



Exploring the promises and challenges of cochleates for drug delivery applications

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Abstract

Background Lipid-based drug delivery systems are widely used to enhance the bioavailability of various pharmaceutical agents. They offer several advantages, including biocompatibility, biodegradability, and low toxicity. However, they are often limited by formulation leakage, oxidation, hydrolysis, and short half-life.

Area covered This review introduces the components of cochleates, mechanisms underlying their formation and absorption, and diverse preparation methods, along with their advantages and disadvantages. Furthermore, it discusses various analytical techniques for characterizing cochleates formulations and their advantages. A comprehensive review of publications on cochleates from the last decade is provided.

Expert opinion Cochleates have the potential to overcome the inherent limitations of lipid-based drug delivery systems, such as susceptibility to oxidation and degradation by digestive enzymes. They offer several advantageous properties, such as enhanced cellular uptake and permeation, improved bioavailability, and low toxicity. In particular, the potential for oral administration and cancer-targeted accumulation make cochleates promising for drug delivery applications. Despite these advantages, challenges like difficulties in optimizing its composition, physical aggregation, and high costs must be addressed. Also, the lack of approved drugs based on this technology is a major limitation.

Keywords Cochleates · Lipid-based formulation · Lipid-based drug delivery system · Absorption enhancement · Bioavailability

Introduction

About 40% of marketed drugs and around 90% of drugs in development encounter challenges related to low aqueous solubility, which poses a significant hurdle in delivering them to target sites in the body (Loftsson and Brewster 2010). For drugs with poor solubility, dissolution is the primary bottleneck to bioavailability, and even a marginal

improvement in the dissolution rate can notably enhance this property. The effectiveness of formulations is closely associated with the rate and extent of drug absorption, particularly for compounds classified as Class II under the Biopharmaceutical Classification System (Pehlivanov 2019; Yoo and Yang 2024). In recent years, lipid-based drug delivery systems (e.g. micelles, liposomes, dendrimers, nanocapsules, and nanospheres) have garnered significant attention for their ability to enhance the solubility and bioavailability of poorly soluble drugs (Ro et al. 2023; Bae et al. 2024; Jeong et al. 2024). These systems are recognized for their biocompatibility, biodegradability, non-toxicity, flexibility, and non-immunogenicity, making them suitable for both systemic and non-systemic administration routes. Despite these advantageous properties, their utility is often limited by the oxidation and hydrolysis of phospholipids, which can result in a short half-life, reduced solubility, and compromised mechanical stability due to formulation leakage and membrane fusion issues (Pawar et al. 2015).

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Cochleates have been developed as an alternative to lipid-based drug delivery systems, and they display several advantages compared to conventional liposomes when orally administered (Li et al. 2015). Cochleates are formed through the cation-induced precipitation of negatively charged liposomes, constituting spiral multilayered structures interconnected by a cationic bridge. This distinctive formulation was discovered by Papahadjopoulos and colleagues during their efforts to obtain negatively charged, large unilamellar vesicles. The term “cochleates” is derived from the Greek word for “snail with a spiral shell”, reflecting the cylindrical folding pattern observed in the respective formulation (Papahadjopoulos et al. 1975).

The objective of this review is to examine the characteristics and applications of cochleate, a novel drug delivery system that addresses the limitations of conventional lipid-based formulations. We present an overview of the components, formation, and absorption mechanisms of cochleate, along with an analysis of their manufacturing methods and their associated advantages and disadvantages. Additionally, we discuss analytical methods for cochleate characterization.

Composition and properties of cochleates

Types of drugs encapsulated in cochleates

Numerous studies have reported the successful encapsulation of vaccines, antibiotics, anticancer drugs, and various other therapeutics in cochleate (Lipa-Castro et al. 2021). Hydrophobic, amphiphilic, and negatively or positively charged drugs can be incorporated into cochleate for delivery applications. The location of the encapsulated drug within cochleate is determined by the physicochemical properties of the given drug (Zarif et al. 2000).

Negatively charged phospholipids

Cochleates are composed of a purified soy-based phospholipid with approximately 75% lipid by weight at a minimum. This lipid can consist of phosphatidylserine (PS), dioleoyl phosphatidylserine (DOPS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylglycerol (PG), and/or a combination of these lipids with others. The head groups of these phospholipids display negative charges at physiological pH. Alternatively, the lipid composition may include phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), dioleoyl phosphatidic acid (DOPA), distearoyl phosphatidylserine (DSPS), dimyristoyl phosphatidylserine (DMPS), and dipalmitoyl phosphatidylglycerol (DPPG) (Sankar and Reddy 2010). Mixtures

containing predominantly negatively charged phospholipids (e.g. a PS/PC mixture with over 50% PS content) can also generate cochleates, although a higher concentration of bridging agents, such as Ca^{2+} , is essential (Li et al. 2015).

Nagarsekar et al observed that the saturation and the chain length of PS fatty acids exert a significant influence on the final morphology of cochleates. Despite the variation in lipid acyl chains, the intermediate structures observed during the formation of cochleates from all phosphatidylserines exhibited high similarities. Furthermore, it was observed that the absence of stack formation below the lipid transition temperature impeded the formation of cochleate structures (Nagarsekar et al. 2016).

Metal cations as bridging agents

Various divalent cations, including Ca^{2+} , Mg^{2+} , Ba^{2+} , and Zn^{2+} , can be used as bridging agents for cochleate preparation. Among these ions, Ca^{2+} has been identified to be the most effective (Landge et al. 2013). Cochleates bridged by Ca^{2+} display smaller dimensions and a higher degree of dehydration than those formed with Mg^{2+} . Additionally, Ca^{2+} -bridged cochleates display more compact and highly ordered structures, thereby considerably lowering the ion concentration needed for bridging (Flach and Mendelsohn 1993). Notably, a synergistic effect between Ca^{2+} and Mg^{2+} has been noted, where Mg^{2+} facilitates aggregation and close apposition of membranes to reduce the Ca^{2+} concentration needed for fusion (Düzgünes et al. 1981). Several researchers have also reported the utilization of biologically active molecules, including tobramycin, polylysine, and oligo-acyl-lysyls, as bridging agents in place of metal cations. This innovative approach has been demonstrated to enable the integration of encapsulation and stabilize cochleates (Syed et al. 2008; Sarig et al. 2011).

Aggregation inhibitor

The presence of Ca^{2+} ions is crucial for the creation and maintenance of the cochleate structure. However, it also induces particle aggregation, which can complicate the separation from by-products. This hinders the evaluation and monitoring of cochleate formation, limits surface modification and functionalization, and impedes the production of well-dispersed particles (Nagarsekar et al. 2017). To address this issue, a patent has recently proposed incorporating milk, casein, albumin, or methylcellulose into the formulation. Various other polymeric materials, such as celluloses, gums, and starches have been proposed as potential aggregation inhibitors, but their effectiveness remains uncertain. The aggregation inhibitors are introduced to both prior to

and after the formation of the cochleates (Mannino et al. 2005).

More recently, it has been revealed that biocompatible low-molecular-weight citric acid can effectively disperse and stabilize cochleates. Citrate is capable of rapidly removing Ca^{2+} from the cochleate surface, which in turn disrupts calcium-dependent cross-linking between particles. Conversely, the extraction of Ca^{2+} bounded in membrane layers is a slower process. Consequently, the removal of surplus citrate following the disassembly of aggregates serves to prevent the curling of the cochleate. Following the removal of the excess citrate, the cochleates retained its structure. While precipitation occurred over time, it was still able to disperse effectively with stirring after a month of storage at 25 ± 1 °C. The dispersing effect of citrate was found to be concentration dependent. At a concentration of 6.2 mM for both DOPS and Ca^{2+} , the optimal citrate concentration range was identified as 200–212.5 mM. At concentrations below this range, the aggregates only partially disintegrated, while concentrations above this range resulted in the transformation of the cochleate into giant vesicles. Therefore, there is a relatively narrow concentration range that acts as an efficient dispersant of cochleate aggregates (Bozó et al. 2017).

Cochleates formation mechanism

Cochleates display an elongated tubular shape and cylindrical morphology derived from small unilamellar vesicles. This shape originates from the interaction between negatively charged liposomes and divalent cations, which initiates dehydration and self-assembly. This is followed by a spontaneous arrangement, resulting in a complex system resembling the shape of a cigar (Nagarsekar et al. 2016). During this process, the proximity of bilayers is dependent on the degree of dehydration of phospholipid head groups. The bilayers roll up to minimize their interaction with water (Santangelo et al. 2000), containing only minimal amounts of water. Under conditions of a 2:1 molar ratio of PS to Ca^{2+} , the lipid bilayers in the cochleates were arranged with a repeat distance of 54 Å (Papahadjopoulos et al. 1975). Cochleates are typically formed from smaller liposomes

rather than larger ones (Zarif 2002). Overall, they comprise three components: the lipid bilayers, the cations, and the therapeutic agent to be delivered (Fig. 1).

While cochleates are commonly characterized as nanostructures with a cigar-shaped appearance, other morphologies have been identified depending on the specific phospholipid and bridging agent employed. Spherical structures have been observed with soya PS, while planar sheets have been noted for palmitoyl-oleoyl phosphatidylserine (POPS) (Marone et al. 2005). In addition, when polylysine is used as a bridging agent, cochleates form a branched, fibrous-like structure (Syed et al. 2008).

Cochleate absorption mechanism

When administered orally, cochleates are absorbed in the intestine, cross the intestinal epithelial barrier, and enter the bloodstream. For routes other than the intravenous route, once the formulation enters the circulation, it is subsequently delivered to the target cells by endocytosis (Pawar et al. 2015). Two hypotheses have been proposed to explain this phenomenon.

First, it has been hypothesized that the elevated tension at the bilayer edges of cochleates drive their interaction with the tissue membrane (Fig. 2A) (Lundbæk et al. 1997). Perturbations induced by calcium in membranes containing negatively charged lipids, along with subsequent membrane fusion events, mediate a crucial mechanism in various natural membrane fusion processes (Papahadjopoulos et al. 1990). Consequently, the calcium-rich and highly ordered membrane of cochleates closely resembles a natural membrane, leading to perturbation and reordering of the cell membrane. This fusion event occurs between the outer layers of cochleates and the membranes of target cells, with the edges of cochleates potentially serving as the initiation site. This process results in the delivery of small quantities of the materials encapsulated in cochleates, or “encocheated,” into the cytoplasm of the target cells. Subsequently, these cochleates may detach from the subjected cells, making themselves available for another fusion event, either with the same cells or different ones (Gould-Fogerite et al. 1998). The fusion of cochleates with cell membranes has

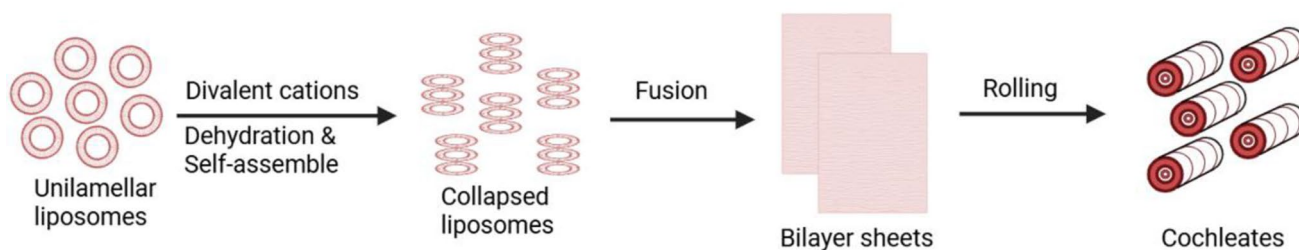


Fig. 1 Schematic illustration of cochleates formation

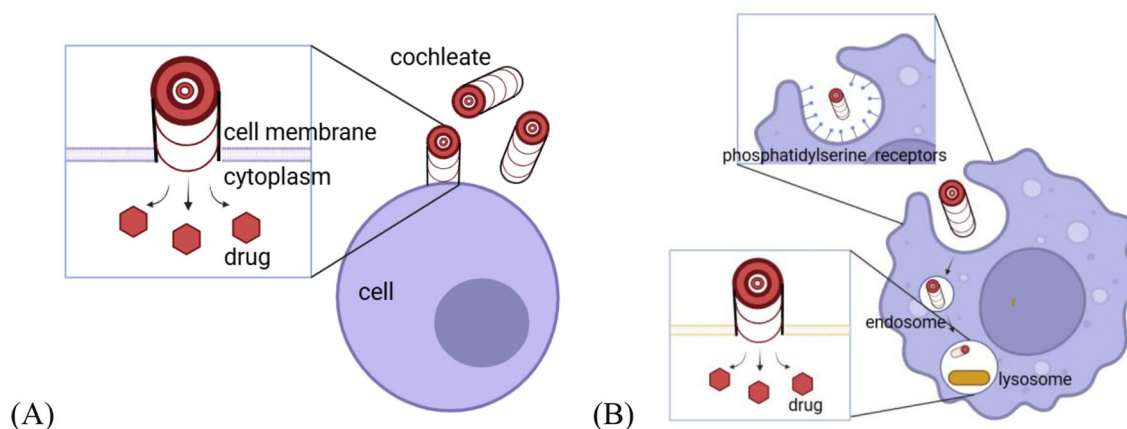


Fig. 2 Schematic illustration of the cell penetration mechanism of cochleates. **(A)** Cell membrane fusion and **(B)** phagocytosis

been confirmed through a bactericidal activity assay tobramycin cochleate, which functions by inhibiting intracellular ribosomes (Syed et al. 2008).

The second hypothesis is phagocytosis (Fig. 2B). Cochleates can specifically target macrophages, which possess membrane PS receptors that mediate phagocytosis. This process facilitates the delivery of encocleated materials into the cytosol of the target cells. Particularly for actively phagocytic cells, cochleates may be endocytosed and subsequently fuse with the membranes of endocytic vesicles (Vijeta et al. 2011). Asprea et al. (2019) reported the encapsulation of fluorescein isothiocyanate into cochleates and demonstrated that this formulation could be phagocytosed by murine macrophages (J774a.1) via confocal microscopy.

Cochleate preparation methods

Several strategies have been developed for cochleate preparation to furnish specific structures and sizes for different applications. The development of these strategies began in the early 2000s (Jin et al. 2000). The conventional approaches for preparing cochleates involve trapping and dialysis methods. These techniques are recognized for generating cochleate structures with large and diverse particle sizes, primarily due to the uncontrolled aggregation of lipid sheets. On the other hand, techniques like hydrogel isolation (Zarif et al. 2003), emulsion-lyophilization (Wang et al. 2014), and microfluidics-based micro-spherical cochleate composites (Nagarsekar et al. 2017) have been developed for application-oriented approaches, enabling the production of cochleates in the nano- and sub-micrometer size ranges. A schematic illustration of each method is shown in Fig. 3, and their advantages and disadvantages are summarized in Table 1.

Trapping method

The trapping method involves adding a calcium chloride solution dropwise to a diluted liposome suspension while stirring (Kristensen et al. 2023). Certain modifications have been introduced to this method. For example, the solvent drip method has been shown to enhance the efficiency of drug loading in cochleates (Mannino et al. 2014). Additionally, modifications like pH elevation through simple mixing of excipients and drugs, as well as the film hydration method, have been introduced to enhance drug loading efficiency (Zarif 2005).

Dialysis method

There are different dialysis methods. Firstly, the “LC (liposomes before cochleates) dialysis method” initially removes detergents from a mixture of lipids and detergents through dialysis, thereby forming liposomes. These liposomes are then further dialyzed against a calcium chloride solution to transform them into cochleates.

Secondly, the “DC (direct calcium) dialysis method” involves removing detergents from a solution containing lipids and materials to be encocleated by dialyzing against a buffer containing calcium. Thus, the removal of detergents and the introduction of calcium occur simultaneously, unlike the sequential introduction used in the LC method (Gould-Fogerite et al. Gould-Fogerite and Mannino 1997). This process leads to the formation of needle-shaped structures, which exhibit larger diameters and lengths compared to the structures obtained through the LC dialysis method (Gould-Fogerite et al. 2000).

In cochleates vaccines containing proteins or peptides, the biological activity of the proteins must be maintained. This is achieved through the use of extraction buffers containing detergents that do not destroy them. In such instances, ionic detergents such as cholate salts, deoxycholate salts,

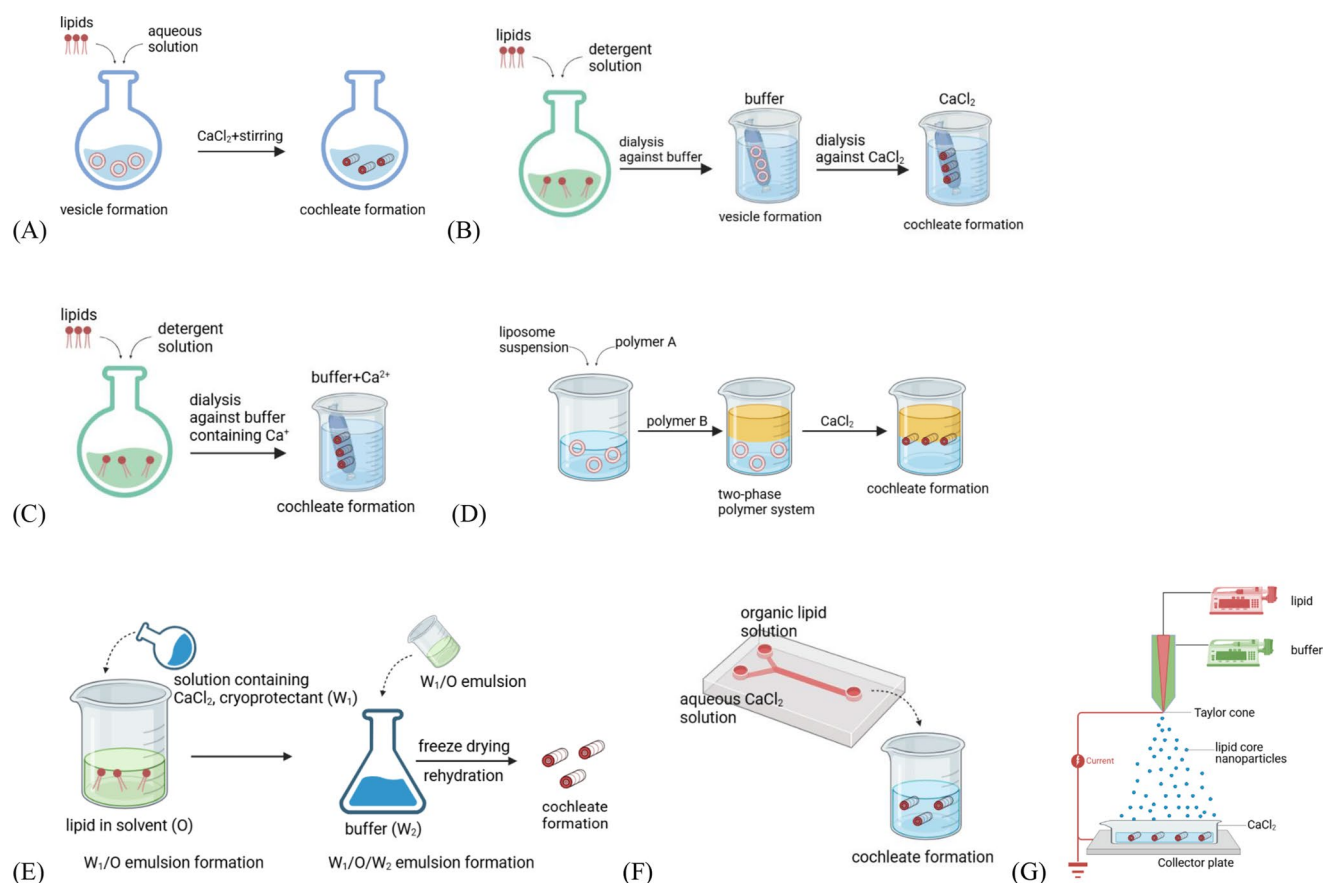


Fig. 3 Cochleates preparation methods: **(A)** trapping method, **(B)** LC (liposomes before cochleates) dialysis method, **(C)** DC (direct calcium) dialysis method, **(D)** hydrogel method, **(E)** emulsion-lyophilization method, **(F)** microfluidic method, and **(G)** EHDA (electrohydrodynamic atomization) method

Table 1 Advantages and disadvantages of manufacturing methods

Method	Advantages	Disadvantages
Trapping and dialysis methods	- fast and easy	- particle aggregation - variable and large particle sizes
Hydrogel and Emulsion-lyophilization methods	- sizes in sub-micrometer range	- multi-step process (complex and time-consuming) - difficult to scale up - potential regulatory issues - broad particle size distributions
Microfluidic method	- reduced random particle aggregation - monodisperse microparticulate system - one-step; reproducibility - easy adaptation for large-scale production	- microfluidic device needed
EHDA method	- one-step; reproducibility - easy adaptation for large-scale production	- electrospray device needed

tion method, **(F)** microfluidic method, and **(G)** EHDA (electrohydrodynamic atomization) method

or nonionic variants containing polyoxyethylene or sugar head groups are employed. Heterogeneous polyoxyethylene detergents like Tween, Brij, or Triton are also commonly used. Among these detergents, the nonionic variants with sugar head groups, such as beta-D-octyl-glucopyranoside, are found to be the most suitable for the maintaining of biological activity of the encochleated proteins (Gould-Fogerite and Mannino 1997).

Hydrogel method

The hydrogel method involves initially mixing a liposome suspension with polymer A (e.g., dextran, polyethylene glycol, or others). This mixture is then added to polymer B (e.g., polyvinylpyrrolidone, polyvinyl alcohol, Ficoll, polyvinyl methyl ether, polyethylene glycol, or others), in which polymer A is non-miscible, resulting in an aqueous two-phase system. Subsequently, a solution of cation salt is introduced into this system, facilitating the diffusion of the cation into the polymer B phase and then into the particles composed of liposome/polymer A, leading to the formation of small-sized cochleates. The polymers are washed out at

the end. This method yields a narrower size distribution compared to the trapping method, with particle sizes typically below 1 μm (Zarif et al. 2003).

Emulsion-lyophilization method

The emulsion-lyophilization method involves multiple emulsions. In brief, the lipids are dissolved in a solvent that serves as the oil phase (O). The inner water phase (W_1) consists of a solution containing a binding agent and a cryoprotectant, while the outer water phase (W_2) is a buffer. The $W_1/O/W_2$ emulsion is subjected to lyophilization, resulting in a dried powder that forms nano-sized cochleates upon rehydration (Wang et al. 2014).

Microfluidic method

Microfluidic method involves disposable syringes attached to the two inlet streams of the microfluidic chip, serving as the reservoirs for the reactor solutions, namely the organic lipid solution and aqueous CaCl_2 . Syringe pumps are employed to precisely control the flow rate and the ratio of the two solvent phases delivered by the two inlet streams. The resulting formulations are then collected from the outlet stream into a tube (Nagarsekar et al. 2017).

Nanoprecipitation is an effective method due to its ability to control the size, shape, and encapsulation efficiency of the final product by adjusting process parameters, such as solvent ratio, flow rate and lipid concentration. To precisely regulate these parameters, a microfluidic reactor can be utilized. This method yields spherical composite microparticles with diameters ranging from 3 to 5 μm .

The impact of the aqueous-to-organic phase ratio on the formation of cochleates was investigated. It was found that an increase of ethanol content is crucial to produce small-sized cochleates. Additionally, the presence of water was identified as a vital factor in enabling the bilayer to curl into nano- cochleate cylinders.

The impact of varying flow rates on particle formation was observed. It was found that lower flow rates resulted in the formation of particles that were typically aggregated and flocculated during the middle stages of cochleate formation. In contrast, higher flow rates led to the production of particles that were significantly more spherical and regular in shape compared to those formed at lower flow rates. However, when the flow rate was excessively high, incomplete particle formation was observed, as the aggregates did not have sufficient time to fully develop a structure.

The effect of lipid concentration on morphology was also investigated. It was found that as lipid concentration decreased, morphology became more regular and uniform in size. Higher lipid concentrations resulted in many

nucleates with small inter-particle distances. This high density of nucleates may cause aggregation before the ‘stack to cochleates’ transformation is complete. Therefore, in the intermediate phase of these particles, cochleates formation or curling of the stack sometimes appears to be interrupted.

EHDA (Electrohydrodynamic atomization) method

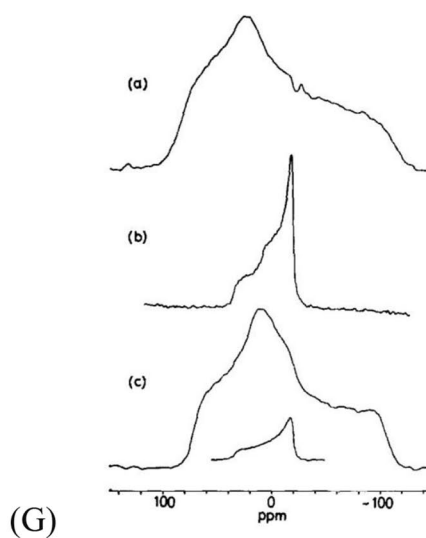
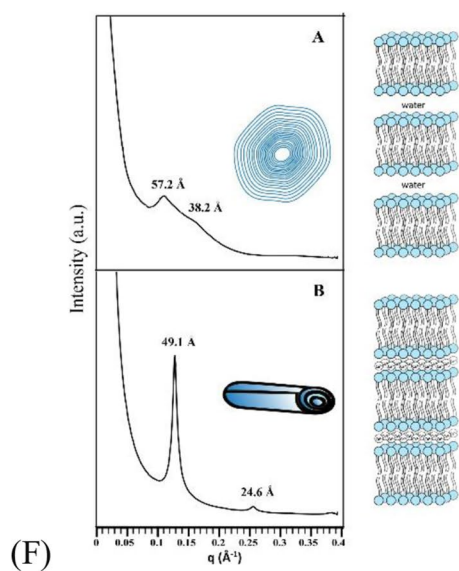
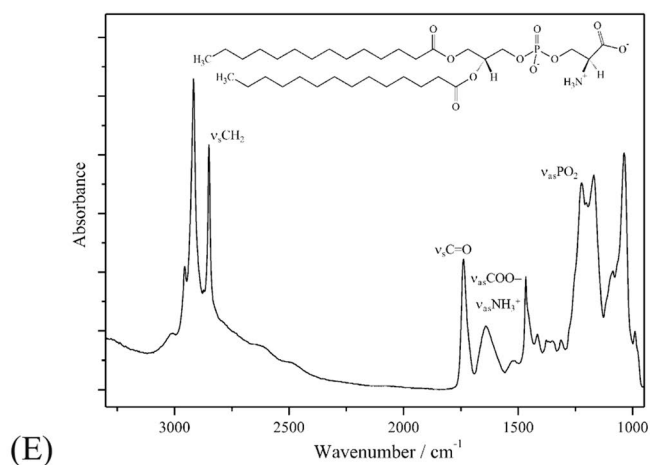
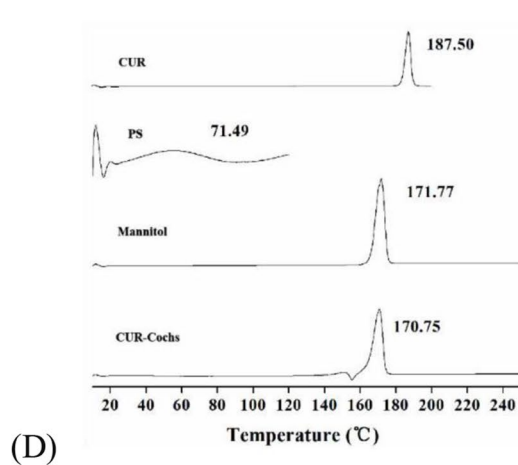
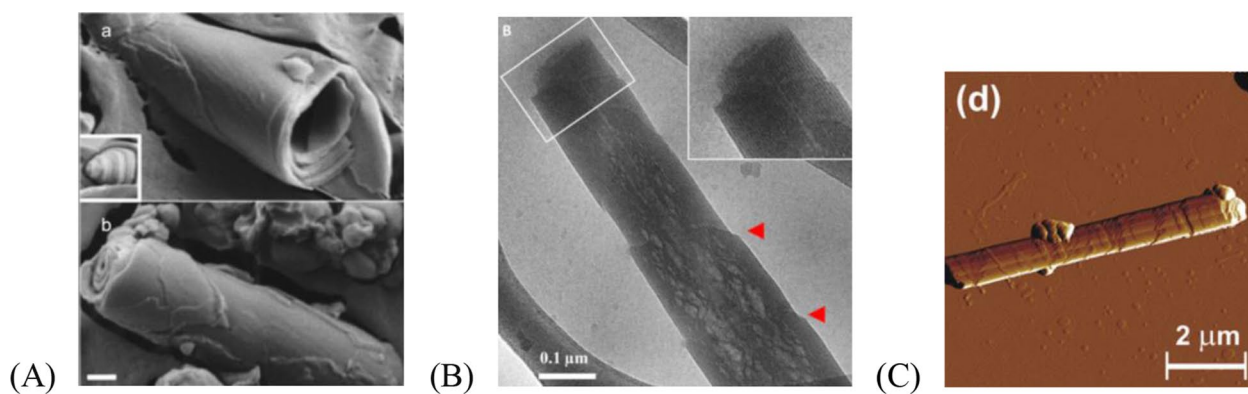
In coaxial electrohydrodynamic atomization, a coaxial nozzle equipped with multiple needles of varying diameters simultaneously dispenses liquids under a high electric potential. The electric field induces a surface charge on the liquid and transforms it into a mist-like form.

The lipids, cholesterol, and the drug to be encapsulated are initially dissolved in an appropriate solvent and loaded into a syringe positioned in the inner section of a coaxial needle/nozzle. The outer section of the needle contains another syringe filled with a compatible aqueous solution. Both liquids are propelled through the coaxial needle, interacting at its tip. Subsequently, they are sprayed into a bath containing a cation solution, such as CaCl_2 , to form cochleates (Jain et al. 2023).

Cochleate characterization techniques

Microscopy

Cochleate morphologies can be evaluated by microscopy. While light microscopy can characterize the overall shape, its resolution is often insufficient to distinguish between cochleates, tubules, or other elongated structures. Therefore, detailed investigations using electron microscopy should be conducted following preliminary screening with light microscopy. Conventional electron microscopy techniques like transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) have been widely utilized to investigate the structural characteristics of cochleates. Among these techniques, the freeze-fracture TEM technique is considered particularly advantageous for assessing morphology of cochleates (Zarif 2005). For SEM and TEM, cochleates are deposited onto glass slides, air-dried, and sputter-coated before imaging. Despite their presumed stability, such dehydration could potentially alter delicate structural features. In this regard, cryo-SEM and cryo-TEM can visualize native structures by preserving the samples in a hydrated condition and maintaining them close to their original bulk state through thermal fixation (Fig. 4A, B) (Hollander and Danino 2015). AFM has been utilized to assess the topological features and surface-hidden structural details of cochleates, revealing their nanomechanical properties and significant resilience (Fig. 4C) (Bozo et al. 2015).



Differential scanning calorimetry (DSC)

DSC has been utilized to monitor crystallization events during the complexation of lipids and binding agents, as well as to investigate the lipid phase, melting, and recrystallization behaviors of drug delivery systems. This technique measures

the heat flow in a sample as it undergoes a controlled temperature change. Pure phospholipids typically exhibit a distinct phase transition from the solid state to the liquid state of acyl chains at a specific temperature, known as the transition temperature. The addition of a binding agent to

Fig. 4 Cochleates characterization: **(A)** Cryo-SEM images of DOPS with CaCl_2 . Hollow and full cochleates (Bar=200nm). **(B)** Cryo-TEM images of DOPS with CaCl_2 . Multilamellar structure (white box) and several wrapping folded layers (red arrowheads) are shown. (A) and (B) were adapted from (Hollander et al. 2015) with permission from Elsevier through the copyright clearance center. **(C)** AFM images of DOPS with CaCl_2 was adapted from (Bozo et al. 2015) with permission from Elsevier through the copyright clearance center. **(D)** DSC diffraction patterns of different samples was adapted from (Chen et al. 2022) with permission from MDPI. **(E)** FT-IR ATR spectrum of hydrated film of DMPS in pH 7.4 HEPES buffer at 20 °C was adapted from (Manrique-Moreno et al. 2016) with permission from Elsevier through the copyright clearance center. **(F)** SAXS patterns of MLV and cochleates formed from soy phosphatidylserine was adapted from (Lipa-Castro et al. 2021) with permission from Elsevier through the copyright clearance center, and **(G)** 81.0 MHz ^{31}P NMR spectra at 30 °C of (a) anhydrous PS, (b) hydrated PS, and (c) hydrated PS with Ca^{2+} added was adapted from (Hope et al. 1980) with permission from Elsevier through the copyright clearance center. SEM, scanning electron microscopy; DOPS, dioleoyl phosphatidylserine; TEM, transmission electron microscopy; AFM, atomic force microscopy; DSC, differential scanning calorimetry; FT-IR ATR, Fourier transform infrared attenuated total reflectance; DMPS, dimyristoyl phosphatidylserine; HEPES, hydroxyethyl piperazine ethane sulfonic acid; SAXS, small-angle X-ray scattering; MLV, multilamellar vesicles; NMR, nuclear magnetic resonance; PS, phosphatidylserine

lipid dispersions increases the transition temperature (Portis et al. 1979).

DSC can also be used to investigate the interactions between cochleates and encapsulated drugs. The melting characteristics of drugs typically change when they are encapsulated within cochleates, and this can be an indicator of the interactions between drugs and phospholipid bilayers. Recently Chen et al. (2022) utilized DSC to analyze the thermal properties of the constituents of cochleates (Fig. 4D). The melting endothermic peaks of PS (lipid), curcumin (drug), and mannitol (lyophilization protective agent) appeared at 71.49 °C, 187.50 °C, and 171.77 °C, respectively. The endothermic peak of curcumin-loaded cochleates appeared at 170.75 °C, suggesting that the formulation altered the overall melting characteristics of the constituents. Additionally, the complete disappearance of curcumin's inherent melting peak at 187.50 °C indicated its non-free state, demonstrating its successful encapsulation within cochleates (Chen et al. 2022).

Fourier transform infrared (FT-IR) spectroscopy

FT-IR is an effective method for probing conformational alterations, membrane dynamics, as well as physical and chemical interactions among lipid molecules, cations, and drugs within both vesicles and cochleates. This technique directly detects ion-induced perturbations at specific phospholipid sites without probing the molecule.

Information obtained in FT-IR studies on cochleate formation predominantly focuses on the stretching vibrations

of three key regions within the phospholipid structure: (1) acyl chain region, (2) carbonyl interfacial region, and (3) polar group region (Arrondo et al. Arrondo and Goni 1998). Figure 4E depicts a comprehensive spectrum of pure DMPS along with its distinctive vibrational bands: the symmetric CH_2 stretching vibration (2850–2855 cm^{-1}), $\text{C}=\text{O}$ stretching mode (1685–1780 cm^{-1}), NH_3^+ antisymmetric bending (1600–1620 cm^{-1}), COO^- antisymmetric stretching band (1620–1640 cm^{-1}), and PO_2^- asymmetric stretching vibration (1220–1260 cm^{-1}) (Manrique-Moreno et al. 2016). Research has been conducted on DMPS and DMPS/DMPC mixtures, both in the presence and absence of Ca^{2+} ions. It has been revealed that upon cochleate formation, the frequency of the methyl symmetric deformation mode near 1,378 cm^{-1} , which was previously thought to be insensitive to changes in lipid morphology, increases by approximately 8 cm^{-1} (Flach and Mendelsohn 1993).

X-ray analysis

X-ray analysis includes small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS). These techniques can distinguish the densely packed bilayers of cochleates from the more spaced-out bilayers of multilamellar vesicles (MLV) (Hui et al. 1983). SAXS provides insights into alterations in the correlation and ordering of adjacent bilayers. Scattering is assessed within a range corresponding to the thickness and periodicity of the bilayers (0.03–0.6 $\text{\AA}^{-1}$) (Bozó et al. 2017). Figure 4F shows a prominent first-order peak, indicating a repeat interlamellar distance of 49.1 $\text{\AA}$, along with second- and third-order reflections that signify multilamellar organization. The observed repeat spacings suggest that the cochleates consist of dehydrated multilamellar phase assemblies (Lipa-Castro et al. 2021). Therefore, SAXS can be used to assess the optimal ratio of negatively charged lipids required for the formation of cochleates when combined with other components (Hui et al. 1983). Moreover, X-ray diffraction can be utilized to elucidate the distinctive features of intermediate structures that form from the gradual reduction of repeat distances during the transition from multilamellar vesicles to cochleates induced by the addition of a bridging agent (Nagarsekar et al. 2016). Meanwhile, WAXS provides insights into the arrangement of acyl chains. In the cochleate phase, multiple sharp reflections appear in the wide-angle scattering region, indicating the formation of a highly organized chain lattice (Ruocco and Shipley 1982).

Nuclear magnetic resonance (NMR)

NMR is versatile for elucidating the molecular environment within phospholipid assemblies and detecting alterations

induced by the addition of divalent cations. In the absence of a binding agent, the ^2H -NMR spectrum of an aqueous lipid suspension exhibits a broad singlet, indicating the presence of excess free water. This broad signal arises from the fast exchange between free and bound water molecules in the hydration shells surrounding the lipid bilayers. Conversely, the complex formed by a binding agent and lipids displays a sharper singlet in the ^2H -NMR spectrum. This suggests an interaction between the two, which may displace water molecules (Hauser et al. 1977).

^{31}P -NMR studies showed that the addition of Ca^{2+} to PS leads to the detection of a “rigid lattice” in the spectrum and a more than tenfold increase in the spin-lattice relaxation time (T_1). This suggests a robust and specific immobilization of the lipid headgroups by Ca^{2+} , resembling the behavior of anhydrous PS (Fig. 4G) (Hope and Cullis 1980).

Cochleate-based drug delivery

Advantageous properties for drug delivery

Cochleates can be produced with relative ease and safety. They can enhance the bioavailability of poorly soluble drugs and protect the loaded drugs from degradation induced by various stimuli, including oxygen, temperature, and light (Nadaf and Killedar 2015). Furthermore, cochleates are safer than other lipid-based formulations due to their oxidation resistance (Shende et al. 2019). Additionally, cochleates can maintain their structures effectively during the freeze-drying process (Mallappa et al. 2020), whereas liposomes can be damaged when freeze-dried (Nugraheni et al. 2020). As phospholipids form the cell membranes of all mammals and are recognized as endogenous materials, cochleates are not immunogenic, toxic, or inflammatory on their own (Gould-Fogerite et al. 2000). Also, the phospholipid-cationic crystal structure of the compound is stable, consisting of spiral lipid bilayers with no internal water molecules. This makes cochleates resistant to degradation within the gastrointestinal tract (Pham et al. 2014). Cochleates have been demonstrated to enhance the loading capacity of various drugs, prolong their release profiles (Khair et al. 2020), augment their potency, and mitigate adverse effects (Batista-Duarte et al. 2016). Cochleates can also enhance drug permeability due to their spiral tubular shape, which functions similarly to a microneedle. The electrochemical forces are concentrated along the longitudinal side of the cochleates, resulting in an exceptionally strong and effective tip (Ağardan et al. 2016). Cochleates have the potential to facilitate the oral administration of drugs that are typically administered intravenously (Castro et al. 2022).

In the study by Munot et al. (2022), it was reported that upon the loading of 5 mg of quercetin, the encapsulation efficiency of liposomes was found to be $74.2 \pm 2.3\%$. In contrast, the encapsulation efficiency of nanocochleates was found to be higher, with an average of $88.62 \pm 4.20\%$. An *in vitro* release study was conducted by dialysis bag diffusion method in phosphate-buffered saline solution (pH 7.4) at 4 hr, 47% of the drug was released from the liposome and 39% from the cochleate, indicating that the cochleate has a controlled release due to its coiled structure (Fig. 5A). Furthermore, *in vitro* anticancer activity experiments utilizing the human mouth cancer cell lines demonstrated that the growth inhibitory 50% concentration of cochleate was $10 \mu\text{g/mL}$ while that of liposome was $20 \mu\text{g/mL}$, which suggests that cochleate exhibits superior anticancer activity (Fig. 5B). Subsequent lyophilisation and testing of the two formulations for stability at $5 \pm 3^\circ\text{C}$ over a period of three months resulted in the conclusion that cochleate demonstrated enhanced stability, while liposome exhibited a substantial decline in encapsulation efficiency due to drug efflux and an augmentation in particle size as a consequence of vesicle fusion. Conversely, no substantial alterations were observed in cochleate (Munot et al. 2022).

In vivo studies with female tumour-bearing Sprague-Dawley rats revealed that the cochleate formulation was the most effective in reducing tumour size when compared to the raloxifene solution, liposome and cochleate formulations (Fig. 5C). This formulation also exhibited the least body weight fluctuation, which is often considered a sign of effective treatment (Fig. 5D) (Ağardan et al. 2016). In another study, male Wistar rats were orally administered amphotericin B, an antifungal agent, in a liposome suspension and a cochleate suspension formulation to monitor its biodistribution. Although blood concentrations of the cochleate were slightly lower than those of the liposome, concentrations in all major organs were high. The elevated concentrations, particularly in the liver and spleen, where *Leishmania* parasites reside, indicate the efficiency of the oral route for the cochleate. Notably, delivering amphotericin B orally—rather than through its current intravenous administration—offers a significant advantage (Castro et al. 2022).

Disadvantageous properties for drug delivery

From the perspective of cellular adhesion, there is evidence that oblate particles adhere more effectively to biological substrates than spherical particles (Decuzzi and Ferrari 2006). From the perspective of intracellular deposition, elongated nanoparticles with a high aspect ratio are more readily intracellularly deposited into HeLa cells (Parakhonskiy et al. 2015). However, contradictory findings have also

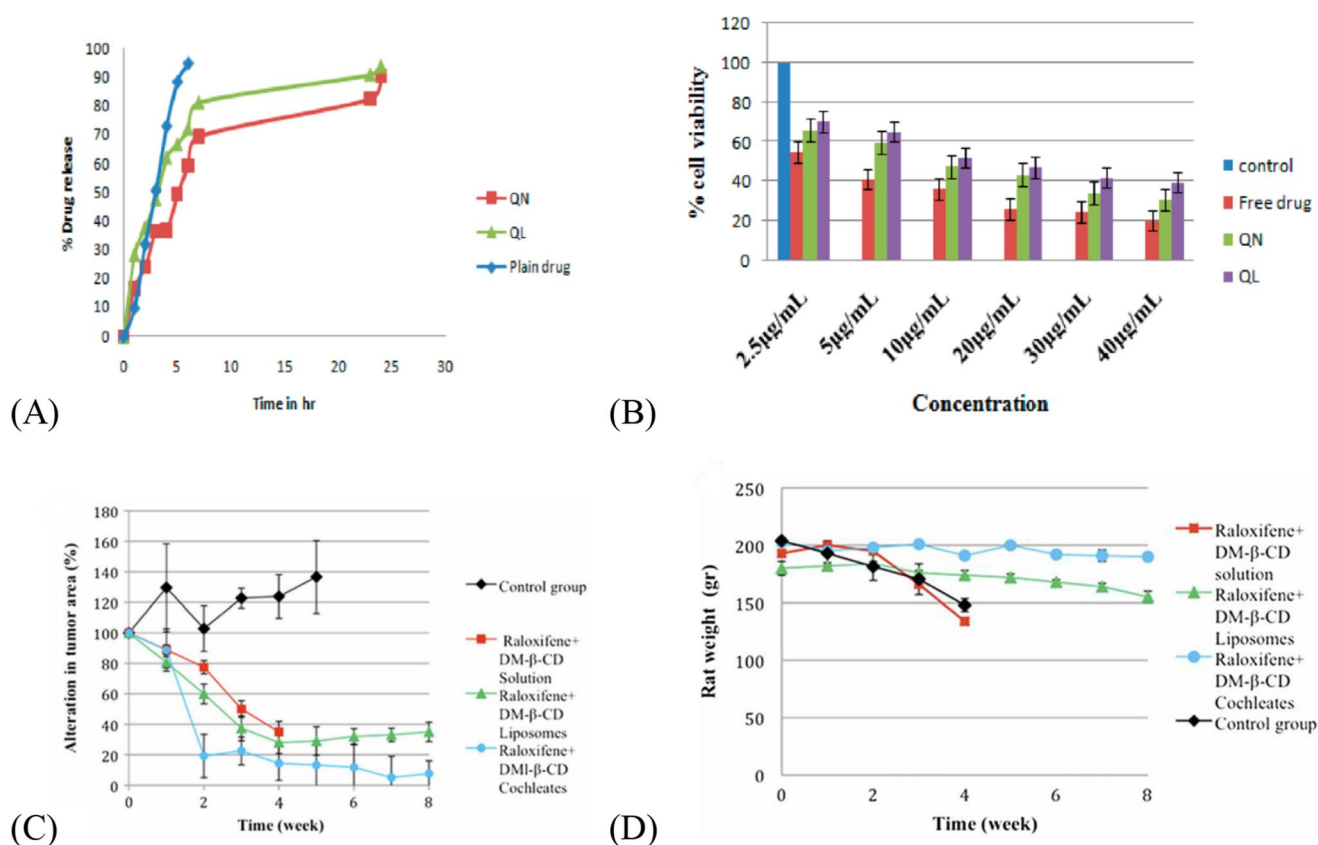


Fig. 5 Experimental data showing potentials of cochleates as drug delivery system: **(A)** in vitro quercetin release from cochleate, **(B)** in vitro anticancer activity in MTT assay were adapted from (Munot et al. 2022) with permission from MDPI, **(C)** alterations in tumor areas

(D) results of rat weight changes weekly were adapted from (Ağardan et al. 2016) with permission from Springer Nature through the copyright clearance center. QN, quercetin-loaded nanocochleates; QL, quercetin-loaded liposomes; DM-β-CD, dimethyl-β-cyclodextrin

been reported, indicating that spherical nanoparticles exhibited the highest percentage of intracellular invasion, with the percentage decreasing as the shape changed in the following order: spherical, cubic, rod, non-spherical nanoparticles (Li et al. 2015). This is due to the absence of fundamental data on the influence of form and shape on the behavior of these objects within the body when in contact with bio fluids and facing different cellular environments, such as particle-cell interactions. To fabricate shape-controlled nanoparticles in a self-assembling manner, they must be carefully designed with molecularly defined structures that can assemble in a structured manner and induce specific predefined geometric shapes and morphologies.

Given the microparticulate nature of cochleate, it is essential to control the particle size, as this may influence the capture by cells of the immune system. The intravenous administration of cochleate has been demonstrated to reduce the level of inhibitory antibody developed against recombinant factor VIII in comparison to liposomes (Kosloski et al. 2011). However, it should be noted that the number of studies in this area is limited. The size range of cochleates is determined by the method of preparation and the conditions

used, but it is essential to control their size and size distribution for administration by various routes. This necessitates the use of aggregation inhibitors and microfluidic or flow focusing devices to produce cochleates with a controlled size, low dispersity and narrow size distribution (Wong et al. Wong and Judeh 2020).

Applications in pharmaceuticals

While hydrophobic or amphipathic molecules are typically preferred as drug candidates, cochleates have been utilized as carriers for various types of drugs (Zarif 2005). Various drug types, including microbial agents, anticancer drugs, and peptides, have been loaded into cochleates. The materials, methods, and benefits of cochleate-based formulations developed over the last decade are summarized in Table 2. Notably, BioDelivery Sciences International, Inc. (BDSI) has developed the patented Bioral[®] drug delivery system based on cochleates. This is an encochleated formulation of amphotericin B, an antifungal treatment for systemic fungal infections, developed for oral administration. Its phase I trial results reported in February 2009 indicated promising

Table 2 The materials, methods, and benefits of cochleates-based formulations developed over the past decade

Drug type	Drug	Materials	Preparation method	Advantages	Reference
Chemical drug	Revaprazan	DMPC, Cholesterol, DCP, Ca ²⁺	Trapping method	- greater resistance to pancreatic enzyme - improved relative bioavailability by approximately 274%	(Goo et al. 2024)
	Beclomethasone dipropionate	PC, PS, Ca ²⁺	DC dialysis method	- less prone to degradation - significant potential for drug delivery applications	(Ghauri et al. 2020)
	Lansoprazole	DMPC, cholesterol, DCP, Ca ²⁺	Trapping method	- acid-protective effects - controlled drug release	(Goo et al. 2021)
	Rosuvastatin	PC, Ca ²⁺	Trapping method	- enhanced permeability by 3.44 times compared to a commercial formulation - the drug release rate in vitro extended over 12 hours, which was significantly better than that of traditional tablet formulations	(Pawar et al. 2021)
	Cyclosporine A	DOPS, Ca ²⁺ , chitosan derivatives	Trapping method	- enhanced bioavailability by nearly 5-fold compared to a commercial formulation - chitosan-induced nanocochleates significantly enhanced gastrointestinal absorption, resulting in up to a 3-fold increase in oral bioavailability	(Liu et al. 2017)
Anti-cancer drug	Glipizide	PC, cholesterol, Ca ²⁺	Electrospray technique	- 2-fold increase in C _{max} and 3-fold increase in AUC following entrapment of glipizide into nanocochleates	(Jain et al. 2023)
	Bupivacaine	Phosphatidic acid, PC, PG, cholesterol, Ca ²⁺ , Mg ²⁺	N.S. ^a	- the potential to overcome production issues associated with other vesicle-based technologies - prolonged retention and sustained release of small hydrophilic molecules at the injection site in vivo	(Aleandri et al. 2022)
	Raloxifene	DPPC, DOPS, Ca ²⁺ , Dimethyl- β -cyclodextrin (DM- β -CD), sodium taurocholate (NaTC)	Hydrogel method	- highest antitumor activity in vitro against MCF-7 (human breast cancer) cells, and in vivo compared to free raloxifene and liposome - highest metalloproteinase-2 enzyme inhibition compared to free raloxifene and liposome	(Ağardan et al. 2016)
	Sorafenib tosylate	DOPS, cholesterol, Ca ²⁺	Trapping method	- 2.18-fold improvement in oral bioavailability - 1.30-, 2-, and 2.18-fold increases in C _{max} , T _{max} , and AUC ($p < 0.05$), respectively - 3.21-, 2.29-, and 7-fold decreases in t _{1/2} , MRT, and volume of distribution, respectively - 2.12-fold enhancement in cell viability in vitro compared to raw material	(Aliwale et al. 2022)
	Fisetin (natural flavonoid)	DMPC, cholesterol, Ca ²⁺	Trapping method	- safety and sustained release - 1.3-fold improvement in anticancer efficacy against MCF-7 (human breast cancer) cells in vitro - 141-fold higher relative bioavailability compared to free fisetin - low tissue distribution	(Bothiraja et al. 2014)
	Paclitaxel (PTX)	PC, cholesterol, Ca ²⁺	Trapping method	- controlled release of PTX - significant improvement in anticancer activity against A549 (human lung adenocarcinoma), OVCAR-3 (ovarian cancer), and MCF-7 (breast cancer) cells in vitro - 14- and 19-fold increases in oral bioavailability in rats compared to nanoliposomes and free PTX, respectively - low tissue distribution - alternative option to the intravenous route	(Pawar et al. 2014)

Table 2 (continued)

Drug type	Drug	Materials	Preparation method	Advantages	Reference
Micro-bial agents		DOPS, cholesterol, Ca ²⁺	Hydrogel method	- protection from degradation at acidic gastric pH - sustained release of PTX in the intestine - low toxicity in vital organs - promising clinical potential for oral delivery of PTX	(Shanmugam et al. 2020)
		DOPS, cholesterol, Ca ²⁺	Modified aqueous hydrogel separation-based method	- excellent pulmonary surfactant action compared to the commercial Taxol formulation - significant ($p < 0.05$) tumor growth inhibition compared to intravenously administered Taxol - significant ($p < 0.05$) reduction in the number of tumor nodules and percent metastasis area covered by melanoma cells in the lung compared to other treatment groups - safely administered as aerosols	(Shanmugam et al. 2020)
	Quercetin (flavonoid)	DMPG, cholesterol, Ca ²⁺	Trapping method	- controlled release of quercetin over 24 h - intact and well protected against hepatic enzymes compared to the free quercetin solution and liposomes - superior anticancer activity compared to liposomes - more stable than other liposome formulations	(Munot et al. 2022)
	Hydroxyamphotericin (HCPT)	DOPS, Ca ²⁺	Trapping method	- 2.3-fold increase in relative oral bioavailability compared to HCPT suspension - favorable intestinal absorption for antitumor activity in vivo - strongest antitumor activity among various liposomes and suspensions - significant increase in HCPT transport across Caco-2 cell monolayers - promising vehicle for oral administration of HCPT	(Zhong et al. 2018)
	Methotrexate	DMPG, cholesterol, Ca ²⁺	N.S. ^a	- controlled release with a 6-fold improvement in oral bioavailability - low tissue distribution	(Chellampillai et al. 2022)
	Doxorubicin (DOX)	DMPG, cholesterol, Ca ²⁺ , folic acid, chitosan	Trapping method	- GI50 (the drug concentration needed for 50% growth inhibition) decreased by 51.64% compared to free DOX - bioavailability increased 4-fold with long circulation time; slower plasma elimination and no sign of tissue toxicity compared to free DOX	(Bothiraja et al. 2018)
	Docetaxel (DTX)	DMPG, cholesterol, Ca ²⁺ , biotinylated chitosan	Trapping method	- GI50 decreased by 88.89% compared to free DTX - bioavailability increased 10-fold with longer circulation time; slower plasma elimination with low tissue distribution compared to free DTX	(Poudel et al. 2018)
	Quercetin, curcumin	PC, cholesterol	Trapping method	- potential for targeted delivery of anticancer drugs - improved in vitro anti-cancer activity - better permeability than a commercial gel	(Meena 2023)
	Amphotericin B	phospholipid rich in PS (Lipoid PSP70), DOPS, Ca ²⁺ , cholesterol, vitamin E	Hydrogel isolation method	- potential for oral administration - drug protected from degradation in the gastric medium	(Lipa-Castro et al. 2021)
	Itraconazole	sodium deoxycholate, Ca ²⁺	DC dialysis method	- improved antifungal effects - immunomodulatory activity both in vitro and in vivo - reduced cytotoxicity vitro	(Batista-Duarte et al. 2016)
		PS, Ca ²⁺	Trapping method	- potential for oral administration	(More et al. 2023)

Table 2 (continued)

Drug type	Drug	Materials	Preparation method	Advantages	Reference
	Rifampicin	Soya lecithin, cholesterol, Ca^{2+} , Bovine serum albumin	Trapping method	- improved permeability	(Yadav and Chaudhary 2016)
	Artemisinin	DOPS, DMPS, Ca^{2+}	Trapping method	- controlled release	(Judeh 2019)
	Famciclovir	PC, cholesterol	Hydrogel, trapping, liposome before cochleate dialysis, direct calcium dialysis and binary aqueous-aqueous emulsion methods	- increased stability - a novel nanocochleate gel of famciclovir	(Kanugo et al. 2023)
	Nelfinavir	DMPG, DOPS, DOctPS, DDPS, Ca^{2+}	Trapping method	- improved oral bioavailability	(Belubbi et al. 2018)
Peptide drug	Artemether, Lumefantrine	Phospholipon 90 H, cholesterol, Ca^{2+}	Trapping method	- effective drug encapsulation in nanoparticles and sustained release properties - compared to free drugs, nanocochleates exhibited reduced tissue distribution and a 20-fold increase in bioavailability in rats	(Mathure et al. 2024)
	Insulin	Soya lecithin, cholesterol, Ca^{2+}	Binary aqueous-aqueous emulsion method	- sustained release - long-term stability - improved in vivo anti-diabetic activity	(Khair et al. 2020)
		DPPE, DMPC, cholesterol, Ca^{2+} , Mg^{2+}	Hydrogel method	- potential for oral administration	(Khaza-eli et al. 2018)
	Salmonella infantis vaccine	Bacterial membrane, Ca^{2+}	Trapping method	- the first step in creating a safe oral S. Infantis vaccine for poultry involves employing an innovative cochleate encapsulation technology	(Aven- daño et al. 2021)

Table 2 (continued)

Drug type	Drug	Materials	Preparation method	Advantages	Reference
	<i>Neisseria meningitidis</i> B protein complex	DOPS, cholesterol, Ca^{2+}	Trapping method	- cochleates did not include the conventional Al(OH) ₃ adjuvant; they elicited a robust Th-1 immune response similar to that of the original vaccine formulation - a lower injection site reaction attributed to the absence of Al(OH) ₃	(Santos et al. 2019)
	<i>Vibrio cholerae</i> antigen	Proteoliposome (protein, LPS), Ca^{2+}	Trapping method	- an effective subunit vaccine comprising only protective antigens without contaminant materials - the formulation enhanced immunogenicity through purified antigens and targeted delivery, showcasing its vaccine development potential	(Tamargo et al. 2021)

^aN.S.: not stated; DCP, dicetyl phosphate; DDPS, 1,2-didecanoyl-sn-glycero-3-phospho-L-serine; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidyl glycerol; DOctPS, 1,2-dioctanoyl-sn-glycero-3-phospho-L-serine; DOPS, dioleoyl phosphatidylserine; DPPC, dipalmitoyl phosphatidylcholine; LPS, lipopolysaccharides; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine

clinical outcomes. Its antifungal efficacy was found to be comparable to that of intravenous administration, with plasma concentrations similar to the intravenous route and a well-tolerated safety profile concerning renal functions (Thornton et al. Thornton and Wasan 2009).

Applications in other industries

In terms of food applications, BDSI has developed nano-cochleates to enhance the cellular delivery of various nutrients, such as vitamins, omega fatty acids, and lycopene. This preserves the colors and flavors of foods, contributing to the concept of superfoods. These advancements are anticipated to offer numerous benefits, including increased energy, improved cognitive functions, enhanced immune responses, and anti-aging effects (Sankar and Reddy 2010).

For cosmetic applications, significant efforts have been dedicated to stabilizing active ingredients and enhancing the transdermal delivery efficiency of functional cosmetics. This often involves creating vesicles that can encapsulate active ingredients using materials like surfactants, lipids, and polymers. Among these systems, lipids are particularly advantageous as they mimic endogenous cell membranes, making them widely used in cosmetic formulations. Cochleates, due to their distinctive spiral rod shape and robust structure, are anticipated to be an excellent delivery system for cosmetic ingredients (Ji et al. 2020).

Future exploration on cochleates technology

In addition to the anticancer drugs, microbial agents, and peptide drugs listed in Table 2, cochleates have also been explored for gene delivery, specifically for transferring transgenes into mammalian cells. Studies using rhodamine-labeled cochleates have demonstrated successful delivery of green fluorescent protein plasmids to both cell lines and primary cells. The results indicate that, in both in vitro and in vivo settings, cochleates are efficiently taken up by macrophages. This suggests that cochleates could serve as a promising, non-toxic, and non-immunogenic approach for transgene introduction (Gibson et al. 2004).

Cochleate research still has a great deal of scope for expansion, with ongoing research able to be directed towards formulation improvements, enhanced drug delivery efficiency, mechanism studies, industrial applications, and the development of new therapeutic areas. In particular, further research is expected in the areas of oral deliverability, mucosal delivery, gene therapy applications, and delivery of antimicrobial and anticancer drugs (Rao et al. 2007; Wang et al. 2014; Zarif and Mannino 2002). In the future, it will be an effective alternative for delivering biological or therapeutic motifs while improving patient compliance. To

get there, further researches are required in relation to the consistent formulation optimization methods, the long-term stability studies, and the development of stable and reproducible processes for clinical and industrialization.

Conclusion

Over the past decade, significant efforts have been devoted to the development of lipid-based drug delivery systems, with cochleates emerging as a promising option due to their unique advantages: increased shelf-life, bioavailability, and efficacy, as well as reduced toxicity and degradation in harsh environments. Although cochleates are limited by particle aggregation, this can be addressed by incorporating aggregation inhibitors. Additionally, the issue of high production costs can be addressed by employing cost-effective techniques. Cochleates have the potential to become effective drug delivery carriers for cancer due to their active uptake by macrophages. Additionally, cochleates can be used for oral, transdermal, nasal, vaginal, and dermal applications, distinguishing them from conventional liposomes and nanoparticles. We envision that cochleates may surpass liposomes in the future with enhanced drug-loading capacity, encapsulation efficiency, and stability.

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Data availability Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Statement of human and animal rights This article does not contain any studies with human and animal subjects performed by any of the authors.

Conflicts of interest All authors (J. Lee, Y.T. Goo, K. Shakhakarmi, M.J. Kang, and S. Lee) declare that they have no conflict of interest.

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References

- Ağardan NBM, Değim Z, Yılmaz Ş, Altuntaş L, Topal T (2016) The effectiveness of raloxifene-loaded liposomes and cochleates in breast cancer therapy. *AAPS Pharm Sci Tech* 17:968–977
- Ahiwale RJ, Chellampillai B, Pawar AP (2022) Investigation of novel sorafenib tosylate loaded biomaterial based nano-cochleates dispersion system for treatment of hepatocellular carcinoma. *J Dispers Sci Technol* 43:1568–1586
- Aleandri S, Rahnfeld L, Chatzikleanthous D, Bergadano A, Bühr C, et al. (2022) Development and in vivo validation of phospholipid-based depots for the sustained release of bupivacaine. *Eur J Pharm Biopharm* 181:300–309
- Arrondo JLR, Goni FM (1998) Infrared studies of protein-induced perturbation of lipids in lipoproteins and membranes. *Chem Phys Lipids* 96:53–68
- Asprea M, Tatini F, Piazzini V, Rossi F, Bergonzi MC et al (2019) Stable, monodisperse, and highly cell-permeating nanocochleates from natural soy lecithin liposomes. *Pharm* 11:34
- Avendaño C, Vidal S, Villamizar-Sarmiento MG, Guzmán M, Hidalgo H, et al. (2021) Encapsulation of cochleates derived from salmonella infantis with biopolymers to develop a potential oral poultry vaccine. *Polymers* 13:3426
- Bae Y, Zeb A, Choi H-I, Ryu J-S, Gul M, et al. (2024) High payload dexamethasone palmitate-loaded solid lipid nanoparticles for enhanced anti-inflammatory effects in acute skin inflammation model. *J Pharm Investig* 54:617–629
- Batista-Duarte A, Lastre M, Romeu B, Portuondo D, Téllez-Martínez D, et al. (2016) Antifungal and immunomodulatory activity of a novel cochleate for amphotericin B delivery against *Sporothrix schenckii*. *Int Immunopharmacol* 40:277–287
- Belubbi T, Shevade S, Dhawan V, Sridhar V, Majumdar A, et al. (2018) Lipid architectonics for superior oral bioavailability of nelfinavir mesylate: comparative in vitro and in vivo assessment. *AAPS Pharm Sci Tech* 19:3584–3598
- Bothiraja C, Rajput N, Poudel I, Rajalakshmi S, Panda B, et al. (2018) Development of novel biofunctionalized chitosan decorated nanocochleates as a cancer targeted drug delivery platform. *Artif Cells Nanomed Biotechnol* 46:447–461
- Bothiraja C, Yojana BD, Pawar AP, Shaikh KS, Thorat UH (2014) Fisetin-loaded nanocochleates: formulation, characterisation, in vitro anticancer testing, bioavailability and biodistribution study. *Expert Opin Drug Deliv* 11:17–29
- Bozo T, Brecka R, Grof P, Kellermayer MS (2015) Extreme resilience in cochleate nanoparticles. *Langmuir* 31:839–845
- Bozó T, Wacha A, Mihály J, Bóta A, Kellermayer MS (2017) Dispersion and

- Chellampillai B, Kashid S, Pawar A, Mali A (2022) Investigation of dimyristoyl phosphatidyl glycerol and cholesterol based nanocochleates as a potential oral delivery carrier for methotrexate. *J Liposome Res* 32:308–316
- Chen L, Yue B, Liu Z, Luo Y, Ni L, et al. (2022) Study on the preparation, characterization, and stability of freeze-dried curcumin-loaded cochleates. *Foods* 11:710
- Decuzzi P, Ferrari M (2006) The adhesive strength of non-spherical particles mediated by specific interactions. *Biomaterials* 27:5307–5314
- Düzgünes N, Wilschut J, Fraley R, Papahadjopoulos D (1981) Studies on the mechanism of membrane fusion. Role of head-group composition in calcium-and magnesium-induced fusion of mixed phospholipid vesicles. *Biochim Biophys Acta Biomembr* 642:182–195
- Flach CR, Mendelsohn R (1993) A new infrared spectroscopic marker for cochleate phases in phosphatidylserine-containing model membranes. *Biophys J* 64:1113–1121
- Ghauri A, Ghauri I, Elhissi AM, Ahmed W (2020) Characterization of cochleate nanoparticles for delivery of the anti-asthma drug beclomethasone dipropionate. In: *Advances in medical and surgical engineering*. Elsevier, Netherlands, pp 267–277
- Gibson B, Duffy AM, Fogerite SG, Krause-Elmore S, Lu R et al (2004) A novel gene delivery system for mammalian cells. *Anticancer Res* 24:483–488
- Goo YT, Jin CH, Kim MS, Han SB, Choi YW (2021) Cochleate formulation enhances the stability of lansoprazole in acidic condition. *Bull Korean Chem Soc* 42:1281–1284
- Goo YT, Kim MS, Choi JY, Sin GH, Hong SH, et al. (2024) A cochleate formulation optimized by D-optimal mixture design enhances oral bioavailability of Revaprazan. *J Liposome Res* 34:31–43
- Gould-Fogerite S, Kheiri M, Zhang F, Mannino RJ (2000) Cochleate delivery vehicles: applications in vaccine delivery. *J Liposome Res* 10:339–358
- Gould-Fogerite S, Kheiri MT, Zhang F, Wang Z, Scolpino AJ, et al. (1998) Targeting immune response induction with cochleate and liposome-based vaccines. *Adv Drug Deliv Rev* 32:273–287
- Gould-Fogerite S, Mannino RJ. Cochleate delivery vehicles. US Patent 5,994,318. United States; 1997 Jul 01.
- Hauser H, Finer E, Darke A (1977) Crystalline anhydrous Ca-phosphatidylserine bilayers. *Biochem Biophys Res Commun* 76:267–274
- Hollander A, Danino D (2015) Cochleate characterization by cryogenic electron microscopy methods: cryo-TEM and Cryo-SEM. *Colloids Surf A Physicochem Eng Asp* 483:187–192
- Hope M, Cullis P (1980) Effects of divalent cations and pH on phosphatidylserine model membranes: a ³¹P NMR study. *Biochem Biophys Res Commun* 92:846–852
- Hui S, Boni L, Stewart T, Isac T (1983) Identification of phosphatidylserine and phosphatidylcholine in calcium-induced phase separated domains. *Biochemistry* 22:3511–3516
- Jain A, Kamble R, Patil S (2023) Electrospray technology as a probe for single step fabrication of glipizide loaded nanocochleates with enhanced bioavailability. *J Dispers Sci Technol* 44:487–495
- Jeong S-J, Song W-Y, Park C-W, Kim D-W (2024) Recent approaches to investigate drug delivery systems through the lymphatic pathway using oral lipid-based formulations. *J Pharm Investig* 54:131–144
- Ji HG, Choi HI, Park YA, Kang YJ (2020) Cochleates using phosphatidylserine/anionic surfactant/calcium. US Patent 11,260,010 B2
- Jin T, Zarif L, Mannino RJ (2000) Nanocochleate formulations, process of preparation and method of delivery of pharmaceutical agents. US Patent 6,153,217
- Judeh Z (2019) Alginate-coating of artemisinin-loaded cochleates results in better control over gastro-intestinal release for effective oral delivery. *J Drug Deliv Sci Technol* 52:27–36
- Kanugo A, Deshpande A, Sharma R (2023) Formulation optimization and evaluation of nanocochleate gel of famciclovir for the treatment of Herpes Zoster. *Recent Pat Nanotechnol* 17:259–269
- Khair R, Shende P, Kulkarni YA (2020) Nanostructured polymer-based cochleates for effective transportation of insulin. *J Mol Liq* 311:113352
- Khzaeli P, Pardakhty A, Dorkoosh F, Karimi-Afshar S (2018) Preparation and physicochemical evaluation of cochleate-based carriers for Insulin. *Int Pharm Acta* 1:128–128
- Kosloski MP, Peng A, Varma PR, Fathallah AM, Miclea RD, et al. (2011) Immunogenicity and pharmacokinetic studies of recombinant Factor VIII containing lipid cochleates. *Drug Deliv* 18:246–254
- Kristensen S, Hassan K, Andersen NS, Steiniger F, Kuntsche J (2023) Feasibility of the preparation of cochleate suspensions from naturally derived phosphatidylserines. *Front Med. Technol* 5
- Landge A, Pawar A, Shaikh K (2013) Investigation of cochleates as carriers for topical drug delivery. *Int J Pharm Pharm Sci* 5:314–320
- Li J, Wang X, Zhang T, Wang C, Huang Z, et al. (2015) A review on phospholipids and their main applications in drug delivery systems. *Asian J Pharm Sci* 10:81–98
- Lipa-Castro A, Nicolas V, Angelova A, Mekhloufi G, Prost B, et al. (2021) Cochleate formulations of Amphotericin b designed for oral administration using a naturally occurring phospholipid. *Int J Pharm* 603:120688
- Liu M, Zhong X, Yang Z (2017) Chitosan functionalized nanocochleates for enhanced oral absorption of cyclosporine A. *Sci Rep* 7:41322
- Loftsson T, Brewster ME (2010) Pharmaceutical applications of cyclodextrins: basic science and product development. *J Pharm Pharmacol* 62:1607–1621
- Lundbæk JA, Maer AM, Andersen OS (1997) Lipid bilayer electrostatic energy, curvature stress, and assembly of gramicidin channels. *Biochemistry* 36:5695–570

- Nadaf S, Killedar S (2015) Novel Liposome Derived Nanoparticulate Drug Delivery System: fabrication and Prospects. *Creative J Pharmaceutical Res* 1:117–128
- Nagarsekar K, Ashtikar M, Steiniger F, Thamm J, Schacher F, et al. (2016) Understanding cochleate formation: insights into structural development. *Soft Matter* 12:3797–3809
- Nagarsekar K, Ashtikar M, Steiniger F, Thamm J, Schacher FH, et al. (2017) Micro-spherical cochleate composites: method development for monodispersed cochleate system. *J Liposome Res* 27:32–40
- Nugraheni RW, Mulyadi NA, Yusuf H (2020) Freeze-dried liposome formulation for small molecules, nucleic acid, and protein delivery. *Syst Rev Pharm* 11:143–151
- Papahadjopoulos D, Nir S, Düzgünes N (1990) Molecular mechanisms of calcium-induced membrane fusion. *J Bioenerg Biomembr* 22:157–179
- Papahadjopoulos D, Vail W, Jacobson K, Poste G (1975) Cochleate lipid cylinders: formation by fusion of unilamellar lipid vesicles. *Biochim Biophys Acta Biomembr* 394:483–491
- Parakhonskiy B, Zyuzin MV, Yashchenok A, Carregal-Romero S, Rejman J, et al. (2015) The influence of the size and aspect ratio of anisotropic, porous CaCO₃ particles on their uptake by cells. *J Nanobiotechnology* 13:1–13
- Pawar A, Bothiraja C, Shaikh K, Mali A (2015) An insight into cochleates, a potential drug delivery system. *RSC Adv* 5:81188–81202
- Pawar AP, Vinugala D, Bothiraja C (2014) Nanocochleates derived from nanoliposomes for paclitaxel oral use: preparation, characterization, in vitro anticancer testing, bioavailability and biodistribution study in rats. *Biomed Pharmacother* 3502:1–9
- Pawar AY, Patil SS, More PS, Jadhav PR, Bhavar SR (2021) Preparation and characterization of nanocochleate by using phosphatidylcholine as lipid carrier for enhancement of permeability and bioavailability of rosuvastatin. *Drug Dev Ind Pharm* 47:2004–2012
- Pehlivanov I (2019) Self-emulsifying drug delivery systems as an approach to improve therapeutic effectiveness of orally administered drugs. *J IMAB* 25:2575–2582
- Pham T, Gueutin C, Cheron M, Abreu S, Chaminade P, et al. (2014) Development of antileishmanial lipid nanocomplexes. *Biochimie* 107:143–153
- Portis A, Newton C, Pangborn W, Papahadjopoulos D (1979) Studies on the mechanism of membrane fusion: evidence for an intermembrane calcium (2+) ion-phospholipid complex, synergism with magnesium (2+) ion, and inhibition by spectrin. *Biochemistry* 18:780–790
- Poudel I, Ahiwale R, Pawar A, Mahadik K, Bothiraja C (2018) Development of novel biotinylated chitosan-decorated docetaxel-loaded nanocochleates for breast cancer targeting. *Artif Cells Nanomed Biotechnol* 46:229–240
- Rao R, Squillante Iii E, Kim KH (2007) Lipid-based cochleates: a promising formulation platform for oral and parenteral delivery of therapeutic agents. *Crit Rev Ther Drug Carrier Syst* 24
- Ro S-Y, Choi H-M, Choi S-H, Lee S-W, Lim S-J (2023) Tricaprin as a membrane permeability regulator: sustained small hydrophilic substance release from liposomes. *J Pharm Investig* 53:539–548
- Ruocco MJ, Shipley GG (1982) Characterization of the sub-transition of hydrated dipalmitoylphosphatidylcholine bilayers: x-ray diffraction study. *Biochim Biophys Acta Biomembr* 684:59–66
- Sankar VR, Reddy YD (2010) Nanocochleate—A new approach in lipid drug delivery. *Int J Pharm Pharm Sci* 2:220–223
- Santangelo R, Paderu P, Delmas G, Chen Z-W, Mannino R et al. (2000) Efficacy of oral cochleate-amphotericin B in a mouse model of systemic candidiasis. *Antimicrob Agents Chemother* 44:2356–2360
- Santos BT, Pérez CF, Bourzac JFI, Nápoles YM, González WR, et al. (2019) Remote induction of cellular immune response in mice by anti-meningococcal nanocochleates-nanoproteoliposomes. *Sci Total Environ* 668:1055–1063
- Sarig H, Ohana D, Epand RF, Mor A, Epand RM (2011) Functional studies of cochleate assemblies of an oligo-acyl-lysyl with lipid mixtures for combating bacterial multidrug resistance. *FASEB J* 25:3336–3343
- Shanmugam T, Joshi N, Ahamad N, Deshmukh A, Banerjee R (2020) Enhanced absorption, and efficacy of oral self-assembled paclitaxel nanocochleates in multi-drug resistant colon cancer. *Int J Pharm* 586:119482
- Shende P, Khair R, Gaud RS (2019) Nanostructured cochleates: a multi-layered platform for cellular transportation of therapeutics. *Drug Dev Ind Pharm* 45:869–881
- Syed UM, Woo AF, Plakogiannis F, Jin T, Zhu H (2008) Cochleates bridged by drug molecules. *Int J Pharm* 363:118–125
- Tamargo S, Thanh TB, Pérez M, Otero O, Oliva H, et al. (2021) Nanocochleates containing N-Octylglycoside extracted *Vibrio cholerae* antigens elicited high vibriocidal antibodies titers after intragastric immunization in a mice model. *Microb Pathog* 156:104902
- Thornton SJ, Wasan KM (2009) The reformulation of amphotericin B for oral administration to treat systemic fungal infections and visceral leishmaniasis. *Expert Opin Drug Deliv* 6:271–284
- Vijeta P, Vivek M, Panwar A, Darwhekar G, Jain D (2011) Nanocochleate: as drug delivery vehicle. *Int J Pharm Biol Sci* 1:31–38
- Wang N, Wang T, Zhang M, Chen R, Deng Y (2014) Using procedure of emulsification-lyophilization to form lipid A-incorporating cochleates as an effective oral mucosal vaccine adjuvant-delivery system (VADS). *Int J Pharm* 468:39–49
- Wong PWK, Judeh Z (2020) Continuous, high-throughput production of artemisinin-loaded supramolecular cochleates using simple off-the-shelf flow focusing device. *M*