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NANOCOCHLEATE: A NOVEL APPROACH FOR DELIVERY OF BIOLOGICAL MOLECULES

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ABSTRACT: Nanocochleate is a novel lipid-based drug delivery system in which the liposomal vesicles are converted into nanocochleate by the addition of Calcium divalent cation. This technology involves encochleation of the drug for overcoming problems such as poor solubility, poor permeability, and poor oral bioavailability. This novel lipid-based formulation approach is applicable for the category of drugs like BCS class III drugs, BCS class IV drugs. Applications of this technology can also be extended to macromolecules as well as small molecule drugs that are lipophilic with poor oral bioavailability. It is appropriate for oral as well as systemic administration of biologically active molecules, including drugs, genes, proteins, peptides, and vaccine antigens. This article covers multiple aspects of Nanocochleate drug delivery system, such as its definition, composition, method of preparation, and mechanism of permeation. The article also elaborates on various advantages, disadvantages, and applications of Nanocochleate drug delivery system. The article highlights the potential of this novel system for oral as well as systemic delivery of chemical and biological drug products.

INTRODUCTION: Successful delivery of biological molecule-based pharmaceutical products is primarily determined by its ability to cross the various barriers in the human body. Various barriers encountered by the delivery system include enzymatic barriers, intestinal epithelial barriers, capillary endothelial barriers, blood-brain barrier (BBB) [1]. The Oral route is the most common and acceptable route for drug delivery due to advantages such as high patient compliance, non-invasiveness, convenience, and acceptability. However, it is challenging to deliver biological molecules like protein and peptides, vaccines, genes, *etc*. by oral route due to reasons such as:

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a. Poor intrinsic permeability of peptides/ proteins across biological membranes
b. Susceptibility to enzymatic attack in GIT
c. Immediate post-absorptive clearance
d. Physical instability [2, 3].

Nanocochleate encapsulates biologically relevant molecule preventing the molecule from degradation caused by gastrointestinal enzymes and environmental factors. Cochleate formulations are easy to formulate, safe, and extremely effective mediators for the *in-vivo* delivery of proteins and peptides. The nanocochleate delivery system overcomes the challenges of oral delivery of biologically relevant molecules, including hydrophobic drugs, genes, proteins, peptides, vaccines, and antigens. Thus, it is a potential drug delivery system for a wide class of drugs [4].

History: Cochleates structures derived from the interaction of liposomes and cations were discovered by Dr. Papahadjopoulos, and his co-worker in 1975

[5]. The term "cochleate" was coined due to the similarity of the structure to a snail with a spiral shell. In the late '80s & 90s', cochleates were used to transport antigens and peptides for vaccine delivery. In the literature, it is reported that the cochleates were initially prepared in micrometers sizes. The cochleate structure formed aggregates of stacked sheets and large size. Cochleates prepared were not always uniform. In 1999, Nanocochleate were introduced to establish smaller, but rather more uniform particles having the particle size between 50 and 100 nm. It was reported that by using the hydrogel method more uniform cochleates can be prepared [6]. The first product encapsulating lipophilic drug was Bioral™ Amphotericin B.[7] This nanocochleate has a diameter of 50 nm, and it consists of crystalline structures with anhydrous interior that encapsulate the drug molecule and protects it from degradation in gastrointestinal tract [8].

Structure of Nanocochleate: Cochleates are cigar like microstructures that are formed from the interaction of small negatively charged liposomes and divalent cations in **Fig. 1**. The cation act as a bridging agent between the lipid bilayers.

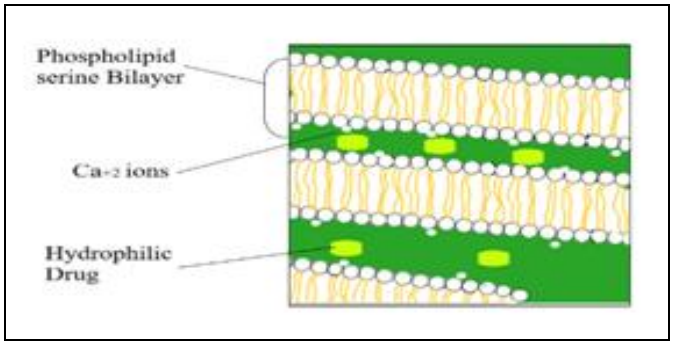

FIG. 1: STRUCTURE OF NANOCOCHLEATE

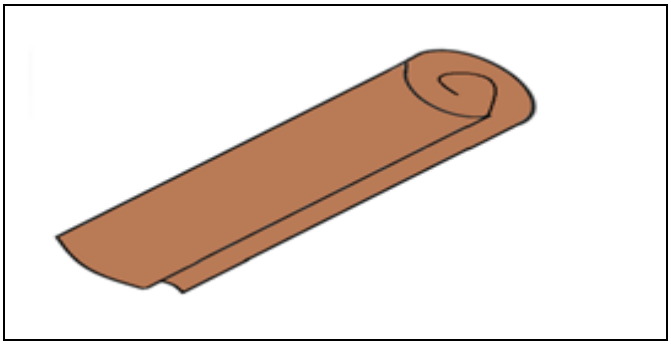

FIG. 2: ROLLED STRUCTURED OF NANOCOCHLEATE

Liposome fusion with the help of calcium forms large multilayer sheets. These liposomes are rolled up through the interaction with the divalent cation in **Fig. 3**. The large multilayer sheets consist of hydrophobic surfaces and, to minimize the water interactions, they tend to roll up in a cigar-like structure in **Fig. 2** [5, 9]. The molecular mechanism is dehydration of the phosphatidyl head group, which is vital in allowing a close approach of bilayers and begin the process of cochleate cylinder formation. The lipid bilayer is aligned in the proximity of 54 Å at a repeating distance [6].

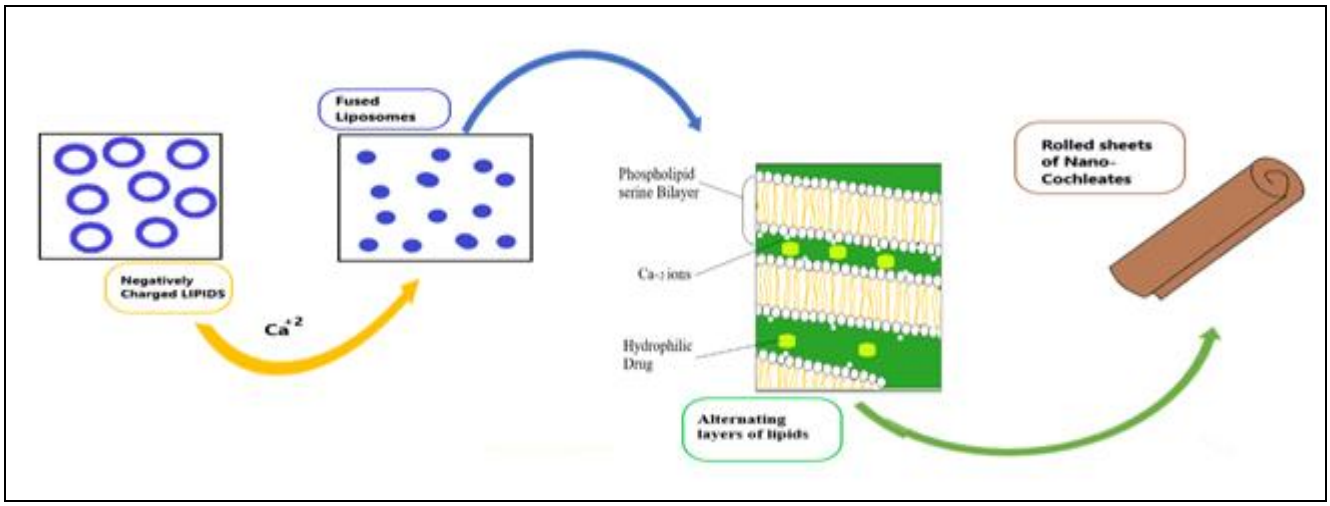

FIG. 3: NANOCOCHLEATE FORMATION FROM THE INTERACTION OF NEGATIVE PHOSPHOLIPIDS AND CATIONIC DIVALENT ION-MOLECULE

Difference between Liposomes and Nano-cochleate: Nanocochleates and liposomes are both lipid-based formulations. Nanocochleates are favoured over other lipid-based formulations because they are less susceptible to oxidation [10]. The difference between liposomes and Nano-

cochleates is shown in **Table 1**. The structure of liposome is represented in **Fig. 4**. Incorporation of biological compounds in Nanocochleate provides high potential for controlled release of drug and for improving oral bioavailability as they are less prone to degradation [11]. Cochleate is an excellent system for delivering hydrophobic substances through the matrix of a lipid bilayer with better stability than liposomes [12].

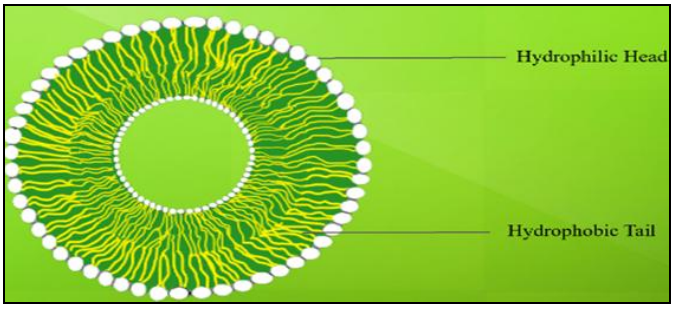

FIG. 4: LIPOSOME STRUCTURE

TABLE 1: DIFFERENCE BETWEEN LIPOSOMES AND NANOCOCHLEATE [13, 14, 15, 16, 17]

Parameter	Liposomes	Nanocochleate
Description	They consist of one or more concentric lipid bilayers, which enclose an internal aqueous volume	Cochleates are solid particulates made of large continuous, lipid bilayer sheets which are coiled with no internal aqueous phase
Composition	Phospholipids, cholesterol	Additionally Cation is required
Nature of internal core	Internal aqueous volume	Non-aqueous internal core
Stability	They are less stable because lipids are more susceptible to oxidation	Stability is more than liposomes as lipids are less prone to oxidation due to coiled structure
Entrapment efficiency	Low entrapment efficiency	High entrapment efficiency

Advantages of Nanocochleate Over other Drug Carrier Systems: The advantages of cochleates are numerous;

1. In comparison to liposomes, cochleates are more stable as they have a non-aqueous inner core and the lipids are less prone to oxidation [12].
2. Lyophilization process is used for increasing the shelf life of formulation and storage at room temperature.
3. Encochleated drugs can be protected from degradation caused due to environmental factors such as sunlight, water, oxygen, temperature, or gastrointestinal enzymes [4, 18].
4. Drugs that are required to be administered parenterally can be administered orally as cochleates *e.g.* Amphotericin B [19].
5. Nanocochleate can also be considered a safer and biocompatible delivery vehicle because the lipid bilayer consists of simple lipids that are naturally occurring, and therefore lipids are non-toxic, non-immunogenic, and non-inflammatory.
6. They enhance the oral bioavailability of a wide spectrum of compounds, such as those with high lipophilicity, genes, vaccines, protein, and peptides biopharmaceuticals products, which are difficult to administer *e.g.* Ibuprofen, Artemisinin [20].
7. They allow efficient incorporation of biological molecules with high molecular weight containing hydrophobic moieties e.g. Insulin Nanocochleate [21].
8. Encochleation of hydrophobic drugs and antigens containing hydrophobic fragment into the lipid bilayer of the cochleate structure is possible *e.g.* Amphotericin B, Clofazimine [8].
9. Permeability of the drug through the intestinal lumen is increased in this form of formulation. *e.g.* nanocochleate formulation of rifampicin shows more than two-fold increase in the apparent permeability over normal rifampicin [22].
10. Vaccines usually contain live-attenuated or inactivated pathogens for the prevention of disease. The vaccine adjuvant delivery system (VADS) was used with the carriers such as virosomes, liposomes, cochleates, which not only protect antigens but deliver them to particular lymphocytes for generating faster, stronger and long-lasting immune response [23].

Disadvantage: The disadvantages of nanocochleate and methods to overcome the disadvantages are mentioned in **Table 2**.

TABLE 2: DISADVANTAGES OF NANOCOCHLEATE AND METHODS TO OVERCOME THEM [6, 24, 25]

Disadvantages	Methods to overcome
Aggregation may happen at times during storage	Use of aggregation inhibitor *e.g.* Bovine serum albumin (BSA), methylcellulose, and casein.
High production costs	Using cost-effective techniques.
Stability problem during storage	The addition of PEG *e.g.* Insulin loaded Nanocochleate were PEGylated, Also, thermostable lipids and polyelectrolyte coating will enhance the shelf-life.

Composition of Nanocochleate Drug Delivery System: The main constituents used in the preparation of Nanocochleates are lipids and cations. Different types of drugs can be encapsulated in Nanocochleate, as shown in **Table 3**.

TABLE 3: COMPONENTS OF NANOCOCHLEATE 10, 26, 27

Class	Examples	Roles
Lipids	Naturally lipids: phosphatidyl-serine (PS), phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylcholine (PC). Synthetic lipids : dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylserine (DSPS), dioleoyl-phosphatidyl-serine (DOPS), dipalmitoylphosphatidylgycerol (DPPG)	Anionic phosphatidylgroup interacts with cation for formation of Nanocochleates.
Cations (divalent)	Zn^{+2}, Ca^{+2}, Ba^{+2}, Mg^{+2}, Fe^{2+}.	
Drug	Protein, peptide, polynucleotide, herbal product, antiviral agent, anaesthetic, vitamin, anticancer agent, immunosuppressant, NSAIDS, tranquilizer, nutritional supplement.	Active

Lipids: Lipid bilayer consists of simple lipids that are naturally occurring, hence, lipids are non-toxic, non-immunogenic and non-inflammatory which makes Nanocochleate a safe and biocompatible delivery vehicle [26]. Mixtures of anionic and zwitterionic lipids can also be used to prepare nano-cochleate [6]. The type of anionic lipid component determines the size and abundance of liposomes and cochleates.

Table 4 gives an overview of lipids used in Nanocochleate delivery systems.

Cations: In cochleate structure, the rolling of the lipid sheets is facilitated by the cations. Divalent cations are preferred over multivalent ones. The outer bilayered structure of the anionic phospho-lipid begins to collapse in the presence of a multivalent cation. It has been reported in the literature that Ca^{2+} has a ten-fold greater intrinsic binding constant than Mg^{2+} for phosphatidylserine. Hence Ca^{2+} forms a more tightly packed, highly ordered and less hydrated structure with phospholipidsthan does Mg^{2+} [28]. It is required in much lower concen-tration than Mg^{2+}. The Ca^{2+} plays an important role in natural membrane fusion phenomena, while other divalent cations are ineffective in most such systems. Hence calcium is the most compatible and most acceptable divalent cation reported for preparing cochleates [24].

TABLE 4: OVERVIEW OF LIPIDS USED IN NANOCOCHLEATE DELIVERY SYSTEMS 18, 29, 30

Lipids	Type of charge	Description
Phosphatidylcholine (PC)	Zwitterionic	✓ Major component of lecithin. ✓ Main functional constituent of the natural surfactants. ✓ Important substrate of acetylcholine. ✓ e.g. Andrographolide, paclitaxel nanocochleates ✓ Commonly used in the preparation of nanocochleates
Phosphatidylserine (PS)	Anionic	✓ play a vital role in phagocytosis ✓ Studies show that PS is very safe and may play a vital part in the assistance of mental functions in the aging brain. ✓ e.g. Andrographolide nanocochleates ✓ Commonly used in the preparation of nanocochleates
Phosphatidylethanolamine (PE)	Zwitterionic	✓ Second most ample phospholipid in plant and animal lipids. ✓ Main lipid component of microbial membranes. ✓ key building block of membrane bilayers

Phosphatidylinositol (PI)	Anionic	✓ Not commonly used in the preparation of nanocochleates ✓ It is a key membrane component hence vital lipid. ✓ It plays an important role in metabolic processes in all plants and animals and some bacteria. ✓ Not commonly used in the preparation of Nanocochleates

Drug: Hydrophobic molecules are encochleated in the tail region of the lipid bilayer of Nanocochleate. When a charged moiety is included within the interspace between the bilayers of cochleates, calcium binds to the phospholipid head group or to the charged drug depending on the ionic condition of the drug. For negatively charged moiety such as DNA, the calcium cation forms bridge between the drug and phospholipid head group in the region. For a cationic drug such as aminoglycoside, direct electrostatic attraction between the drug and negatively charged phospholipid takes place. Here calcium acts by complexing two head groups of two bilayer [6, 24].

Salient Features:

1. Formation of Nanocochleate from liposomes is independent of the transition temperature of the lipid.
2. They are formed by the fusion of small liposomes rather than larger ones.
3. It involves the use of simple phospholipids hence it can be considered a safer and biocompatible delivery vehicle.
4. They can resist structural changes induced due to heating above the Transition temperature of the lipid.
5. Cochleates can be converted into liposomes after the addition of a Calcium chelator *e.g.*, EDTA [6].

Methods of Preparation: Small unilamellar negatively charged liposomes are condensed to form a cochleate, which consists of a series of solid lipid bilayers. Large sheets are prepared from the fusion of negatively charged phospholipid liposomes in the presence of calcium. For the generation of the cochleate, the first step is to prepare the liposome and then to treat it with a divalent cation to modify the spherical vesicle into a cochleate cylinder by one of the following methods.

1. Hydrogel method
2. Trapping method
3. Liposome before cochleates dialysis
4. Direct calcium (DC) dialysis method
5. Binary aqueous-aqueous emulsion system

1. Hydrogel Method: Steps involved in the formation of Nanochocleates by Hydrogel method are as follows:

Step 1: The small unilamellar drug-loaded liposomes are prepared by methods such as film hydration method followed by sonication.

Step 2: Drug-loaded liposomes are then added to Polymer- A *e.g.* dextran (molecular weight (MW) 200,000-500,000), Polyethylene glycol (MW 3400-8000).

Step 3: Polymer B solution is prepared *e.g.* Polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polyvinyl methyl ether (PVME).

Step 4: The liposome-Polymer A suspension is mixed with Polymer B solution by using injection. The two polymers being immiscible with each other form an aqueous two-phase system of polymers. This can be attained mechanically through a syringe pump at a suitably monitored rate, ideally at a rate of 1 to 10 ml/min.

Step 5: The cationic cross-linkage of the polymers is achieved by adding a solution of a divalent cation salt. It helps to generate nano-size cochleate.

Step 6: The formed cochleates are bathed in a buffer containing a positively charged molecule, which helps to eliminate polymer.

The addition of a calcium cation to the bathing buffer makes sure that the cochleate structures are intact throughout the washing step and continue to remain as precipitates [19, 27].

The drugs namely Amphotericin B, Nelfinavir have been explored to make cochleates by given method [31]. **Fig. 5** represents the Hydrogel method in flow chart.

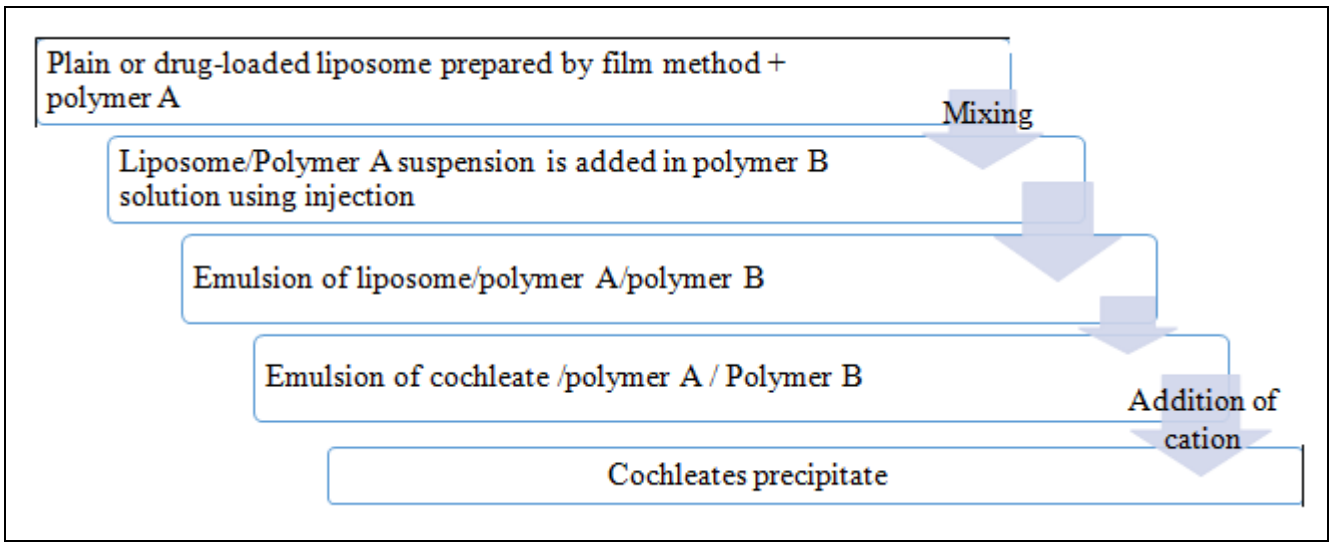

FIG. 5: HYDROGEL METHOD

2. Trapping Method: This method is useful for the encochleation of both hydrophilic and hydrophobic molecules. It consists of the formulation of the liposomal suspension, which encloses the drug either in the aqueous layer of liposome for hydrophilic drug or intercalated within the bilayers for hydrophobic drug. The Liposome can be prepared by either addition of water to phospholipid powder or by adding water phase to a phospholipid film. An assembly of cochleates is formed by the dropwise addition of calcium in liposomal suspension [32, 33]. The cochleates made by the trapping method present higher aggregation compared with other methods, which can be determined by electron microscopy after freeze-fracture. The process is explained as follows:

Step 1: Liposomes are prepared form Phospholipids such as phosphatidylserine by vortexing the solution for 15 min.

Step 2: Prepared liposomes are then separated from the above solution by filtration.

Step 3: Trapping solvent and the hydrophobic drug is added to the separated liposomes, *e.g.* of trapping solvent is ethanol, dimethylsulfoxide.

Step 4: Calcium chloride solution is added dropwise to the solution of Step-3 as a result of which the crystalline cochleates are precipitated.

Step 5: The resulting cochleates are washed with calcium-containing buffer to remove the residual solvent [6]. Examples of drugs explored by this method include Paclitaxel, Fisetin, Quercetin.

In the modified trapping method, DOPS (dioleoyl-phosphatidylserine) is dissolved in ethanol. Calcium Chloride ($CaCl_2$) is added and homogenized at 13000 rpm for 5 min. It is further stirred for 1 h [34, 35].

The trapping method is explained with the help of the flow chart in **Fig. 6**.

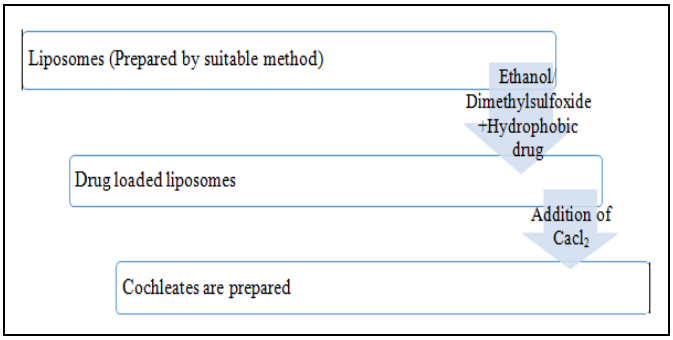

FIG. 6: TRAPPING METHOD

3. Liposome before Cochleates Dialysis: This method is used for preparing small-sized cochleates comprising of lipids, detergent, biologically relevant molecule, and cation. The objective of adding detergent is to disrupt the liposomes. Initially, the mixture is dialyzed using a buffer. Then Calcium chloride is added to form the cochleates. The detergent is removed by double dialysis. The procedure is described in the following steps:

Step 1: An aqueous suspension is prepared using the lipid-detergent mixture.

Step 2: Polymer A *e.g.* Polyethylene glycol (PEG), dextran or Phosphatidylserine is mixed with suspension prepared in step 1.

Step 3: The detergent-lipid/polymer A suspension is added to a solution comprising of polymer B *e.g.*, Polyvinylpyrrolidone (PVP), polyvinylalcohol (PVA), polyvinyl methyl ether (PVME). Polymer A and polymer B are immiscible and form a two-phase polymer system.

Step 4: A solution of a cationic moiety is added to the two-phase polymer system.

Step 5: The two-phase polymer system is washed to remove the polymer [31, 36].

The drugs *viz*., griseofulvin and cyclosporine were tried to make cochleates by given method [34]. The method produced cochleates of size 50-100nm.

4. Direct Calcium (DC) Dialysis Method: Intermediate liposome formation is absent in Direct calcium (DC) dialysis method when compared with Liposome before cochleates dialysis method. Large size cochleates are formed by using this method.

The mixture of lipid and detergent is directly dialyzed against calcium chloride solution. In this method, a competition between the removal of detergent from the detergent/lipid/drug micelles and the condensation of bilayers by calcium results in needle-shaped large dimensional structures.

Step 1: Phospholipid and cholesterol (9:1 weight ratio) are mixed in extraction buffer.

Step 2: Non-ionic detergent is added with a selected concentration of API and the solution is vortexed for 5 min.

Step 3: The clear solution prepared in step 2 is then dialyzed against three changes of buffers at room temperature.

Step 4: The final dialysis is performed in 6 mM Ca^{2+} solution, although 3 mM Ca^{2+} is sufficient.

The resultant white calcium phospholipid is DC cochleate [36].

5. Binary Aqueous-Aqueous Emulsion System: The method is based on the incompatibility between two-phase systems of Polymer solutions both of which are aqueous and immiscible with each other. This method does not require organic solvent.

Step 1: Liposomes are formed by high pH or film method.

Step 2: Liposomes are mixed with a polymer A *e.g.* Dextran.

Step 3: The dextran/liposome phase is then introduced into a second, non-miscible, polymer such as PEG.

Step 4: Calcium is then added in step 3 solution, which diffuses gently from one phase to another resulting in Nanocochleates. Later the gel is washed with the physiological buffer [37].

It is used to prepare cochleates of size less than 1000 nm [38].

Route of Administration: [37, 39] Oral drug delivery is the most preferred route for the delivery of nanocochleate as it increases patient compliance.

Different route of administration and dosage form for nanocochleate preparation are mentioned in **Table 5**.

TABLE 5: DIFFERENT ROUTES OF ADMINISTRATION AND DOSAGE FORM FOR NANOCOCHLEATE PREPARATION

Route of administration	Dosage form
Oral administration	Capsules, tablets, lozenges, powders, cachets, pills, granules, solutions, suspension and emulsion.
Topical or Transdermal administration	Powders, ointments, pastes, lotions, gels, sprays, solutions, creams, patches and inhalants.
Parenteral administration	Sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior use.

Mechanism of Drug Release Post Administration: After Oral administration of formulation, the absorption occurs *via* intestinal epithelial cells. It delivers the active drug molecules to the blood vessel.

After intravenous administration, the nanocochleate reaches directly into the blood circulation and it fuses with the cell membrane to deliver the drug into the cell cytoplasm [34].

Extensive studies were reported on the interaction between negatively charged phospholipids and calcium. Many naturally occurring membrane fusion events require the interaction of calcium with anionic phospholipids. Calcium induces disruption and reordering of membranes containing negatively charged lipids, as a result of which membrane fusion between the exterior layer of the cochleate and the cell membrane occurs. It is an important mechanism in many natural membrane fusion processes [24, 37].

The cell membrane of macrophages and neutrophils contains phosphatidylserine (PS) receptors which play a vital role in phagocytosis. When nano-cochleate comes close to a cell membrane, phago-cytosis occurs, which results in the delivery of a small amount of the encochleated material into the cytoplasm of the target cell [10].

Characterization of Nanocochleate:

Determination of Particle Size Distribution: The laser diffraction technique uses Malvern 2000SM (Malvern, UK) to measure the mean particle size of dispersed cochleates. Its analysis is done at an angle of detection of 90° and at a temperature of 30±2°C. The mean vesicle size is expressed in terms of volume mean diameter D, which is the average diameter of a sphere having volume same as that of the particle under measurement [40, 41].

Structure and Morphology of the Cochleates by Transmission Electron Microscopy (TEM): The morphology of the nanocochleate can be determined by using transmission electron microscopy (TEM). A drop of diluted sample is placed onto a carbon-coated copper grid to form a thin liquid film. Then the excess solution is removed, the sample is examined and photographed with a Zeiss EM 109 transmission electron microscope at an accelerating voltage of 80 Kv [42, 43, 44].

Drug Content: The dispersed nanocochleate suspension is centrifuged at 15,000 rpm for 40min at 25°C. The free drug Concentration in the supernatant can be determined by a method such as UV-Vis spectrophotometry after suitable dilution [26, 45].

Density: The density of nanocochleate is measured by helium or air with a gas pycnometer. The estimate achieved with air and helium is far more distinct due to the specific surface area and porosity of the structure [26].

Specific Surface Area: The specific surface area of lyophilized nanocochleate is measured with the help of a sorptometer.

Equation: $A = 6/\rho d$

Where A is the specific surface area, ρ is the density, and d is the diameter of the cochleate. In some cases, the measured and calculated specific surface areas fairly tally, while in some other cases, it will generate a small difference in the measured values because of residual structure [26].

Surface Charge Determination: The nature and intensity of nanocochleate surface charge determine its interaction with the biological environment and its electrostatic interaction with bioactive compounds. The surface charge can be estimated by evaluating the particle velocity in an electrical field. Laser diffractometry like Velocimetry or Laser Doppler Anemometry is used to determine the velocities of nanocochleate [26].

Entrapment Efficiency (EE) of Nanocochleate: An aliquot of 100 µl of cochleates is taken into centrifugation tubes. To each tube, 60 µl of pH 9.5 EDTA and 1ml of ethanol is added while vortexing. The resulting solution is clear and colorless. The samples are suitably diluted, and absorbance was measured to calculate entrapment efficiency as per equations [46, 47].

Entrapment efficiency = Amount of API present in cochleates / Total amount of API

Cochleates Cell Interaction Study: To examine the interaction of cochleates with cell membrane, 2% fluorescent lipid is mixed with negatively charged lipids to form fluorescent cochleates. When cochleates interact with the cell membrane involving a fluorescent lipid transfer, cell surfaces become fluorescent and can be observed under fluorescent microscopes [10]. Villa *et al.*, reported in a study that cell surfaces become fluorescent when exposed to nanometre-sized cochleates [48].

Stability Study: For stability study, cochleates dispersions can be maintained at a temperature of 2 to 8 °C and 25±2 °C/60% RH for 3 month. The stability of the formulation is determined in terms of change in entrapment efficiency (%EE) and particle size of cochleates [47, 49].

***In-vitro* Drug Release Study:**

Diffusion Cell Method: In the diffusion cell method, double chambers diffusion cells on a shake stand are usually used. The Millipore low protein binding membrane is kept between the two chambers. The receptor chamber contains phosphate buffer; donor chamber is filled with the formulation. The receptor compartment is assayed for the released drug at different time intervals by using standard analytical methods [50].

Application of Nanocochleate:

Delivery of Factor VIII: Factor VIII is a glycoprotein. It reacts with factor IX to form a sequence of reactions that initiates the formation of a blood clot. The absence of factor VIII is called as Haemophilia. It causes an increase in clotting time which increases the bleeding and can cause the death of the patient. Factor VIII shows poor solubility and poor stability because of its protein nature. So, antibodies are developed against such proteins when administered directly into the body. Cochleates are prepared to reduce the toxic effect and immune response such that no antibodies are produced. Matthew *et al*., concluded that cochleates containing factor VIII escape rapid RES mediated clearance observed with PS liposomes. The low immunogenicity of factor VIII and delayed-release property make this formulation a viable delivery system [28, 34].

Oral Mucosal Vaccine Adjuvant-Delivery System (VADS): Vaccines usually contain live-attenuated or inactivated pathogens for the prevention of diseases like polio, rubella, tetanus, measles, mumps, *etc*. The vaccine adjuvant delivery system (VADS) was made with cochleates that protect antigens as well as deliver them to particular lymphocytes for generating faster, stronger and long-lasting immune responses. Cochleates are generally engulfed by antigen-presenting cells (APCs) through receptor-mediated phagocytosis, which in turn results in antibody production. Conventional methods for the preparation of vaccines involve several time-consuming processes that hamper cochleates.

Wang *et al*., in a study, prepared adjuvant lipid-A incorporated cochleates (LACs) entrapping a BSA (antigen bovine serum albumin) using the emulsification-lyophilization technique. The study concluded that LACs induce a mixed response against antigens to establish both systemic and mucosal immunity against pathogens [51].

ApoA1 Formulation: ApoA1 (natural lipoprotein) is a crucial HDL considered to be the most essential in enzymatic esterification of cholesterol and act as a carrier for the same to reach the liver, which protects the blood vessels from atherosclerosis. Parenteral administration of ApoA1 lipoprotein increases the HDL ability to act as a carrier for cholesterol to liver and safeguard from atherosclerosis. Since ApoA1 is a protein, it shows poor intrinsic permeability. It is degraded by GIT enzymes and undergoes rapid post-absorptive clearance.

Nanocochleate oral formulation of ApoA1 can increase patient compliance and bring a change in the therapeutics of various heart diseases and atherosclerosis arising from Low-Density Lipoprotein levels and high blood cholesterol [34].

Delivery of Antifungal Agents: Amphotericin B (AmB) is an antifungal agent that shows a narrow therapeutic index and adverse drug effects, specifically nephrotoxicity, which limits its use. Santangelo *et al*., concluded that AmB loaded cochleates are highly effective for the oral delivery of AmB, which is currently possible through injectable formulations only. It shows it improved stability and efficacy at low doses and improved patient compliance [52].

Topical Drug Delivery: Ketoconazole (KCZ) is an antifungal drug and usually used in the treatment of fungal infections such as athlete's foot, ringworm, and candidiasis. Landge *et al*. presented a study in which ketoconazole is successfully entrapped in cochleates and used for dermal and transdermal delivery of drugs [49].

Delivery of Antibacterial Agent: The antibacterial agent like clofazimine, aminoglycosides, beta-lactam/beta-lactamase combinations, vancomycin, imipenem, and ethionamide are prescribed for long term therapies, thereby produce toxicity and drug resistance in the host. It was reported in a study that free Clofazimine was 500 times more toxic than Clofazimine cochleate hence clofazimine cochleate decreases the toxicity and improved the bactericidal activity [6]. Rifampicin shows variable absorption in the presence of other anti-TB drugs; hence it is

required to be given in a high dose. To overcome this problem, Rifampicin nanocochleate was prepared. Yadav *et al.,* reported that Rifampicin nanocochleate showed a significant increase in the apparent permeability of drug [22].

Delivery of Fisetin: Fisetin is a natural flavonoid showing anticancer properties. The challenges in the delivery of Fisetin are poor aqueous solubility and extensive *in-vivo* metabolism. To overcome this problem Fisetin loaded nanocochleate was prepared. Bothiraja *et al.* reported that Fisetin-loaded nanocochleate exhibited higher drug loading, sustained release, and improved *in-vitro* anticancer potency of Fisetin *via* intraperitonal route. Nanocochleate enhanced systemic bioavail-ability with low tissue distribution [53].

Delivery of Insulin: Magnetocochleate can be developed by fused lipid microstructure which is embedded with ferrite nanoparticles. They are used to encapsulate biologically relevant macro-molecules. It is reported that insulin is encapsulated in magnetocochleate by changing the lipid phase transition from the fluidic lamellar phase to the gel phase at pH 2. Dwivedi *et al.*, reported that magnetocochleate is a potential method for delivery of macromolecules through subcutaneous injection. Encapsulation of drugs by magnetocochleate increases its bioavailability, stability, and shelf life [54]. A review of some more drugs used in nanocochleate and their application is compiled in **Table 6**.

TABLE 6: DRUGS USED IN COCHLEATES AND THEIR APPLICATIONS

Drug	Category	Route of administration	Method of preparation	Application
Paclitaxel [55]	Anti-cancer	Oral	Trapping method	Enhanced oral bioavailability with low tissue distribution than its free form and nanoliposomes.
Andrographolide [18]	Anti-inflammatory, Anti-diabetic	Oral, Parentral	Trapping method	Enhanced oral bioavailability, increased stability, high % EE.
Influenza Glycoprotein [56]	Anti-influenza	Oral	LC dialysis method	Induce antibody and cell mediated responses, systemically and on mucosal surfaces.
HIV-1 Proteins DNA [56]	Anti-HIV	Oral	DC dialysis method	Small quantities of encochleated DNA are required for inducing antigen specific response.

Commercial Status of Bioral® Technology: BioDelivery Sciences International, Inc. (BDSI) is a biopharmaceutical company engaged in developing clinically significant formulations of proven therapeutics. The Company in collaboration with the University of Medicine and Dentistry, New Jersey, and e Albany Medical College patented Bioral ® (cochleate) drug delivery technology which encochleates selected drugs or therapeutics. Systemic fungal infections can be treated by using the company's lead Bioral® formulation. It is an encochleated version of AmB (which the Company refers to as CAMB). Bioral® AMB formulation has the potential to deliver the drug through the oral route. After the completion of preclinical testing, IND application was submitted by BDSI to the FDA for CAMB in December 2006, which was accepted by the FDA [6]. The products under development using Bioral® technology are shown in **Table 7**.

TABLE 7: THE PRODUCTS UNDER DEVELOPMENT USING BIORAL® TECHNOLOGY

Bioral® technology	Application	Phase	Stage
Bioral® Amphotericin	Antifungal	Under preclinical trials	Inhouse commercialization
Bioral® NSAIDS and COX-2 inhibitors	Anti-inflammatory	Under preclinical trials	Available for licensing
Bioral® paclitaxel	Oncology	Under preclinical trials	Available for licensing
Bioral® siRNA therapeutics	Infectious diseases/ cancer	Under preclinical trials	Available for licensing

Future Prospects: Nanocochleate demonstrates applications in the delivery of various biologically relevant molecules such as antigens, proteins, polypeptides, polynucleotides, vitamins, minerals, and amino acids. Cochleates can be used for the delivery of photolabile drugs, drugs susceptible to oxidation, for masking bitter taste and odor of actives. Cochleates technology can focus on the use

of anionic marine lipids [57]. There is a future scope for investigation for alternative routes of administration such as intranasal, transdermal, and vaginal. It also shows potential as a carrier for targeted delivery of the drug-using targeting ligand. The delivery of the drug by active targeting can be achieved using ligand targets or magneto-cochleates in cancer, diabetes, tuberculosis, neurological diseases [54]. Gene transfer into a genome of a defective cell or hematopoietic cells can be done by combining a DNA plasmid and proteins with cochleates. Many genetic disorders can be cured using this technology platform.

CONCLUSION: Nanocochleate is a unique lipid-based system that overcomes the limitation of liposomes. It is used to encapsulate the drug molecules and protect it from the environmental harsh conditions with improved stability and shelf life. The encapsulation of peptide drugs in vesicular systems offers several advantages such as reduced toxicity, increased stability, and long circulation time. It is a promising tool for delivering the API genes, protein, peptides, vaccines, and antigens. Nanocochleate formulations show broad range of therapeutic applications such as peptide delivery, large DNA constructs, and plasmids (Bioral DNA vaccines and Bioral gene therapy); Bioral$^{(TM)}$ nano-cochleate technology; magneto-cochleates, oral delivery of amphotericin B (bioral amphotericin B), *etc*.

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