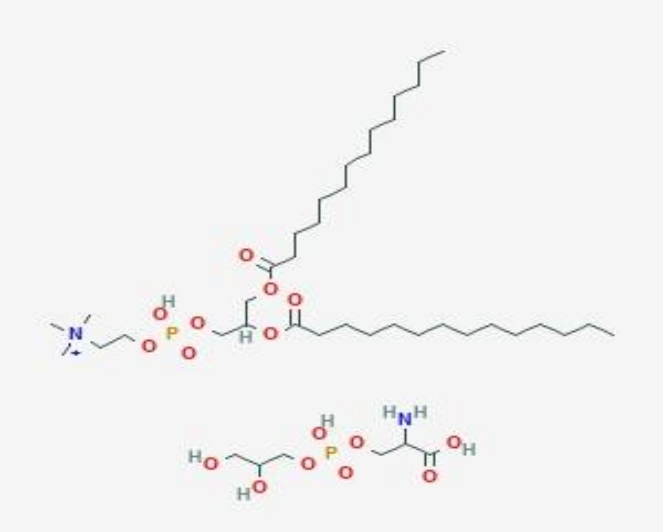

Figure2: Chemical structure of liposome [16]**.**

The most popular method in preparing liposome is thin-filmhydration method, the lipids that used in the preparation with the active ingredient were dissolved in chloroform in a round flask, a thin film appeared on the flask wall after the evaporation of chloroform using a rotary Evaporator, the thin film was hydrated with a phosphate buffer (pH=7), followed by mixing the whole suspension by vortex at a warm temperature until the thin lipid film was completely dissolved and the active ingredient was encapsulated by the phospholipid complex [10].

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2.2 Types of Liposomes

Liposomes consist of three types: Cationic, anionic and neutral liposomes[17].

2.2.1 Cationic Liposomes

Cationic lipids are amphiphilic molecules of three main parts; positively charged polar heads, a linker and hydrophobic tails. Polar heads may contain single or multiple functional groups and can form complexes with an anionic cargo, forming electrostatic interactions. For instance, negatively charged phosphate groups react spontaneously to form condensed cationic lipoplexes, when brought together with a positively charged liposome or lipid species [17].

Lipoplexes are formulated using excess positively charged lipids to DNA/RNA ratio to create a positively charged complex that will make an interaction between surface proteoglycans and the cationic lipoplex system, which will then help with cell binding, fusion of anionic endosomal membranes and endocytosis. Liposomes have lower DNA entrapment efficiency, lower extent of cellular internalization, and efficiency in protecting DNA in the cytoplasm during nuclear entry. Cationic lipids create a natural electrostatic contact

between the lipid and the genes, which improves its encapsulation efficiency and size exclusion [18]. These liposomes have low serum stability because they are attracted to plasma proteins forming a corona layer on their surface. Then, interacts with the serum-anionic proteins by electrostatic forces when introduced into a host cell. Finally, the carriers dissociate and degrade. Similarly, cationic liposomes activate cellular pathways, particularly, the pro-apoptotic and pro-inflammatory cascades.)[19].

2.2.2 Anionic liposome

The anionic phospholipid head groups, phosphatidic acid, phosphatidyl glycerol, and phosphatidylserine, are found naturally in cellular membranes. The anionic systems are more stable in suspension and plasma media as they have a lower aggregation tendency than neutral and cationic types [19]. The stability decreases when administered into the bloodstream but is still stable in other biological environments compared to the positively charged carriers. Modifications are required to overcome the electrostatic repulsion with the negatively charged nucleic acid [20]. The introduction of anionic lipids to a large industrial scale is difficult. Hence, the structure is complex and its production cost is high. Toxicity could be decreased by mixing ratios of different types. There is an increasing interest in developing anionic liposomes as carriers for transdermal drug delivery because of their property in enhancing penetration through the skin, they diffused into the dermis and the hair follicles, the rapid penetration of anionic liposomes would increase permeation of cargo through the skin [21-23].

2.2.3 Neutral Liposomes

This type is formulated using zwitterionic, where the charge is neutral physiological pH but could be changed according to the surrounding environment. Lipids like phosphatidylcholine and cholesterol are neutral [18]. The low physical stability is due to their aggregation tendency.

On the other hand, the neutral type exerts longer circulation time and lower cytotoxicity. This refers to their null interaction with negatively charged proteins in the serum and blood. These are shown in tumours and sites of inflammation due to their ease in adding targeting strategies than cationic liposomes [20]. Adding cholesterol in liposome formulation will stabilize the vesicle, improve rigidity, and prevent aggregation and packed structures from infiltrating or attracting serum proteins, it also improves blood circulation time, neutral liposomes are nontoxic or of very low toxicity as they are found naturally in the body cells[21].

2.3 Mechanisms of Liposomal Drug Delivery

Liposomes could improve antibiotics efficiency and efficacy by their drug delivery mechanism, they have the ability to prevent, target, and control antibiotic release makes them useful in fighting infections by different mechanisms like inducing of apoptosis and stimulating of autophagy, liposomes then digested in the macrophage's phagosome, this will leads to releasing of its drug, they induce body defense mechanism, liposomes also mediate enzyme and drug uptake, this mechanism decrease drug toxicity[22].

3. Mechanisms of Antibacterial Action of Liposomes

3.1 Enhanced Drug Delivery and Targeting

Connecting of legend with the surface of the liposome shall enhance the specificity of the target and alter the kinetics of the encapsulated active constituent. Many liposomes are available in the pharmaceutical market, they showed higher

efficacy than the ordinary formulations, lower drug toxicity, and lower bacterial resistance.

Liposomes could be added to opsonins and ligands to increase the site-specificity, liposome poorly accumulates in soft tissues like the kidney, liver, and heart, resulting in a site avoidance effect, the drug produces restricted effects at the desired site after adding of legends and reduces the possibility of drug-related toxicities, Moreover, it enhances the antimicrobial efficacy by protecting the encapsulated drug from enzymatic hydrolysis, such as for penicillin and cephalosporin. Owing to their lipid content, permeation across the pathogens' cell membrane is enhanced and cellular concentration of the antibiotics is increased, consequently, the required dose and the accompanying toxicity would decrease[23].

3.2 Liposome Overcome Bacterial Resistance

Liposomes lower bacterial resistance, so increase the antibacterial activity of the drug that it encapsulates, liposomes increase antibiotic pharmacokinetics by improving the circulation time of the drug in the blood stream, and ligands could be added targeting specific bacteria [23].

3.3 Synergistic Effects with Antibiotics

Co-encapsulation of two antimicrobial agents into liposomes provides a higher contact time of the proper concentration at the infected area. The liposomal carrier will moderate the distribution of the drugs in the tissues and intensify the drug amount in the infected site[24].

4. Liposomal Encapsulation of Antibacterial Agents examples

Klebsiella pneumoniae the responsible bacteria of unilateral pneumonia were found to be more sensitive to a single dose of gentamicin or ceftazidime after encapsulated into the liposomal delivery system than the free drugs. The novel deliver system contributes to increasing the target localization of the encapsulated antibiotics. For example, a single dose of 5 mg/kg of free gentamicin failed to rescue any of the experimental animals, while an equivalent dose of encapsulated gentamycin ensured the survival of 70% of the animals [24].

Moreover, polymyxin B showed higher bactericidal when encapsulated than the free form. Its bactericidal activity was tested on *P. aeruginosa*, *Bordetella bronchiseptica*, *E. coli*, *Acinetobacter lwoffii, K. pneumoniae*, and *Acinetobacter baumannii*. Formulated polymyxin B reduced minimum inhibitory concentration 4–16 times compared to the free antibiotic. The undesirable effects, such as nephro, oto, and neuromuscular toxicities were decreased when administering the liposomal formulated medicine to the bloodstream [23].

As previously described, liposomes have shown promise as a drug delivery method for antibiotics. Their structure allows them to fuse with the microbial cell's outer membrane, facilitating the delivery of drugs directly to the cytoplasm [24][25]. This characteristic makes liposomes a useful and adaptable tool for raising the effectiveness of antimicrobial treatments, especially in addressing difficulties such as minimizing adverse effects, infections with biofilm formation, and treatment resistance. [26]. For example, fusogenic liposomes, including cholesterol hemi-succinate have been shown to boost the antibacterial activity of vancomycin towards various vancomycin-resistant bacteria [27].

4.1 Liposomes in the treatment of gram-negative bacterial infections

G-ve bacteria possess a complex outer membrane that acts as a formidable barrier, restricting the entry of antibiotics and altering

their interaction with the bacterial cell wall. This barrier is a key contributor to antibiotic resistance [25]. Nevertheless, as previously mentioned, liposomes can potentially fuse with the outer membranes of bacteria, causing structural disruption and potentially enhancing membrane permeability. The fluidity of the liposomes can be increased, and fusogenic phospholipids can be incorporated to further enhance the fusion process [29]. Examples of approved liposomal formulations were shown in Table 1.

G-ve bacteria, which are leading contributors to worldwide morbidity and mortality rates, are generally more resistant to antibiotics than G+ve bacteria [30]. In contrast to G+ve bacteria, most G-ve strains, aside from those lacking lipopolysaccharides (LPS), are encased in an LPS layer [31]. LPS plays a dual role: it stimulates the immune system and contributes to antibiotic resistance by insulating the bacterial cell it surrounds [32]. Actually, strains lacking LPS are less virulent and more sensitive to drugs [31]. It is, therefore, unsurprising that G-ve bacteria dominate the World Health Organization's list of antibiotic-resistant pathogens [32].

Table 1: An overview of liposomal formulations used against G-ve bacteria (- means not active, + means active, and ++ means highly active)

Liposome used	Bacteria	Active component	Dose	Efficacy	References
Both anionic and Cationic with or without PEGylation	*Pseudomonas aeruginosa*	-	0 –8.5 µmol/mL	Anionic liposomes (-) Cationic liposomes (+) PEGylated cationic liposomes (++)	[33]
Surface cationic lysozymal liposome		Gentamicin	10 µL	Liposomal gentamicin with surface cationic lysozyme (++) Control (-)	[34]
Cationic liposomes		Tobramycin with anti-biofilm peptide	(4-32) µg/mL	Liposomal tobramycin with or without peptide (++)	[35]
Neutral liposomes: NLG Negatively charged liposomes: NLG-1 and NLG-2.		Gentamicin	0.125–0.5 mg/L.	*P.aeruginosa*: NLG and NLG-1 (++) Control and NLG-2 (+)	[35]
Cationic Liposomes + photodynamic therapy		DVDMS	10 µg/mL	Cationic liposomal DVDMS with Photodynamic therapy (++) Control (-)	[36]

Photodynamic liposome		Perfluorohexane and photosensitizer	100 µL	Photodynamic liposome (++)	[37]
Rhamnosome nano-vesicles	*Escherichia coli* & *Pseudomonas aeruginosa*	Nisin Z	*E.coli*: 25 µg/mL *P.aeruginosa*: 100 µg/mL	Rhamnosomal Nisin Z (++) Control (+)	[38]
Thermal Liposomes	*Escherichia coli*	Tungsten sulfide quantum dots and vancomycin	50 µg/mL	Liposome with NIR (++) Control (-)	[39]
Cationic liposomes DPPC:Chol:DOTAC -Lipo 1 (1:0.5:0.3) -Lipo 2 (1:0.5:0.5) -Lipo 3 (1:0.5:0.8) -Lipo 4 (1:0.5:1.2)		Methylene blue	0.02 % *w/v*	Lipo 1&2&3 (+) Lipo 4 & Control (-)	[40]
Neutral liposomes		Azithromycin or azithromycin/ N-acetylcysteine (LAN)	2.5– 3 µg/mL	**Strain SA057:** AZI and LAN (++) Free drugs+ **Strain SA10:** AZI (+) LAN and Control (++)	[41]
Negative liposomes with rigid bilayers (CL-3), propylene glycol liposomes (PGL-2), or deformable		Azithromycin	(1.26–45.65) µg/mL	DPGL-2 (++) CL-3 and PGL-2 (+)	[42]
propylene glycol liposomes (DPGL-2)					
Solid liposomes		Clove oil	0.5 mg /mL	Liposome (+)	[43]
Liposomal Chitosan + ultrasound microbubbles (USMBs)	*Acinetobacter baumannii*	Polymyxin B	8 µg/mL	USMBs with CLPs (++) Control (+)	[44]
Silver sulfadiazine liposomes (AgSD-NLs@Cur)		Curcumin	7.8 µg/mL	LED plus AgSD-NLs@Cur (++) Control (-)	[45]
Neutral liposomes		Thymoquinone (TQ)	2 µg/mL for TQ and 4 µg/mL for liposomal TQ	Control (++) Lip-TQ (+)	[46]
Neutral liposomes	*Salmonella typhimurium*	Geraniol	0.10%	Liposomal Geraniol (+)	[47]

4.2 Liposomes in the treatment of G-+ve bacterial infections

Infections caused by G+ve bacteria, including Staphylococcal strains, including *S. aureus, S.epidermidis,* and *S. saprophyticus*, Streptococcal strains, such as pneumoniae, oralis, and mutans, *Cutibacterium acnes*, *Bacillus subtilis, Mycobacterium avium*, *M. avium* subsp. hominissuis, *M.abscessus*, and *Listeria monocytogenes*, represent a significant public healthcare burden . This challenge is exacerbated by the growing antibiotic resistance among G+ve strains [48]. G+ve bacteria are a major cause of Intensive care unit (ICU) infections, as well as being the leading pathogens responsible for infections related to the skin, soft tissues, and medical devices [49]. Table 2 provides a summary of studies exploring the use of liposomal formulations used for targeting G+ve bacteria.

Table 2: An overview of liposomal formulations used against G+ve bacteria (- means not active, + means active, and ++ means highly active)

Bacteria	Liposome used	Active component	Dose	Efficacy	References
MRSA	Glycosylated cationic liposomes	Trans-resveratrol	1.2 mM	Galactosylated liposomes (++) Mannosylated liposomes (+) Glucosylated liposomes (-)	[50]
	Mannosylated liposome	Platensimycin	0.5 to 8 µg/ mL	Mannosylated liposome and control (+)	[51]
	Neutral, deformable, propylene glycol, and cationic liposomes	Azithromycin	0.5–8 µg/mL	Cationic liposomes (++) neutral, deformable, and propylene glycol liposomes (+)	[52]
MSSA	Stealth liposomes	Nafcillin	PEGylated (0.5 µg/mL) unPEGylated (1 µg /mL)	PEGylated liposomes (++) UnPEGylated liposomes (+)	[53]
Staphylococcus aureus	Neutral liposomes	Azithromycin	4 µg/mL	Against biofilm formation (+) Against preformed biofilm (-)	[54]
S. pneumonaie	Stealth or sodium cholate liposomes	Endolysin MSlys	4 µM	Stealth liposomes (++) Sodium cholate liposomes (+)	[55]
Listeria monocytogenes	Liposomal chitosan	Gentamicin	20 µg /mL	Liposomal Chitosan (+)	[56]

4.3 Role of liposomes in treating biofilm-associated infections

Biofilms, complex communities of bacteria encased in an Extracellular polymeric substance (EPS) matrix, represent a significant challenge in clinical settings due to their enhanced resistance to antibiotics and their tendency to form on a wide variety of

biotic and abiotic surfaces [29]. Most chronic human infections, including chronic sinusitis, otitis media, endocarditis, cystic fibrosis, and diseases associated with implants or devices, are mediated by biofilms [57]. Eradicating biofilm-forming infections is the goal of various treatment strategies [58]. Bacteria exist in two survival conditions: the planktonic condition (free living bacteria) and the sessile condition (attached bacteria without a footstalk), also known as biofilms [59]. The transition from planktonic to biofilm includes an intricate and permanent process. It begins with the bacterial cells attaching themselves to a surface, the process known as seeding, then planktonic cells aggregating on the seeds, followed by the production of EPS and quorum sensing molecules, development of the final biofilm structure, and ultimately, the release of planktonic cells as the mature biofilm matrix disperses [60]. Figure 3 shows the biofilm growth cycle.

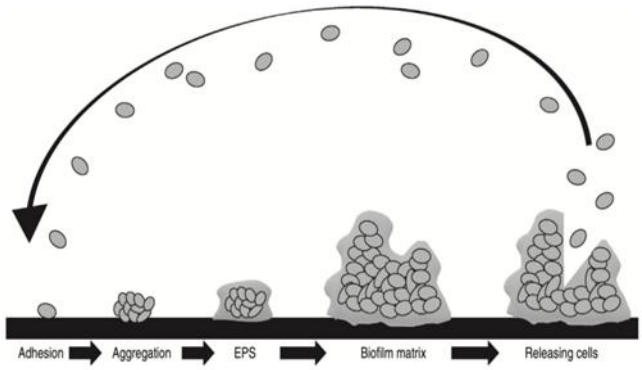

Figure 3: Biofilm growth process steps. Cellular adhesion followed by aggregation, EPS production, biofilm matrix development, and biofilm cell release [63]

Bacteria have formed biofilms on a wide range of surfaces, including Barriers, flooring, drainage, plastic containers, glass surfaces, edibles, mouth cavities, intestines, contact lenses, blood vessel grafts, and heart valves. These biofilms, embedded in an extracellular polymeric substance (EPS) matrix, allow bacterial DNA exchange and survive under harsh conditions such as starvation, dehydration, mechanical stress, and extreme pH, and are not limited to UV rays exposure. Compared to their free-floating (planktonic) counterparts, bacteria within biofilms are up to 1,000 times more antibiotic-resistant [29].G+ve bacteria, are able to form biofilms, which are accountable for chronic infections perceived in clinical practice [61]. Biofilms pose significant challenges to therapy. The biofilm matrix shields bacterial cells from the external environment, preventing antibacterial compounds from penetrating the interior. Additionally, the EPS on the biofilm surface can absorb antimicrobial agents, reducing their bioavailability. Liposomes emerged as a carrier system to deliver medications to the site of biofilm-mediated infections. Liposome versatility enables carrying various medicines with minimal toxicity, non-immunogenicity, and target delivery, and the ability to fuse with the biofilm matrix and cell membranes, significantly enhancing the efficacy of antibacterial drugs and reducing the likelihood of infection recurrence [62].

Liposomes can be engineered with functionalising compounds and membrane stabilisers, including proteins, cholesterol, and lipid-polymer conjugates, to achieve site-specific targeting, biocompatibility, and extended circulation period [63]. For instance, by modifying the surface using covalently bound polymers, liposomes can be directed to particular tissue targets [64]. pH-responsive poly (β-amino ester) gives liposomes pH sensitivity by imparting a positive charge to the surface which makes targeting in acidic situations easier, like inside a biofilm matrix. [65][66]. The development of pH-responsive liposomes that can target the acidic environments within biofilms offers another promising avenue for improving treatment outcomes. These liposomes can be engineered to release their cargo more effectively in the acidic conditions found within biofilm matrices, thereby increasing the local concentration of the antimicrobial agent and enhancing its efficacy [67].

Recent studies found that liposomes, particularly those with a cationic charge, exhibit strong adsorption onto hydroxyapatite, the primary mineral component of tooth enamel, due to the presence of a salivary pellicle. Therefore, they are increasingly used in treating oral infections, particularly periodontitis [68]. The negatively charged salivary pellicle coats the enamel, facilitating the adhesion of cationic liposomes [69]. Studies have shown that liposomes loaded with antimicrobial agents such as triclosan and benzylpenicillin are more effective at eradicating oral biofilms formed by G+ve bacteria, including *S. salivarius*, *S. sanguis*, and *S. oralis*, compared to the drugs in their free form [70][71]. The interactions between the liposomes and the biofilm matrix are driven by electrostatic forces, which can be modulated by the liposome's surface charge [72][73].

The delivery of chlorhexidine and triclosan was enhanced by introducing cationic liposomes, therefore, the antimicrobial activity against dental biofilms increased [74]. These formulations target biofilms more effectively than free drugs because they can fuse with the biofilm matrix and disrupt the bacterial cell membranes [75]. In addition to their use in oral infections, Liposomes have also been explored for the treatment of biofilm-associated infections on medical devices and in other clinical settings [29]. For example, liposomal vancomycin has been investigated for its ability to penetrate and eradicate biofilms formed by ***S. aureus*** on vascular grafts and other implant surfaces [29]. The encapsulation of vancomycin in liposomes enhances its delivery to the biofilm and increases its efficacy against resistant strains of **S. aureus**, including MRSA [30].

While liposomes show great promise in managing biofilm-associated infections, several challenges remain. One significant limitation is the potential aggregation of liposomes with salivary components, such as proline-rich proteins and divalent ions like calcium and magnesium. This aggregation can reduce the stability and effectiveness of the liposomal formulations. Future research may focus on modifying the surface of liposomes with negatively charged polymers, such as pectin, to prevent aggregation and enhance their stability in biological environments [24].

Moreover, *P. aeruginosa* is a versatile opportunistic microorganism known for causing infections in various parts of the body, including the lungs, bloodstream, urinary tract, and skin [75]. One of the primary challenges in treating these infections is the bacterium's ability to create

dense, sticky biofilms, which are often resistant to multiple drugs [76][77]. This resistance makes conventional antibacterial less effective, prompting the exploration of alternative strategies, such as liposomal carriers to enhance the activity of against. *aeruginosa* biofilms [78].

Scientists developed fusogenic liposomes to deliver tobramycin to pulmonary mucoid biofilms formed by *P. aeruginosa*. The studies demonstrated that the liposomal formulation of tobramycin was significantly more effective at eradicating biofilms compared to the drug in its free form, which showed no substantial effect. [79]. In a subsequent study, a liposomal system of tobramycin, bismuth-ethanedithiol, and alginate lyase was manufactured to increase the susceptibility of *P. aeruginosa* biofilms to these drugs. This is achieved by the inhibition property of alginate, which provides stability and adherence to surfaces to biofilms and contributes to antibiotic resistance [80]. Nevertheless, despite these promising results, complete biofilm eradication was not achieved within three days of treatment, likely due to the good stability of the liposome formula caused by elevated cholesterol amounts, which restricted the release of tobramycin to the necessary levels for full elimination [80]. Further research by Omri et al., (2002) tested the antibacterial effects of polymyxin B liposomes towards lung biofilms of P. aeruginosa in a rat model, with results indicating that the liposomal drug achieved more inhibition of biofilms, reducing the bacterial load compared to a lower reduction of the free form [81]. Additionally, the liposomal formulation led to five times increase in drug buildup in the pulmonary cells without being found in the blood or kidneys, indicating a reduction in the associated toxicity, particularly nephrotoxicity (Omri et al., 2002). Additional research corroborated these findings, showing that liposomal delivery of polymyxin B produced significantly better cell membrane permeability and biofilm-suppressing activity towards resistant P. aeruginosa [82].

5. Challenges and limitations

5.1 Manufacturing and scale-up challenges

Developing successful liposomal drugs remains challenging despite the availability of numerous liposomal therapies. Creating an efficient liposomal formula requires the simultaneous optimization of several elements [24]. Liposomes are manufactured in various strategies, such as injection, film and ultrasonic dispersion, freeze-drying, high-speed shearing, and extrusion [83]. A multiple-stage process complicates the quality control across batches, particularly for factors like particle size, size distribution, surface charge, and antibiotic release behaviour. One of the primary obstacles is the complexity of encapsulating antibiotics. [24]. Different studies have reported varying methods as most efficient for encapsulating specific antibiotics, but the use of different formulations to determine encapsulation efficiencies has introduced biases, leading to inaccurate outcomes [84].

Because laboratory-scale liposome preparation techniques are so intricate and costly, it isn't always feasible to scale up the procedures [85]. While various strategies exist for creating liposomes on a lab scale, there are very few techniques available that are used commercially to produce liposomes with the necessary essential characteristics [86][86][87]. Among these, ethanol injection with extrusion is the most widely utilised technique for parenteral liposome production on a large scale due to its reproducibility in terms of particle size and polydispersity index (PDI), as well as the preference for Ethanol, which involves

solvent diffusion, compared to chloroform, which involves solvent evaporation [89]. The kinetics and biological distribution of liposomes are influenced by particle size, impacting their efficacy, thus necessitating stringent regulation of particle size, which makes the extrusion a vital process [90].

Manufacturing liposomes at the industrial scale is lengthy and arduous, involving multiple unit activities and exhaustive related evaluation and assessment tests. The ordinary procedure includes buffer preparation, filtration, phospholipid solution preparation, lipid hydration, extrusion, diafiltration, dilution, sterile filtration, and final filling. Each step's in-process controls add to the overall process complexity. Quality control typically includes testing for filter integrity, measuring particle size and zeta potential, and controlling pH at crucial stages, phospholipid content assessment, pH, related compounds analysis, bulk drug product analysis, bioburden evaluation, and visual examination [91]. Further complexities, such as active loading in liposomal doxorubicin or freeze-drying after finishing the production, further complicate the process [92]. For instance, the ethanol injection technique, then extrusion of a large-scale liposome production method for either lipo- or hydrophilic medications, entails roughly nine-unit procedures. Each operation necessitates labor-intensive and time-consuming in-process quality control. Investigators have been committed to finding answers to those challenges, and some promising progress has been made. For example, techniques such as ethanol injection, membrane dispersion, and Shirasu porous glass membranes have been shown to facilitate vast liposome production and offer a broader selection of lipids in comparison with conventional methods [93].

5.2 Stability and storage issues

The challenging drawback of antibiotics carried by liposome formulation is the limited shelf life, which negatively affects its stability. Hydrolyzing susceptible bonds such as the ester-bond and oxidizing the unsaturated carbon tails are among the chemical instability issues. Besides, physical factors affect the stability of the liposomal formulation. [94]. These reactions can happen to synthetic and organic phospholipids, with the potential for peroxidation in unsaturated acyl chains, such as those found in egg and soybean phosphatidylcholine [87]. Natural-source phospholipids. Additionally, they display structural variation in their acyl chains, leading to variations in liposome stability and composition. The primary determinant of liposomal drug durability in vitro is lipid content, with temperature of storage being a crucial factor. Protection from oxidation could be achieved by the addition of antioxidants or using freeze-drying, and storage at low temperatures can help inhibit hydrolysis [95].

Physical instability includes drug leakage from lipid vesicles, particularly during the lipid phase transition, where membrane permeability and content leakage are highest. This issue is more pronounced in liquid than gel phases [96]. Liposomal drug stability is particularly problematic during in-vivo administration, where stability is generally minimal and highly dependent on interactions between liposomal membranes and elements found in bodily fluids [97]. Lipid transfer from the liposome membrane to blood lipoproteins can alter liposome characteristics and trigger drug release. This effect is especially noticeable in preparations of liposomes that contain short-chain lipids or fluid membranes, where content release occurs within minutes following IV injections. Adding cholesterol can enhance the stability and

fluidity of liposome vesicles, reducing in-vivo medication leakage, but this may also slow down drug release during treatment [98]. Additionally, the leakage of the active constituents from liposomes with an overall zeta potential in vivo is a limiting issue since the best antibacterial activity of liposomal drugs in vitro is detected with fluid liposomes or cationic liposomes [99][100].

Negatively charged lipids in the vesicles facilitate the binding of proteins in the serum with the surface of the liposomal vesicles, leading to good adsorption due to electrostatic attraction [101]. Conversely, positively charged liposomes do not attract bovine serum proteins [102]. Another issue related to the physical factors is the fusing and aggregating of the manufactured liposomes, which can alter the liposomal overall size and affect the drug's effectiveness in vivo [103].

Researchers have been actively working to address these restrictions, as well as, a few progresses have been made. For example, stabilizers like 2-morpho-linoethanesulfonic acid have been discovered to shield liposomes for a maximum of one year against phospholipid decomposition [104]. Hydrolysis and oxidation of phospholipids are usually mitigated by making neutral-pH liposome dispersions, along with adding antioxidants. [105]. Using fatty acids with long hydrocarbon chains and cholesterol can increase the stiffness of the two-layer design, raise the transition temperature, and inhibit premature cargo leakage. However, these measures could unintentionally reduce medication release during therapy. [106]. Reverse-evaporation can be used to optimize the liposome manufacturing process and increase the encapsulation efficiency for water-soluble medicines. However, this may increase toxicity since the finished liposome contains remaining organic solvents. [107]. Alternatively, freeze-drying can improve encapsulation but may cause size variations and cargo leaks, making it preferable to store liposomes in fluid form using capping agent to decrease aggregation and improve chemical and biological characteristics [108]. Finally, liposomal antibiotic formulations intended to be administered intravenously must meet sterilization specifications, which, because of the delicate nature of lipids, cannot be met through radiation, heating, or chemical methods. [109]. As a result, Industrial manufacturing frequently uses mechanical filtering though, it does not ensure that viruses are eliminated [107].

5.3 Regulatory and clinical hurdles

Liposomes have several constraints that need to be overcome before they can be effectively translated into clinical use. For example, cationic liposomes are generally harmful to human cells, as they can damage membrane integrity, leading to contraction of cells and vacuolization of cytoplasm and, at high levels, even cell lysis and necrosis [108][109][110]. This toxicity restricts their use in therapeutic settings in treating various bacterial infections, like those in the urinary system, lungs, and mouth. Conversely, anionic liposomes tend to have short circulation times in vivo because of their rapid elimination. [65]. Additionally, Liposomes face additional typical challenges, including inadequate solubility and stability (as their particles tend to fuse and aggregate), expensive production costs, challenges in scaling up for industrial production, and susceptibility to phospholipids hydrolysis and/or oxidation. [25].

Compared to unbound antibiotics, liposomes have A more intricate destiny, yet in vivo behavior is not fully understood. The interactions among the liposome,

antibiotic, substances and other auxiliary components such as stabilizers, surfactants, and ligands are still not well understood. [111]. For example, there is disagreement over the timing and mechanism of antibacterial release from liposomes in vivo. A thorough understanding of the physiological outcome of liposomes would not only benefit their design in scientific wise but also accelerate their clinical translation. [24].

To overcome physiological delivery barriers like the blood-brain barrier (BBB), functional substances like carbohydrates, proteins, and antibodies are frequently added to liposomes, enabling active targeting through ligand-receptor interactions [112]. Although preliminary investigation often yields promising results, there remains a notable discrepancy between preclinical findings and clinical implementations. Despite extensive studies on using liposomal formulation for active targeting, effective clinical implementation has not been achieved yet. [113]. Therefore, even with considerable advancements in liposome anticancer treatment, the optimization of multiple elements is necessary for liposomal delivery methods for antibiotics to attain an elevated therapeutic index [24].

Promising outcomes have been reported recently regarding the utilization of liposome-based delivery methods in treating both G-ve and G+ve bacteria, particularly strains like P. aeruginosa, E. coli, and S. aureus, which have been the focus of most studies. Nevertheless, research should also include additional clinically significant bacteria, such as Mycobacterium tuberculosis and Neisseria gonorrhoeae. There are still gaps in the data that need to be addressed. For example, many studies have only evaluated the liposomal compositions' antibacterial efficacy in-vitro, lacking in-vivo information. It is also important to determine whether the antibacterial effects observed in controlled environments would persist in real-world conditions, where factors such as temperature, humidity, the accessibility of nutrients, pH, the use of detergents, and contact with other microbes play a role [114]. Real-world scenarios are often more complex than laboratory conditions (Makhlouf et al., 2023) [29].

Moreover, most studies focus on single bacterial strains, but few evaluate the efficacy of liposomal preparations to combat several strains bacterial communities, which are common in environmental samples [115]. Even less research have looked into how liposomal compositions work in clinical cases or murine model [116]. While various research have assessed the biocompatibility of these systems, just a limited number have explored hemo-compatibility, systematic toxicity, and immune system interactions. Critical criteria for determining the clinical viability of using these combinations as antibacterial agents [118].

The process of encapsulating drugs significantly impacts the therapeutic effectiveness of liposomes. Liposomal formulas are beneficial just if they contain a sufficient drug concentration without excessive lipid content, as high lipid doses may be harmful and may affect the liposomal formulations pharmacokinetics [103].

6. Recent advances and Innovations

6.1 Nano liposomes and Targeted drug delivery

Nanoliposomes have materialized as an influential tool for targeted drug delivery, offering significant benefits of enhancing treatment efficacy and minimizing adverse events. By precisely directing drugs to the infection site or disease, nanoliposomes can

improve drug concentration at the target site, increase bactericidal efficacy, enhance drug uptake, and reduce the toxicity of antimicrobial agents. [119][120][121][122]. Active or passive performances delivering drugs with targeted liposomal delivery. Passive targeting relies on the enhanced permeability and retention (EPR) effect, allowing liposomal drugs to accumulate in tumors [123]. Active targeting involves modifying the liposome formula with directing molecules such as monoclonal antibodies, peptides, or glycoproteins to direct the drug to specific cells or tissues [124][125]. To further improve targeting and circulation time, the surface of conventional liposomes can be modified with water-soluble polymers like polyethene glycol (PEG), which offers shields towards biological interactions and reduces identification by the mononuclear phagocyte system. [126].

Active targeting of liposomal antibiotics shows encouraging results. For instance, rifampicin with specific ligands such as maleylated bovine serum albumin (MBSA) and O-steroyl amylopectin (O-SAP) have been developed for target release within infected pulmonary macrophages [127]. Similarly, liposomes containing isoniazid and rifampicin with an active-targeting substance like O-SAP have been explored for targeted drug delivery [128]. Several liposomes have seen successful commercialization, with 13 FDA-approved liposomes and treatments based on lipids currently available and more still being studied clinically [129]. Notably, DOXIL, the first FDA-approved Nano formulation, exemplifies the potential of targeted liposomal delivery with its PEG -coated, stealth design and superior loading efficacy, exhibiting significantly greater drug concentrations in cancerous tissues than in free DOX [130]. Antibodies, particularly of the IgG group, are well investigated as ligands for targeting liposomes to tumors, with transferrin receptors (TfR) and transferrin itself being effective in guiding liposomes to and within tumor cells through endocytosis [131][132][133]. These advances underscore the critical role of nanoliposomes in the evolving landscape of targeted drug delivery.

Table 3: Studies on PEGylated Liposomes Employed in Active Targeting

Targeting ligand	Medication	Outcomes	References
Anti-HER2 mAb	Doxorubicin	-Enhanced antitumor efficacy compared to nontargeted liposomes in vivo -Comparable accumulation of targeted and nontargeted liposomes in tumors in a nude mouse model with HER2-overexpressing cells	[134]
Folate	Doxorubicin	Significantly greater antitumor effect in mice with lung carcinoma when using folate ligand in PEGylated and masked folate-linked liposomes, compared to non-PEGylated liposomes, after intravenous administration.	[135]
EGFR	Doxorubicin, epirubicin, or vinorelbine	• Greater anticancer effects in vivo compared to nontargeted liposomes; • No change in tumour accumulation in MDA-MB-468 overexpressing EGFR-naked mice	[136]
Thiolated Herceptin	Paclitaxel	• Improved in vivo anticancer activity against BT-474 and increased in vitro cellular uptake	[137]
Anti-MT1-MMP antibody	Doxorubicin	• Enhanced absorption by HT1080 fibrosarcoma cells overexpressing MT1-MMP in vitro and more successful tumour growth suppression in vivo	[138]
Transferrin	Doxorubicin	Increased doxorubicin levels in tumours and lowered doxorubicin levels in the heart and kidneys of mice. • Increased intracellular absorption of entrapped doxorubicin by HepG2 cells.	[139]

6.2 Liposomal vaccines and immunotherapies

Liposomes have gained significant traction as versatile carriers in the manufacturing of vaccines, particularly in immunotherapies. Liposomes and liposome-derived nanovesicles, such as virosomes as well as archaeosomes, have become crucial components in the formulation of vaccines. Their ability to enhance immune responses and provide targeted delivery has led to a marked increase in interest for liposome-based vaccines [140].

One of the key advantages of using liposomes, archaeosomes, and virosomes in vaccine delivery systems is their versatility and plasticity. The components and formulation process can be tailored to achieve specific characteristics, including lipid choice, zeta potential, size distribution, and adjuvant or antigen entrapment and placement. This flexibility allows for the co-formulation of various kinds of adjuvants, enabling the design of liposomal vaccines customized for individual applications. [141].

Despite the potential of liposome-based vaccines, developing effective vaccines against cancer-associated antigens presents unique challenges. While most malignancies alter host proteins which have antigenic properties, the immunological reaction to these antigens is often weak, and cancer itself can induce immunosuppressive consequences. Tumor-associated antigens, such as viral proteins like HPV and overproduced proteins such as MUC1, HER2/neu and telomerase are targets for vaccine development. [142]. Table 4 lists few recent formulations of experimental vaccines based on liposome formulations.

Table 4: Instances of experimental vaccinations based on liposomes

Liposome type	Antigen	Adjuvant	Tumour model	Results	References
DC-chol/PC/pegDSPE/protamine small unilamellar vesicles (SUV) with surface-linked anti-CD20 (rituximab)	Bcl-2 antisense oligonucleotide (G3139) encapsulated within	Bcl-2-targeted antisense G3139 as an antisense therapy	Targeted B-cell selection in Raji B-cell lymphoma in NOD-SCID mice	Decreased G3139's harmful immunostimulatory effects. 80% survival as a result of Bcl-2 downregulation and TLR9-driven immunostimulation inhibition	[143]
Pegylated PC/cholesterol liposomes containing α-GC and octa-arginine-SA	Lipid antigen α-galactosyl ceramide (α-GC)	α-GC-mediated NK T cell activation with splenic targeting	B16 lung metastases of melanoma	Reduction of lung metastases by 65%	[144]
Nanoliposomes	Encapsulated tetanus toxoid helper peptide + tumour associated antigen ESO-1	Adjuvant coadministration of MAP-IFN-γ and palm-IL-1	Targeting Fcγ receptors on DCs in vitro	Maximum immunological response while using nanoliposomes as opposed to soluble antigens	[145]
Complex of DOTAP-PIC-liposomes	Hepa 1–6 cell lysates	polyriboinosinic: polyribocytidylic acid (poly I:C, PIC)	Hepa 1-6, subcutaneous	elevated IFNγ and CTL response specific to the tumour; significant tumour growth suppression with DOTAP-PIC + antigen	[146]

Hybrid liposomal systems

Hybrid systems were developed to optimize the conventional liposomes in pharmacokinetics and dynamics. While traditional liposomes offer significant advantages in terms of biocompatibility and drug encapsulation, they often confront difficulties such as inadequate chemical and physical stability, cargo loss while encapsulated, insensitivity to stimuli, and quick blood clearance. To overcome these limitations, integrating adaptable, useful biopolymers with liposomes has become an attractive strategy. [147].

Hybrid formulations of biopolymer and liposomes represent the 2nd version of liposomes. They combine the desirable characteristics of biodegradability with biocompatibility and are developed for use in various fields, including the food industry, cosmetics, and medications. [148]. Among the most often researched systems involves liposomes with surface modifications, where polymers are deposited or coated onto liposome surfaces. This surface modification can lead to modifications to the lipid chain sequence, fluidity of the membrane, surface electrical charge, structure, and size of the particles, resulting in improved physiological stability enhanced targeting at specific sites, and prolonged circulation time. [149][150].

The biopolymer-included liposome is a different type of hybrid formulation in which phospholipid bilayer permeability and fluidity are altered by inserting amphiphilic biopolymers, including N, N-dimethylhexadecyl carboxymethyl chitosan, within the bilayer [151]. Additionally, hydrophobic medications' therapeutic effects can be increased by adding cyclodextrin (CD)/guest inclusion complexes to the liposome vesicles' hydrophilic interior [152][153][154][155].

Beyond surface-modified and biopolymer-included liposomes, liposomes can also be contained in a variety of polymeric matrices, including films, hydrogels, and nanofibers. The encapsulated liposomes' functions and structural stability are maintained by these hybrid formulas, in addition to providing synergistic benefits by combining the advantages of 2 different delivery vehicles. Consequently, the range of liposome applications has expanded to cover areas of wound dressings, tissue remodeling, topical disease treatments, and active packaging. 156], [157][158], [159], [160].

Table 5: Biopolymer-incorporated liposomes to release different bioactive substances

Incorporated biopolymer	Active component	Liposome Formulation	Outcomes	References
DCMC	Curcumin	DCMC, soyLC	Compared to traditional liposomes, the hybrid liposomes showed improved storage stability, longer prolonged curcumin release, and increased curcumin bioavailability.	[161]
Casein	Curcumin, β-carotene	SoyLC	Curcumin and β-carotene were stabilised more effectively by casein-liposomes than by conventional liposomes or casein micelles.	[162]
β-CD	Curcumin	DPPC, cholesterol	Comparing amino-modified β-CD complexes with traditional liposomes, the encapsulation efficiency of the former into pH gradient-performed liposomes had been over five times higher.	[137]
β-CD	Lycopene	SoyLC, cholesterol	The improved formula showed strong cardio-protective effects and maintained lycopene release to a maximum 49.6% in 12 hours.	[163]
γ-CD	Resveratrol	DPPC, cholesterol	In contrast to traditional liposomes and free resveratrol, which have a drug release of around 50%, the liposomal formula demonstrated full resveratrol release within 24 hours.	[164]
β-CD	Curcumin	EYPC, cholesterol	The double-loaded liposomes were a potential delivery system for improving curcumin's stability and effectiveness of entrapment without compromising the liposomal bilayer's integrity.	[165]

7. Case studies and clinical trials

7.1 Review of successful clinical trials

This section reviews various successful clinical trials that have explored the effectiveness of liposomal formulations as antibiotic carriers in treating bacterial infections. Initial findings by Peyman et al. (1988) [166] documented the successful use of liposomes to treat persistent intraocular inflammatory conditions treated with one intravitreal dosage of several medications. Following these initial successes, Phase I/II clinical studies began to investigate liposomal antimicrobials. Previous studies explored the use of gentamicin carried by liposomes in treating Mycobacterium avium, or Mycobacterium intracellular complex (MAC), bacteremia in patients with HIV. Gentamicin enclosed in typical large unilamellar vesicles (LUVs) showed reduced bacterial colony counts in blood samples, though no complete eradication was achieved. Notably, liposomal gentamicin did not induce bacterial resistance and had limited nephrotoxicity. The studies concluded that while liposomal gentamicin was effective, further research is needed to optimize treatment duration, dosing, and combination therapy. [167] [168]

Other studies investigated liposomal aminoglycosides, finding them useful only when combined with other drugs. In phase

II trials, MiKasome® (liposomal amikacin) demonstrated significantly greater accumulation in serum and sputum compared to free amikacin, with improved pharmacokinetics and reduced toxicity. However, despite these benefits, the elimination of Mycobacterium tuberculosis was not observed, and further investigation into dosing and early bactericidal activity was recommended. [169] [170]

The efficacy of MiKasome® in treating pulmonary tuberculosis was further tested in South Africa [171]. While the liposomal formulation did not exhibit early bactericidal activity, it was noted that liposomal amikacin's performance was hindered by the extracellular presence of mycobacteria and an acidic microenvironment. Krieger et al. (1999) [172] align with other studies that found that MiKasome® was effective for urinary tract infections, although its development was halted following NeXstar Pharmaceuticals' acquisition by Gilead Company.

These clinical trials illustrate the promise of liposomal antibiotic formulations in enhancing treatment outcomes, though continued research is essential to address existing challenges and optimize therapeutic strategies.

7.2 Case studies demonstrating efficacy

The following case studies highlight recent research on the effectiveness of liposomal formulations in combating bacterial infections. Buzia et al. (2020) [177] conducted microbiological research to evaluate 3 formulations containing benzoyl peroxide & tretinoin encapsulated within liposomes. These formulations were investigated on G+ve bacteria, G-ve bacteria, and some fungal strains. The ointment showed the most potent antibacterial efficacy, particularly against G-ve and G+ve bacteria, both for control bacterial strains and strains isolated from patients with acne or infected supradermatosis. Among the tested formulations, the 0.05% tretinoin liposomal gel achieved the best results in a case study involving 10 participants with varying acne levels [178].

Perrie et al. (2013) [179] aimed at assessing positively charged liposomes as adjuvants for vaccines and determining their physico-chemical properties that enhance efficiency of vaccines, particularly through depot formation at the injection site and subsequent immunological reaction. The study verified that trehalose 6,6'-dibehenate and dimethyldioctadecylammonium bromide-based positive liposomes produced efficient depots at the point of injection, which stimulated and promoted robust immunological cellular and humoral responses. Key elements contributing to this effect included a strong cationic charge, the antigen's electrostatic attachment to the liposome, and the formation of stiff bilayer vesicles by using lipids that have elevated transition temperature. While reducing rhe vesicle dimensions did not improve drainage from the site of injection, decreasing the cationic charge by substituting the cationic lipid with a neutral lipid or PEGylation lowered the development of depots as a result and diminished Th1- immunological reactions, though Th2-type reactions was less affected. Those findings underscore the importance of physicochemical properties in modulating immunological reactions for particulate- adjuvants [180].

7.3 Comparative analysis with conventional therapies

The effectiveness of liposomal formulations in comparison to conventional antibiotic therapies has been a focus of

recent research. Rukholm et al. (2006) [180] conducted a study comparing the bactericidal activity of free and liposomal formulation of gentamicin towards P. aeruginosa strains. The liposomes, composed of DMPC and cholesterol in a 2:1 ratio, had an average size of 426.24 ± 13.55 nm and an encapsulation efficiency of 4.52 ± 0.53%. Notably, the isolated strain that was highly resistant to gentamicin displayed a significant reduction in the minimum inhibitory concentration (MIC) from 511 mg/L for the free gentamicin to just 33 mg/L for the liposome. The researchers suggested that the potential merging of liposomes with P. aeruginosa's outer cell membrane was the cause of this significant variation, which likely enhanced the antibiotic's penetration. Additionally, the time-kill studies showed that liposomal gentamicin at 1, 2, or 4 times the MIC either matched or outperformed the free gentamicin. For example, liposomal gentamicin at four times the MIC eradicated bacteria completely within 6 hours, compared to 24 hours for the free form. The study indicated that the nanoliposome formula offered better killing time and stronger antibiotic efficacy when used towards *P. aeruginosa* [180].

In another study, Halwani et al. (2008) [124] explored the use of liposomes as a drug delivery system for co-administering two substances to stop the development of biofilms and bacterial resistance. They developed a liposomal complex comprising gallium and gentamicin to enhance the efficacy of gentamicin against P. aeruginosa. Gallium has been shown to effectively inhibit *P. aeruginosa* growth and biofilm formation. The study revealed a significant difference in MICs and minimum bactericidal concentrations (MBCs) between liposomal complex and free antibiotic as opposed to significantly higher antibiotic level for the antibiotic-resistant PA-48913 pseudomonas strain. When comparing the lowest concentration at which conventional medications can eradicate biofilms to liposomal complex, the latter was the only formulation that completely eradicated biofilms and blocked quorum sensing molecules at extremely low concentrations. The toxicity profile of gallium was also assessed using A549 lung epithelial cells. The researchers noted that the toxicity assessment of gallium was significantly altered when encapsulated in liposomes, resulting in increased cell viability compared to free gallium. This novel liposome system with a particle size of 336 ± 34 nm, demonstrated the potential to optimize gentamicin delivery while minimizing gallium toxicity, making it a potential strategy to eradicate antibiotic-resistant *P. aeruginosa* in both planktonic and biofilm states [124].

Additionally, another study developed a liposomal linolenic acid nanoformulas and examined its bactericidal efficacy against *H. pylori* strains that were resistant to antibiotics [178]. The liposomes, prepared using techniques for needle extrusion and sonication with egg phosphatidylcholine and cholesterol, had an average diameter (89 ± 32) nm and a surface charge (−79 ± 42) mV. The liposomal system showed similar antimicrobial efficacy to free linolenic acid in suppressing spiral and coccoid *H. pylori* types. Moreover, the liposomal system was able to eliminate 7 isolated *H. pylori* strains and a metronidazole-resistant *H. pylori* strain. In a laboratory setting, no drug resistance was observed with the liposomal system at various below bactericidal levels, whereas fast emergence of medication resistance developed using both metronidazole and free linolenic acid. This new formula represents a potentially

effective antibacterial nano formula for treating *H. pylori* strains resistant to antibiotics [178].

8- Future Prospects and Research Directions

8.1 Emerging Trends in Liposomal Antibacterial Applications

Liposomes were recently developed for targeting biofilm [181]. Most microorganisms biosynthesize polymeric matrix to attach to the cell surface and enclosed in a matrix of primarily polysaccharide material. [182]. The main restriction for effective activity against microbes is the biofilm formation that increases the resistance despite increasing the concentration of the therapeutic agent. Delivering these agents via nanoliposomes improves their delivery to the target bacterial cells in biofilms.

Conclusion

The growth burden of antibiotic resistance, along with the increasing threat of the emergence of health pandemics, the risky consequences of untreated infections, and the overwhelmed health economy are all factors to consider by researchers and scientists to warranty the discovery of new antimicrobial agents or to enhance the activity of the available conventional medicines. Nano-liposomal systems were developed as a novel delivery system that ensures the delivery of potent antimicrobial agents in a reduced dose, lower cost, increased safety regimen, higher selectivity, targetting the active site even in active or passive way even in the presence or absence of site directing ligands providing better overall efficacy.

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