NANOCOCHLEATES: A NOVEL APPROACH FOR DRUG DELIVERY

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ABSTRACT
Nanocochlate is a unique tailor based system used for microencapsulation and delivery of therapeutics by entrapping them in supramolecular assemblies composed of negatively charged phospholipids and a divalent cation. It is a unique multilayered structure widely used for oral and systemic delivery of wide variety of molecules including genes, vaccines and antigens. This article highlights the history, manufacturing methodology, materials used, stability aspects, characterization, applications and advantages of nanocochlate drug delivery systems over other vesicular systems. In a whole nanocochlate represents a unique technology, suitable for oral and systemic delivery of important chemical and biological therapeutics and promises a potential drug delivery system encouraging the future researchers to explore and advance in this new area of delivery technology.

Keywords: Nanocochlate, liposomes, protein and peptides, vesicular system.

INTRODUCTION
In the past few decades, considerable attention has been focused on the development of new drug delivery system (NDDS). An ideal NDDS should fulfill two basic requirements:
It should deliver the drug at a rate directed as per the needs of the body, over the period of treatment and It should channel the active ingredient to the site of action.
The novel drug delivery system is most suitable and approachable in developing the delivery system which improves the therapeutic efficacy of new as well as pre-existing drugs, thus providing controlled and sustained drug delivery to the specific site. Conventional dosage
forms are unable to meet any of these requirements. At present, no available drug delivery system behaves ideally\textsuperscript{[1]}. Now-a-days vesicles as a carrier system have become the vehicle of choice in drug delivery\textsuperscript{[2]}. Encapsulation of the drug in these vesicular structures is an system which predicts to prolong the existence of drug in the systemic circulation\textsuperscript{[3]}. The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers. Vesicles consist of a diverse range of amphiphilic building blocks. They can be formed when these building blocks are confronted with water\textsuperscript{[4]}. Biologic origin of these vesicles was first reported in 1965 by Bingham, and was given the name Bingham bodies\textsuperscript{[5]}. The ultimate aim is to control degradation of drug and its loss, prevention of harmful side effects and increase the availability of the drug at the affected site\textsuperscript{[6]}. For the treatment of intracellular infections, conventional chemotherapy is considered ineffective, because of limited permeation of drugs into cells. This can be overcomed by the use of vesicular drug delivery systems\textsuperscript{[5]}. Vesicular drug delivery system has some of the advantages like:

1. Bioavailability is greatly improved, especially in the case of poorly soluble drugs.
2. Both hydrophilic and lipophilic drugs can be incorporated.
3. Delays elimination of rapidly metabolizable drugs and thus eventually function as sustained release systems\textsuperscript{[7]}.
4. Reduces the cost of therapy.
5. It provides an efficient method for drug delivery to the site of infection leading to reduced drug toxicity\textsuperscript{[8]}.
6. Reduces dose-related side effects.
7. Maintains therapeutic concentrations of drugs for longer time duration, thereby decreasing dosing frequency\textsuperscript{[7]}.

**TYPES OF VESICULAR SYSTEM**

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<thead>
<tr>
<th>Vesicular system</th>
<th>Description</th>
<th>Disadvantages</th>
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<tr>
<td>Liposomes</td>
<td>They consist of one or more concentric lipid bilayers, which enclose an internal aqueous volume\textsuperscript{[9]}</td>
<td>Leakage and fusion of encapsulated drug or molecules, Phospholipids undergoes oxidation and hydrolysis, Short half-life\textsuperscript{[10]}</td>
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<td>Niosomes</td>
<td>Drug is encapsulated in an vesicle, which itself is composed of a bilayer of non-ionic surface active agents\textsuperscript{[11]}</td>
<td>Vesicles aggregation, fusion &amp; leaking leading to physical instability, Hydrolysis of medicament\textsuperscript{[5]}</td>
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<tr>
<td>Liposomes</td>
<td>Pharmacosomes</td>
<td>Transferosomes</td>
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<td>Vesicle-based delivery systems particularly liposomes were quite successful in delivery of drugs across the membrane, because of their structural similarity with the cell membrane. Liposomes were first described by British haematologist, Dr. Alec Bangham in 1961 at the Babraham Institute in Cambridge. Liposomes were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. Liposomes resembled to the structure of plasma membrane. From the microscopic images, first evidence was obtained for the bilayer lipid structure of cell membrane. The word liposome was derived from two Greek words: lipo means &quot;fat&quot; and soma means &quot;body&quot;; it is so named because its composition is primarily of phospholipid.</td>
<td>They are defined as colloidal dispersions of drugs covalently bound to lipids and may exist as ultrafine vesicular, micellar or hexagonal aggregates. Covalent bonding is required to protect the leakage of drugs. Surface and bulk interaction of lipids with drugs is necessary. On storage it undergoes fusion and aggregation leading to hydrolysis.</td>
<td>They are specially optimized, ultraflexible lipid supramolecular aggregates, which consists of inner aqueous compartment surrounded by lipid-bilayers. Chemically unstable due to oxidative degradation. Formulations are expensive. Difficult to maintain purity of natural phospholipids.</td>
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Liposomes are considered to be simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule.\textsuperscript{[24]}

![Fig. 1: Basic liposome structure.](image)

**Advantages of liposomal drug delivery system:**
Provides selective passive targeting to tumor tissues, increases efficacy and therapeutic index of drug molecule, increases stability via encapsulation, reduces toxicity of the encapsulated agents, shows site avoidance effect, improves pharmacokinetic parameters of drug molecule (reduced elimination, increased circulation life times), imparts flexibility to couple with site specific ligands to achieve active targeting\textsuperscript{[25]}, help to reduce exposure of sensitive tissues to toxic drugs\textsuperscript{[24]}, liposomes are biocompatible, completely biodegradable, non-toxic, flexible and non-immunogenic for systemic and non-systemic administrations.

**Types of liposomes**\textsuperscript{[26-31]}
Liposomes are classified on the basis of their structural parameters, method of preparation, composition and applications.

[a] **Based on structural parameters**
1. Uni-Lamellar Vesicles:
   - Small Uni-lamellar Vesicles(SUV): Size ranges from 20-40 nm
   - Medium Uni-lamellar Vesicles(MUV): Size ranges from 40-80 nm.
   - Large Uni-lamellar Vesicles(LUV): Size ranges from 100-1000 nm.
2. Oligo-Lamellar Vesicles(OLV): These are made of 2 to 10 lipid bilayers surrounding a large internal volume.
3. Multi-Lamellar Vesicles (MLV): They compose of several lipid-bilayers. Their arrangement can be ONION-like concentric spherical bilayers of LUV/MLV enclosing a large number of SUV etc.

[b] Based on method of liposome preparation
1. REV:- Single or Oligolamellar vesicles made by Reverse-Phase Evaporation Method.
2. MLV-REV:- Multi-lamellar Vesicles made by the Reverse-Phase Evaporation Method.
3. SPLV:- Stable Pluri-Lamellar Vesicles.
4. FATMLV:- Frozen & Thawed MLV.
5. VET:- Vesicles prepared by Extrusion Technique.
6. DRV:- Dehydration-Rehydration Method.
7. BSV:- Bubblesomes.

c] Based on composition & application
1. Conventional Liposomes (CL):- Neutral or negatively charged phospholipids & cholesterol.
2. Fusogenic Liposomes (RSEV):- Reconstituted Sendai Virus Envelopes.
3. pH sensitive Liposomes:- Phospholipids such as phosphatidyl-ethanolamine (PE) or 1,2 dioleoyl phosphatidyl-ethanolamine (DOPE).
4. Cationic Liposomes:- Cationic lipids with DOPE.
5. Long Circulatory (Stealth) Liposomes (LCL):- They have Poly-Ethylene Glycol (PEG) derivatives attached to their surface to decrease their detection by phagocyte system. The attachment of PEG to liposomes decreases the clearance from blood stream & extends circulation time of liposomes in the body. The attachment of PEG is also known as “Pegylation”.
6. Immuno-Liposomes:- CL or LCL with attached monoclonal antibody or recognition sequence.

Architect of liposomes[32]
1. Phospholipids: Glycerol represents more than 50% of the weight of lipid in the biological membranes. Thus glycerol containing phospholipids are most common in use. These are derived from phosphatidic acid. For stable liposomes, saturated fatty acids are generally used, whereas unsaturated fatty acids are less common in use. Examples of phospholipids are:- phosphatidyl Choline(Lecithin)(PC), phosphatidyl Serine(PS), phosphatidyl Glycerol(PG), phosphatidyl Inositol(PI).
2. **Sphingolipids**: They are present in animal & plant cells. They contain three characteristic building blocks, fatty acid molecule, sphingosine molecule and a head group (simple alcohol, choline or complex carbohydrates). Most common sphingolipids are sphingomyelin & glycosphingolipids.

3. **Sterols**: Cholesterol & its derivatives are used in liposome preparation. They serve following functions in liposomes, decreasing fluidity, reducing permeability of membrane to water soluble molecules and stabilizing membrane in presence of biological fluids. In the absence of cholesterol, liposomes tend to interact with plasma proteins. These proteins extract the phospholipids from liposomes, thereby depleting the outer monolayer of vesicles creating physical instability. Cholesterol reduces this type of interaction.

4. **Synthetic phospholipids**

Examples of saturated phospholipids are dipalmitoyl phosphatidyl choline(DPPC), distearoyl phosphatidyl choline(DSPC), dipalmitoyl phosphatidic acid(DPPA), dipalmitoyl phosphatidyl glycerol(DPPG).

Examples of unsaturated phospholipids are, dioleoyl phosphatidyl choline(DOPC), dioleoyl phosphatidyl glycerol(DOPG).

5. **Polymeric materials**: Synthetic phospholipids containing diacetylenic group in the hydrocarbon chain when exposed to U.V. light polymerizes & leads to formation of polymerized liposomes. These liposomes have high permeability barriers to entrapped drugs.

6. **Polymer bearing lipids**: Stability of repulsive interactions is governed by repulsive electrostatic forces. This repulsion can be induced by coating surface of liposome with charged polymers. Non-ionic & water compatible polymers like polyethylene oxide, polyvinyl alcohol & poly-oxa-zolidines can be used for coating liposomal surface.

7. **Cationic lipids**

- DODAB/C- Di-Octa-Decyl Dimethyl Ammonium Bromide/Chloride.
- DOTAP- Di-Oleoyl Propyl Trimethyl Ammonium Chloride.
Methods of liposome formation

![Diagram of liposome formation](image)

Fig. 2: Mechanism of liposome formation\(^{[33]}\).  

![Diagram of liposome preparation](image)

Fig. 3: General scheme for liposome preparation & drug loading\(^{[34]}\).

![Diagram of liposome preparation methods](image)

Fig. 4: Methods of liposome preparation\(^{[34]}\).

Therapeutic applications of liposomes\(^{[24]}\)

a) Liposome are used for drug/protein delivery vehicles: For controlled and sustained drug release, enhanced drug solubilization, altered pharmacokinetics and biodistribution, enzyme replacement therapy and biodistribution, enzyme replacement therapy and lysosomal storage disorders.
b) Liposome in tumor therapy is used as a carrier of small cytotoxic molecules & vehicle for macromolecules as cytokines or genes.

c) Liposomes are used in Gene Delivery for gene and antisense therapy as well as genetic (DNA) vaccination.

d) Liposome are used as immuno-adjuvants, immuno-modulator & in immuno-diagnosis.

e) Liposome are also used as artificial blood surrogates.

f) Liposome are used as radio diagnostic carriers.

g) Liposome are used in several cosmetics and dermatology products.

h) Liposome is also used in enzyme immobilization and bioreactor technology.

Limitations of liposomal drug delivery\textsuperscript{[24]}

Cost of production is high, phospholipid may sometimes undergo oxidation & hydrolysis like reaction, short half-life, low solubility, poor mechanical stability due to leakage & fusion of the formulation, low entrapment efficiency. So the need of the hour was to develop an formulation to answer the above limitations of liposome based vesicular drug delivery systems. Cochleates are the vesicular system which could satisfy the present needs of the market.

**COCHLEATE**: Various formulation modifications with liposomes allowed the development of a new class of drug vehicles called “COCHLEATE”\textsuperscript{[35]}. Cochleates are solid particulates made of large continuous, lipid bi-layer sheets rolled up in a spiral structure with no internal aqueous phase. This technology was able to answer the challenges of oral delivery of different kind of biological molecules, especially the hydrophobic ones. Cochleates differ from liposomes in having water-free interior, rod-shape & rigid stable structure\textsuperscript{[36]}.

![Fig. 5: Structural difference between liposomes & cochleates.](image-url)
These unique characteristics make cochleates a great platform for delivery of drugs that were having poor bioavailability\[^{36}\]. It is most versatile technology for the delivery of a wide range of drugs and molecules such as proteins and peptides, polynucleotide, antiviral agent, anaesthetic, anticancer agent, immunosuppressant, steroidal anti-inflammatory agent, non-steroidal anti-inflammatory agents, tranquilizer, nutritional supplement, herbal product, vitamin. Thus it provides a potential delivery system for the wide class of drugs\[^{37}\].

**History:** Cochleates were discovered in 1975 by Dr. Dimitrious Papahadjoupoulos and his co-workers as precipitates formed by the interaction of negatively charged phosphatidylserine and calcium. He named these cylindrical structures "COCHLEATE". In Greek, it means “SHELL” because of their rolled-up form\[^{38}\]. In the late ‘80s & ‘90s, cochleates were used to transport antigens and peptides for vaccine delivery. Cochleate structure is either aggregates of stacked sheets formed by trapping method or large size needles-like structures formed by the dialysis method\[^{39}\]. In 1999, cochleates were introduced to develop smaller, but rather more consistent particles. It was demonstrated that by using a binary phase system, such as two non-miscible hydrogels; cochleates can be formed that display a small mean particle of less than 500 nm. These nanocochleates were highly suitable for the encapsulation of hydrophobic drugs\[^{40}\].

In the initial time, cochleates were prepared in micrometer sizes\[^{41}\] either by direct addition of multivalent ion solution to liposome’s solution or by the dialysis method. However, the particle size could not be reduced to nanometer range. Perhaps in recent past a new method named “Hydrogel-Isolated cochleation” to prepare nanometer-sized cochleates was coined\[^{42}\].

**Basics:** Cochleate and nanocochleate are cigar like spiral rolls formed of negatively charged phospholipid bilayers, which are rolled up through the interaction with multivalent counter ions(Ca\(^{2+}\) or Zn\(^{2+}\)) as bridging agents between the bilayers. They roll-up in order to minimize their interaction with water\[^{39}\]. They possess little or no aqueous phase. The entire nanocochleate structure is a series of solid layers. Thus even if the outer layers of nanocochleate are exposed to harsh environmental conditions or enzymes, the encapsulated drug molecules will remain intact within the interior\[^{43}\]. As a particulate system, cochleates possess unique properties like superior mechanical stability and better protection for encapsulated drugs compared with liposomes due to their solid matrix. These solid particles are so flexible that they can readily convert to liposomes by extracting the bridging counter
ions out of the inter bilayer spaces\textsuperscript{[44]}. Nanocochleates contain both hydrophobic and hydrophilic surface which makes it suitable for encapsulation of both hydrophobic drugs like amphotericin B and clofazimine and amphiphilic drug like doxorubicin. The loading capacity of the cochleates depends upon the physical chemistry of the drug to encapsulate, whereas the particle size of the complex formed depends on the process used to encapsulate\textsuperscript{[36]}. Such unique properties have made cochleates an ideal system for delivering insoluble ingredients which can be loaded in the matrix of a phospholipid bilayer while avoiding the instability problem of liposomes\textsuperscript{[44]}.

Fig. 6: Nanocochlate formation by interaction between negative lipids and cations\textsuperscript{[45]}.

**Components of nano-cochlate drug delivery system\textsuperscript{[46]}:**

The three major components used in preparation of nanocochleates are:- API, Lipids and Cations.

**Table No 2: Components of nanocoachlate drug delivery system**

| LIPIDS | Phosphatidyl Serine[PS], Phosphatidic Acid[PA], Di-Oleoyl Phosphatidyl Serine[DOPS], Phosphatidyl Inositol[PI], Phosphatidyl Glycerol[PG], Phosphatidyl Choline[PC], Di-Myristoyl Phosphatidyl Serine[DMPS], Phosphatidyl Ethanolamine[PE], Di-Phosphatidyl Glycerol[DPG], Dioleoyl Phosphatidic Acid (DOPA), Di-Stearoyl Phosphatidyl Serine[DSPS], Di-Palmitoyl Phosphatidyl Glycerol[DPPG]. |
| CATIONS | Zn\textsuperscript{2+} or Ca\textsuperscript{2+} or Mg\textsuperscript{2+} or Ba\textsuperscript{2+} |
Table No 3: Brief description for some lipids used in nanocochleate delivery systems

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidyl choline(PC)</td>
<td>It is the major component of lecithin. It is the main functional constituent of the natural surfactants and the body's foremost reservoir of choline, an essential nutrient[^{47}].</td>
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<tr>
<td>Phosphatidyl ethanolamine (PE)</td>
<td>1,2-Diacyl-glycero-3-phosphahtidyl ethanolamine is the second most abundant phospholipid in animal and plant lipids. It is the main lipid component of microbial membranes. It is a key building block of membrane bilayers[^{48}].</td>
</tr>
<tr>
<td>Phosphatidyl inositol (PI)</td>
<td>It is an important lipid as it is a key membrane constituent. Also it is an participant in essential metabolic processes in all plants and animals and in some bacteria[^{49}].</td>
</tr>
<tr>
<td>Di-phosphatidyl glycerol(DPG)</td>
<td>Also named as Cardiolipin. It is found exclusively in certain membranes of bacteria(plasma membrane &amp; hydrogenosomes) and mitochondria of eukaryotes.</td>
</tr>
<tr>
<td>Phosphatidyl glycerol(PG)</td>
<td>It is a constituent of cell membranes typically present at 1-2% concentrations in most animal tissues. It is an important precursor of cardiolipin. It is found in all bacteria types.</td>
</tr>
</tbody>
</table>

**Methods of nanocochleate preparation:** Nanocochleates are derived from liposomes which are suspended in an aqueous two-phase polymer solution, enabling the differential partitioning of polar molecule based-structures by phase separation. The liposome-containing two-phase polymer solution, treated with positively charged molecules such as Ca++ or Zn++, forms a nanocochleate precipitate of a particle size less than one micron. The process may be used to produce nanocochleates containing biologically relevant molecules\[^{50}\]. There are several methods for cochleate preparation:-

1. **Hydrogel method:** This method comprises of following steps:
   
   **Step1:** A suspension of small unilamellar liposomes or biologically relevant molecule-loaded liposomes is prepared. This can be achieved by standard methods such as sonication or microfluidization or other related methods.
   
   **Step2:** The liposome suspension is mixed with polymer A such as dextran (mol wt-200,000-500,000), Polyethylene glycol (mol wt- 3400-8000) or Phosphatidylerine.
Step3: Preferably by injection, the liposome/Polymer A suspension is added into polymer B such as poly vinyl pyrrolidone, poly vinyl alcohol, ficoll (mol wt- 30,000-50,000), and poly vinyl methyl ether (PVMB)(mol wt- 60,000-160,000) in which polymer A is not miscible, leading to an aqueous two-phase system of polymers. This can be achieved mechanically by using a syringe pump at an appropriate controlled rate, for example a rate of 0.1 ml/min to 50 ml/min, and preferably at a rate of 1 to 10 ml/min.

Step4: A solution of cation salt is added to the two-phase system of above step, such that the cation diffuses into polymer B and then into the particles comprised of liposome/polymer A, allowing the formation of small-sized cochleates.

Step5: To isolate the cochleate structures and to remove the polymer solution, cochleate precipitates are repeatedly washed with a buffer containing a positively charged molecule, and more preferably, a divalent cation. Addition of a positively charged molecule to the wash buffer ensures that the cochleate structures are maintained throughout the wash step, and that they remain as precipitates(fig:7)[51].

Fig. 7: Hydrogel isolation method[42].

2. Liposome before cochleates(LC)/dialysis method: In this method mixture of lipid and detergent are used as the starting material and the removal of detergent is made by double dialysis[52]. The detergent is added to disrupt the liposomes. The method comprises the
following steps: **Step1:** An aqueous suspension containing a detergent-lipid mixture is prepared.

**Step2:** The detergent-lipid suspension is mixed with polymer A such as dextran (mol wt-200,000-500,000), Polyethylene glycol (mol wt- 3400-8000) or Phosphatidyl-Serine.

**Step3:** The detergent-lipid/polymer A suspension is added into a solution comprising polymer B such as poly vinyl pyrolidone, poly vinyl alcohol, ficoll (mol wt- 30,000- 50,000), and poly vinyl methyl ether (PVMB) (mol wt- 60,000-160,000), wherein polymer A and polymer B are immiscible, thereby creating a two-phase polymer system.

**Step4:** A solution of a cationic moiety is added to the two-phase polymer system.

**Step5:** Washing the two-phase polymer system to remove the polymer(fig.8)\(^{[51]}\)

3. **Direct calcium (DC) dialysis method:** Unlike LC method this method dose not involves the intermediate liposome formation and the cochleates formed were large in size. The mixture of lipid and detergent was 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.

**Step1:** Mixture of phosphatidylserine and cholesterol (9:1 wt ratio) in extraction buffer and non-ionic detergent was mixed with a preselected concentration of API and the solution was vortexed for 5 minutes.

**Step2:** The clear, colorless solution was then dialyzed at room temperature against three buffers.

**Step3:** The final dialysis is done in 6 mM Ca\(^{2+}\) solution and buffer concentrations are maintained compatible to cochleate formation. The resulting white calcium-phospholipid is DC cochleate\(^{[50]}\).

4. **Binary aqueous-aqueous emulsion system:** In this method small liposomes were formed by either high pH or by film method, and then the liposomes are mixed with a polymer, such as dextran. The dextran/liposome phase is then injected into a second, non-miscible, polymer (i.e. PEG). The calcium was then added and diffused slowly from one phase to another forming nanocochleates. By this method the cochleates formed are of particle size less than 1000 nm(fig.9)\(^{[50]}\).
5. **Trapping method:** This method is useful for the encapsulation of hydrophilic and hydrophobic molecules\(^{39, 52}\). It consists of preparation of the liposomal suspension containing the drug either in the aqueous space of liposome (when hydrophilic) or intercalated in between the bilayers (when hydrophobic). A step of addition of calcium follows, and an aggregate of cochleates are formed. The cochleates made by the trapping method present higher aggregation compared with other methods. This has been demonstrated using electron microscopy after freeze-fracture(fig.10)\(^{53}\).

6. **Solvent drip method:** It consists of preparing a liposomal suspension separately based on soy PS and a hydrophobic or amphipathic cargo moiety solution. Solvent for hydrophobic drug can be selected from DMSO or DMF. The solution is then added to liposomal suspension. Since the solvent is miscible in water, a decrease of the solubility of the cargo moiety is observed, which associates at least in part with the lipid-hydrophobic liposomal bilayers. The cochleates are then obtained by addition of calcium and the excess solvent is being washed\(^{54}\).

**Route of administration**

Nanocochleate drug delivery vehicle allows an efficient oral delivery of drugs. An alternative route of administration can be parenteral, rectal, topical, sublingual, mucosal, nasal, ophthalmic, subcutaneous, intramuscular, intravenous, transdermal, spinal, intrathecal, intra-articular, intra-arterial, sub-arachnoid, bronchial, lymphatic, and intrauterine administration, intravaginal or any other mucosal surfaces\(^{42}\).

**Dosage forms for nanocochleate administration:** For oral administration: capsules, cachets, pills, tablets, lozenges, powders, granules, or as a solution or a suspension or an emulsion, for topical or transdermal administration: powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants, for parenteral administration: sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use\(^{55}\).

**Stability of nanocochleate formulations**

Encapsulation of the drug molecules in the interior provides protection and stability to the entire formulation. Because the entire structure is a series of solid lipid bilayers, components within the interior of this structure remain intact, even though the outer layers of it may be
exposed to harsh external environmental conditions or enzymes. The interior is essentially free of water and resistant to penetration by oxygen which has been resulted into increased shelf-life of the formulation. Nanocochleates may be lyophilized to a powder and stored at room temperature or 40°C. Lyophilized cochleates can be reconstituted with liquid prior to in-vitro use or in-vivo administration. Lyophilization has no adverse effects on cochlate’s morphology or functions\(^5\)\(^4\).

**Safety & compatibility of nano-cochlate formulations**

Phosphatidyl-Serine(PS) and calcium which are safe, simple, naturally occurring substances which makes nanocochlate a safe and biocompatible delivery vehicle as its an natural component of all biological membranes concentrated mostly in the brain. Soyabean Phosphatidyl-Serine is also an alternative source & its inexpensive, available in large quantities and suitable for use in humans. Nanocochleates which are composed of anionic lipids are non-inflammatory and bio-degradable\(^5\)\(^4\).

**Mechanism of drug delivery through cochlate formulation:** Absorption after oral administration takes place from intestine. Nanocochleates cross across the digestive epithelium and deliver their active drug molecules into blood vessel. In case of other route (except IV) they cross across the associated cell and reach into circulation. After reaching into circulation they are delivered to targeted cell(fig.11)\(^5\)\(^0\).

![Fig. 8: Absorption mechanism of nanocochlate](image)

The recently proposed hypothesis states that when lipid bi-layer structure of nanocochleates fuses with the cell membrane, then the contents of nanocochleates are delivered into cells, thus release of the drug occurs(fig.12)\(^3\)\(^6\).
Nanocochleate first comes into close approximation to a natural membrane, a perturbation and reordering of the cell membrane is induced, resulting in a fusion event between the outer layer of the nanocochleate and the cell membrane. This fusion results in the delivery of a small amount of the encochleated material into the cytoplasm of the target cell. The nanocochleate may slowly fuse or break free of the cell and be available for another fusion event, either with this or another cell\textsuperscript{[50]}.

**ADVANTAGES:** Below are few advantages of nano-cochleate formulation over the other delivery systems. Nanocochleates have a non-aqueous structure and therefore:

1. They are more stable than liposomes because the lipids in nanocochleates are less susceptible to oxidation. They maintain structure even after lyophilization, whereas liposome structures are destroyed by lyophilization\textsuperscript{[50]}.

2. With administration of live vaccine, there are many life threatening risk associated like allergies, inversion of vaccine effect to wild infection etc. These can be neglected by use of nanocochleates.

3. Multiple administrations of high doses of cochleate formulations to the same animal shows no toxicity and do not result in either the development of an immune response to the cochleate formulation.

4. They exhibit efficient incorporation of biological molecules, particularly with hydrophobic moieties into the lipid bilayer of the cochleate structure.

5. They have the potential for slow or timed release of the biological molecule *in-vivo* as nanocochleates slowly unwind or otherwise dissociate.

6. They have a lipid bilayer matrix which serves as a carrier and is composed of simple lipids which are found in animal and plant cell membranes, so that the lipids are non-toxic, non-immunogenic and non-inflammatory.
7. They improve oral bioavailability of a broad spectrum of compounds, such as those with poor water solubility, protein and peptides etc.
8. They reduce toxicity, stomach irritation and other side effects of the encapsulated drug.
9. They encapsulate or entrap the subject drug within a crystal matrix rather than chemically bonding with the drug.
10. They provide protection from degradation to the encochleated drug caused by exposure to environmental conditions such as sunlight, oxygen, water and temperature.

CHARACTERIZATION OF NANO-COCHLEATE FORMULATION

Particle size determination: The mean particle size of the liposomal dispersion and cochleates dispersion can be determined by laser diffraction technique using Malvern analyzer. Analysis is to be carried out at 30±2°C temperature keeping angle of detection 90°C\[56]\.

Entrapment efficiency(EE): One hundred micro liters of cochleates is aliquoted into centrifugation tubes. To each tube 60 µl pH 9.5 EDTA and 1ml of ethanol is added while vortexing. Absorbance of the resulting solution is determined using spectroscopic technique and entrapment efficiency is calculated using below mentioned equation\[56]\.

\[
\text{ENTRAPMENT EFFICIENCY} = \frac{\text{AMOUNT OF DRUG PRESENT IN COCHLEATES}}{\text{TOTAL AMOUNT PRESENT}}
\]

Stability study: Cochleates dispersions can be kept at 2-8°C and 25±2°C/60% RH for a period of 3 months to check their stability. The stability of the vesicles is determined in terms of change in particle size and percent entrapment efficiency\[56]\.

Cochleates-cell interaction: To examine the interaction of cochleates with cell membrane, 2% fluorescent lipid in addition to the negatively charged lipid is used to form fluorescent liposomes\[57, 58]\. When cochleates interact with cell membrane involving a fluorescent lipid transfer, cell surfaces become fluorescent under fluorescent microscopes as illustrated in fig. 13-A and B.
Fig. 10-A & B Mammalian skin cells exposed to the poly-lysine fluorescent nanocochleate.

Specific surface area: The specific surface area of freeze-dried nanocochleate can be determined with the help of a sorptometer. The equation given below can be used to calculate specific surface

\[ A = \frac{6}{\rho d} \]

Here A is the specific surface area, \( \rho \) is the density and d is the diameter of the cochleate\textsuperscript{[41]}. 

Surface charge: The nature and intensity of the surface charge of nanocochleate determines their interactions with the biological environment as well as their electrostatic interaction with bioactive compounds. The surface charge can be determined by measuring the particle velocity in an electric field. Laser light scattering techniques such as Laser Doppler Anemometry or Velocimetry (LDA/LDV) are used as fast and high-resolution techniques for determining nanocochleate velocities. The surface charge of colloidal particles can also be measured as electrophoretic mobility. The composition of charge decides the bio-distribution of drug carrying nanocochleate. Generally, the electrophoretic mobility of nanocochleate is determined in a phosphate saline buffer and human serum. The phosphate saline buffer (pH 7.4) reduces the absolute charge value due to ionic interaction of buffer components with the charged surface of nanocochleate. \textsuperscript{[58]}

In vitro release study: The \textit{in vitro} release profile of nanocochleates is determined using standard dialysis, diffusion cell or modified ultra-filtration techniques. Phosphate buffer with
double chamber diffusion cells on a shake stand is generally used. A Millipore, low protein-binding membrane is placed between the two chambers. The donor chamber is filled with nanocochleates and the receptor compartment is assayed at different time intervals for the released drug using standard procedures. The modified ultra-filtration technique is also used to determine the in vitro release behavior of nanocochleates. Here nanocochlate is added directly into a stirred ultra-filtration cell containing buffer. At different time intervals, aliquots of the medium are filtered through the ultra-filtration membrane & assayed for the released drug using standard procedures\textsuperscript{[59]}.

**APPLICATIONS**

1. For delivery of antibiotics:- Cochleate delivery system has potential for the delivery of antibacterial agents such as Aminoglycosides and Anti-TB drugs\textsuperscript{[60, 61]}. As to illustrate the Clofazimine entrapped cochleates exhibit a greater decrease in toxicity versus free clofazimine and had a higher efficacy in killing intracellular M. Tuberculosis than free clofazimine. This shows that encapsulation of clofazimine in cochleates potentiates the antimicrobial efficacy of the drug\textsuperscript{[62]}.

2. As carriers for topical delivery:- This novel structure reported to be more stable than liposomes, aids in dermal and transdermal release of the active constituent. Ketoconazole loaded cochleates retained antifungal activity with better permeability across the skin\textsuperscript{[56]}.

3. Nanocochleates can provide a good platform for the delivery of formulations by oral administration and can bring a revolution in the treatment of atherosclerosis and heart diseases originating from high blood cholesterol and LDL levels.

4. Nanocochleates have the ability to stabilize and protect an extended range of micronutrients and the potential to increase the nutritional value of processed foods.

5. Nanocochleates have been used to deliver proteins, peptides and DNA for vaccine and gene therapy applications.

6. Nanocochleates showed potential to deliver Amphotericin B, a potential antifungal agent, orally and parentally having a good safety profile with reduced cost of treatment. The prepared cochleates of amphotericin B showed improved stability and efficacy at low doses. They showed improved patient compliance & the formulation was approved by USFDA.

7. Bio Delivery Sciences International(BDSI), an US based company have developed nanocochleates which can be used to deliver nutrients such as vitamins, omega fatty acids more efficiently to cells, and lycopene without affecting the color and taste of food which makes the concept of super foodstuffs a reality\textsuperscript{[35, 63-65]}.
CONCLUSION
Nano-cochleate drug delivery system provides an unique platform for delivery of wide range of oral & systemic therapeutics including drugs, genes, and vaccine antigens. Encochleation helps to improve the efficiency of the final product by enhancing the qualities of formulation, increasing processing and shelf-life stability, enhancing bioavailability, reducing toxicity, and increasing efficacy. As nanocochleate possesses unique multilayered structure, it protects active agents inside which are to be carried. Thus nanocochleates defeats the disadvantages of other drug delivery systems. There is tremendous increase in patent filing and publications of nanocochleates indicating growing industrial interest as well as academic interest in the area of drug delivery. Thus nanocochleate drug delivery system is gaining more importance in pharmaceutical development for transfer of suitable & desired drug molecule into body with good potential.

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