

## Nanocochleate: Novel Bypass of Conventional Drug Delivery System

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### ABSTRACT

Nanocochleate is a novel lipid based drug delivery system offering systemic and oral delivery of various charged drug molecules. It is formed by negatively charged lipid bilayer. Nanocochleate can encapsulate drugs which are hydrophobic, positively charged, negatively charged and poor orally bioavailable. It is also very much beneficial for oral absorption for peptide drugs that possess a net positive charge. Nanocochleate is beneficial for molecules having binding sites inside the cell. The property that nanocochleates can facilitate cross membrane diffusion for charged and impermeable molecules finds wide application in drug delivery. Nanocochleate have been proven better than liposomes and more compatible with body environment. They differ from liposomes by its structure and compatibility to the body. Liposomes are lipid bilayer with aqueous inner environment and unlike liposomes nanocochleates are having charged lipid bilayer without aqueous environment and are multilayer lipid matrix i.e. Cochleate.

Various methods of its formulation, structure, merits demerits and wide range of application are described in this article.

**Keywords:** Nanocochleates, Lipid, Liposomes, Phospholipid, Cholesterol

### INTRODUCTION

Recently, many drug delivery platforms have emerged and are present in either in preclinical stage or in an advanced clinical trial with the intent of trying to demonstrate efficient oral absorption. Reported strategies to improve drug absorption through cross membrane diffusion included pro-drug analogue design, application of absorption enhancers and enzyme inhibitors and delivery by using lipid-based systems. Various approaches have been reported for oral delivery of tissue-impermeable drugs .for example i) converting a drug to lipophilic pro-drug, ii) conjugating a drug with lipophilic moieties, and iii) encapsulating a drug into particulate systems.

Particulate systems may offer good protection of delicate biological agents with no need for chemical modification of the molecules themselves. However, absorption of particles by the intestines is generally less than 1%. Because of the structural similarity of liposomes (phospholipid bilayer vesicles) to cellular membranes, the material had once been regarded as an ideal system for delivering therapeutic agents and attracted considerable research interest. However, utilization of liposomes to improve oral absorption of hydrophilic and hydrophobic drugs remains unsuccessful mainly due to their poor mechanical stability, low-drug loading capacity, and probably the lack of mechanism to facilitate

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cross membrane diffusion in intestine. To overcome this stability problem, several alternative lipid bilayer systems have been reported, namely, stealth liposomes [1], polymerized liposomes, polyethylene glycol coated liposomes, lipo-beads, and cochleates.

In particular, cochleate technology was shown to be effective in the therapeutic oral delivery of the hydrophobic drugs, which is negatively charged phospholipids bilayer rolled up by interaction with multi-cationic metal ions to form a rigid spiral rod. The nanocochleate drug delivery vehicle is based upon encapsulating drugs in a multi-layered, lipid crystal matrix (a cochleate) to potentially deliver the drug safely and effectively. It is different from liposome in that it has a water-free interior, a rod shape and a rigid structure. Freeze fracture electron microscopy reveals a typical cochleate cylinder characterized by the elongated shape and by the tight packed bilayers [2]. These unique characteristics make cochleates a great platform in delivery of drugs that were not orally bioavailable. Various formulations with liposomes have allowed the development of a new class of delivery vehicles called cochleates. These are stable, lipid based delivery formulations whose structure and properties are very different from liposomes. Liposomes are composed of lipid bilayer membranes with aqueous space bounded by the lipid bilayer. This lipid bilayer is susceptible to attack by harsh environment conditions like pH, lipase degradation and temperature. Cochleates protect the entrapped molecules from harsh environmental conditions. They are stable to lyophilisation and can be reconstituted with liquid prior to administration. Cochleate is most versatile technology for the delivery of a wide range of drugs and fragile molecules such as proteins and peptides. Cochleates are calcium-phospholipid structures composed of naturally occurring materials that “wrap” around the drug or nutrient being introduced into the body. Nanocochleates consist of a purified

phospholipid that contains at least about 75% by weight of lipid which can be phosphatidyl serine (PS), dioleoylphosphatidylserine (DOPS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidyl glycerol (PG) and /or a mixture of one or more of these lipids with other lipids. Additionally or alternatively, the lipid can include phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), dioleoylphosphatidic acid (DOPA), distearoyl phosphatidylserine (DSPS), and dimyristoyl phosphatidylserine (DMPS), dipalmitoyl phosphatidylglycerol (DPPG). A multivalent cation, which can be  $Zn^{+2}$  or  $Ca^{+2}$  or  $Mg^{+2}$  or  $Ba^{+2}$  and a drug, which can be protein, peptide, polynucleotide, antiviral agent, anaesthetic, anticancer agent, immunosuppressant, steroidal anti-inflammatory agent, non-steroidal anti-inflammatory agents, tranquilizer, nutritional supplement, herbal product, vitamin and/or vasodilatory agent. Thus it is proving to be potential carrier for the wide class of drug for the therapeutics

#### **MATERIAL USED FOR NANOCOCHEATES [3, 4]:**

Nano cochleates is a negatively charged phospholipids bilayer rolled up by interaction with multi-cationic metal ions to form a rigid spiral rod. Cochleates are calcium-phospholipid structures composed of naturally occurring materials that “wrap” around the drug or nutrient being introduced into the body.

Nanocochleates consists of

1. A purified soy based phospholipid that contains:-

At least about 75% by weight of lipid which can be phosphatidyl serine (PS), dioleoyl phosphatidylserine (DOPS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidyl glycerol (PG) and /or a mixture of one or more of these lipids with other lipids.

2. Additionally or alternatively, the lipid can include-

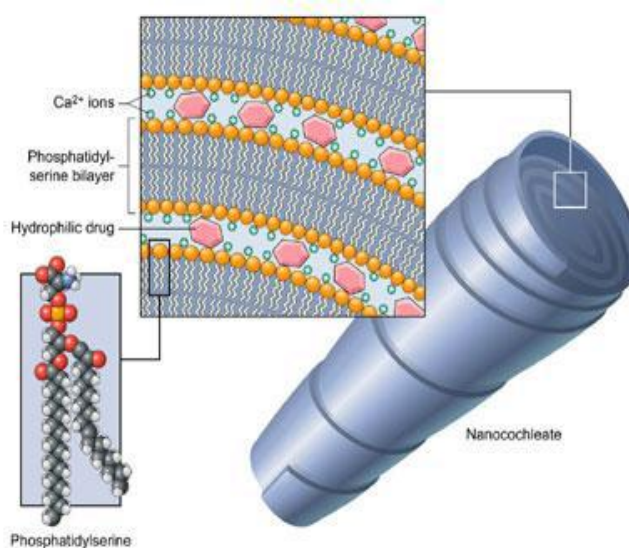
Phosphatidylcholine (PC), phosphatidyl ethanolamine (PE), diphosphatidylglycerol (DPG), dioleoyl phosphatidic acid (DOPA), distearoyl phosphatidylserine (DSPS), and dimyristoyl phosphatidylserine (DMPS), dipalmitoyl phosphatidylglycerol (DPPG).

3. A multivalent cation, which can be:-

Zn<sup>+2</sup> or Ca<sup>+2</sup> or Mg<sup>+2</sup> or Ba<sup>+2</sup>

4. A drug which can be:-

Protein, peptide, polynucleotide, antiviral agent, anaesthetic, anticancer agent, immunosuppressant, steroidal anti-inflammatory agent, non-steroidal anti-inflammatory agents, tranquilizer, nutritional supplement, herbal product, vitamin and/or vasodilatory agent. Thus it is proving to be potential carrier for the wide class of drug for the therapeutics.



**Figure 1: Nanococheate Structure**

#### METHOD OF PREPARATION <sup>[5]</sup>:

##### **Method 1 (hydrogel method):**

This method comprises of following steps:

**Step 1-** A suspension of small unilamellar liposomes or biologically relevant molecule-loaded liposomes is preparing. This can be achieved by standard methods such as sonication or micro fluidization or other related methods.

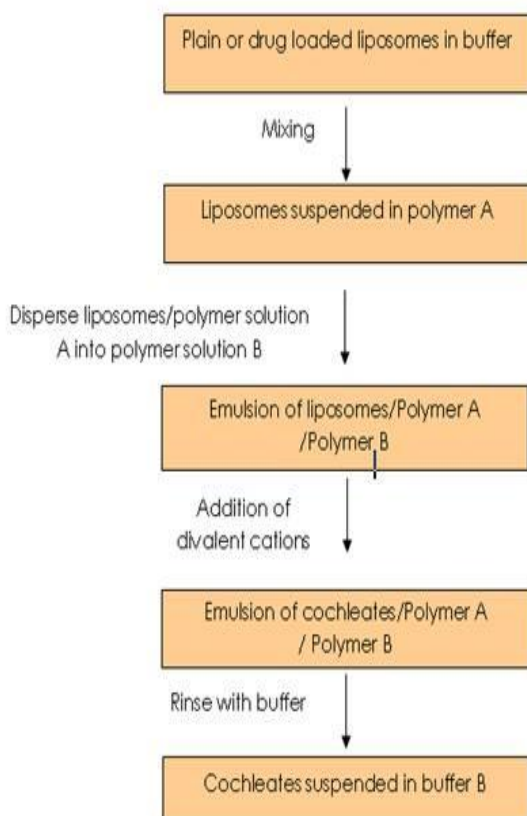
**Step 2-** The liposome suspension is mixed with polymers such as dextran (mol wt-200,000-500,000), polyethylene glycol (mol wt- 3400-8000) or phosphatidylserine.

**Step 3-** Preferably by injection, the liposome/polymer a suspension is added into another 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 nonmiscible, 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.

**Step 4-** A solution of cation salt is added to the two-phase system of step 3, such that the cation diffuses into polymer b and then into the particles comprised of liposome/polymer a allowing the formation of small-sized cocheates.

**Step 5-** Now 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.



**Figure 2: Hydrogel Method**

**Method 2 (liposomes before cochleates (lc) dialysis method):**

A second method for preparing the small-sized cochleates comprises detergent and a biologically relevant molecule and cation. 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 phosphatidylserine.

**Step3-** the detergent-lipid/polymer a suspension is added into a solution comprising 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), 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-** now wash the two-phase polymer system to remove the polymer.

**Method 3 (direct calcium (dc) dialysis method):**

Unlike lc method this method does not involve 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 polynucleotide and the solution was vortexed for 5 minutes.

**Step2-** the clear, colourless solution which resulted was dialyzed at room temperature against three changes of buffer.

**Step3-** the final dialysis used is 6 mM  $Ca^{2+}$ , and buffer concentrations are maintained compatible to cochleate formation. The resulting white calcium-phospholipid precipitates have been termed dc cochleates.

**Method 4 (trapping method):**

This method involves the formation of phosphatidylserine liposomes followed by drop

wise addition of a solution of calcium chloride. Liposomes can be generated by either addition

of water to phospholipid powder or by adding the water phase to a phospholipid film.

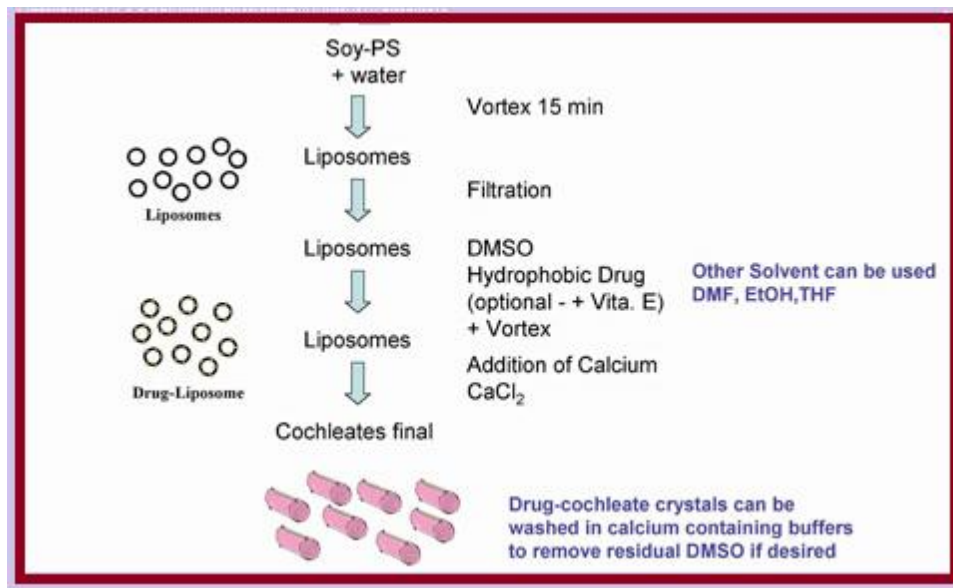


Fig. 3: Schematic presentation of trapping method.

**Method 5 (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, after which the gel is washed out. The nanocochleates proved to promote oral delivery of injectable drugs. By this method the cochleates formed are of particle size less than 1000 nm.

**STABILITY:**

Nanocochleates are stable system which is lipid based delivery formulations. Their structure and properties are very different from liposomes. Their unique structure provides protection from degradation for encapsulated molecules. The entire cochleate structure is a series of solid layers, components within the interior of the cochleate structure

remain intact, even though its outer layers may be exposed to harsh environmental conditions or enzymes, such as in the stomach. Because of this unique properties of nanocochleates were used to mediate and enhance the oral bioavailability of a broad spectrum of biopharmaceuticals, including compounds with poor water solubility, such as Amphotericin B<sup>[6, 7]</sup>. They can be reconstituted with liquid prior to administration as they are stable to lyophilisation.

**ADVANTAGES:**

1. They are more stable than liposomes because the lipids in nanocochleates are less susceptible to oxidation. They maintain structure even after lyophilisation, whereas liposome structures are destroyed by lyophilisation.
2. They exhibit efficient incorporation of biological molecules, particularly with hydrophobic moieties into the lipid bilayer of the cochleate structure.
3. They have the potential for slow or timed release of the biologic molecule in vivo as

nanocochleates slowly unwind or otherwise dissociate.

4. 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.

5. They are produced easily and safely. <sup>[8]</sup>

6. By the use of nanocochleates iv drugs to be administered orally (e.g. Amphotericin B, a potent antifungal).

7. They improve oral bioavailability of a broad spectrum of compounds, such as those with poor water solubility, and protein and peptide biopharmaceuticals, which have been difficult to administer.

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 adverse environmental conditions such as sunlight, oxygen, water and temperature.

#### DISADVANTAGES <sup>[8, 9]</sup>:

1. They require specific storage condition.
2. Sometimes aggregation may occur during storage; this can be avoided by the use of aggregation inhibitor.
3. The cost of manufacturing is high.

#### APPLICATIONS <sup>[10-13]</sup>:

1. Development of a nanocochleates based apoa1 formulation for the treatment of atherosclerosis and other coronary heart diseases hypercholesterolemia, a condition associated with high levels of low-density lipoproteins (LDL), and low levels of high density lipoproteins (HDL), is universally accepted as a major risk factor for atherosclerosis and other cardiovascular diseases. The inverse relationship between HDLs and heart diseases is well documented. HDL facilitates the

cholesterol efflux from peripheral cells and, after enzyme-mediated cholesterol esterification, transports cholesteryl esters to the body.

Apoa1 (a naturally existing lipoprotein) is an important HDL believed to be the most important in enzymatic esterification of cholesterol and then its transport to the liver, thus protecting the vessels against atherosclerosis. Infusion or intraperitoneal administration of apoa1 enhances the HDL ability to transport cholesterol to lever and protect against

Atherosclerosis but the major limitation for the use of apoa1 as pharmacological/therapeutical agents has been the need for parenteral administration, as apoa1 is a protein, it is rapidly degraded by GIT enzymes and so it is not delivered to blood as intact molecule.

So nanocochleates can provide a good platform for the delivery of apoa1 by oral preparations and can bring a revolution in the treatment of atherosclerosis and other heart diseases originating from high blood cholesterol and LDL levels.

2. Biogeode nanocochleates have the ability to stabilize and protect an extended range of micronutrients and the potential to increase the nutritional value of processed foods.

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

4. 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. Delmas et. al. Investigated benefits of cochleates containing Amphotericin By using orally administered doses ranging from 0 to 40 mg/kg of body weight/day for 14 days in a murine model of systemic aspergillosis. The administration of oral doses of camb (20 and 40

mg/kg/day) resulted in a survival rate of 70% and a reduction in colony counts of more than 2 logs in lungs, livers, and kidneys. Orally administered camb shows promise for the treatment of aspergillosis.

5. Use of cochleates in the delivery of antibacterial agents:

Cochleates would have the advantage of reducing the toxicity and improving the bactericidal activity. For aminoglycosides and linear or cyclic peptides, cochleates should allow oral administration. The proof of principle of the efficacy of anti-TB cochleates was achieved using clofazimine as an antibacterial drug model.

6. Nanocochleates can deliver omega-3 fatty acids to cakes, muffins, pasta, soups and cookies without altering the product's taste or odour.

7. Bio delivery sciences international 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 colour and taste of food which makes the concept of super food stuffs

A reality and these are expected to offer many different potential benefits including increased energy, improved cognitive functions, better immune function, and antiaging benefits.

**Table 1. Marketed preparation**

| Name                     | Trade name   | Company                        | Indication  |
|--------------------------|--------------|--------------------------------|---|
| Liposomal Amphotericin B | Abelcet      | Enzon                          | Fungal infections   |
| Liposomal Amphotericin B | Ambisome     | Gilead sciences                | Fungal and protozoal infections   |
| Liposomal cytarabine     | Depocyt      | Pacira (formerly skyepharma)   | Malignant lymphomatous meningitis   |
| Liposomal daunorubicin   | Daunoxome    | Gilead sciences                | Hiv-related kaposi's sarcoma  |
| Liposomal doxorubicin    | Myocet       | Zeneus                         | Combination therapy with cyclophosphamide in metastatic breast cancer             |
| Liposomal iriv vaccine   | Epaxal       | Berna biotech                  | Hepatitis a   |
| Liposomal iriv vaccine   | Inflexal v   | Berna biotech                  | Influenza   |
| Liposomal morphine       | Depodur      | Skyepharma, endo               | Postsurgical analgesia  |
| Liposomal verteporfin    | Visudyne     | Qlt, novartis                  | Age-related macular degeneration, pathologic myopia, ocular histoplasmosis        |
| Liposome-PEG doxorubicin | Doxil/caelyx | Ortho biotech, schering-plough | Hiv-related kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer |
| Micellular estradiol     | Estrasorb    | Novavax                        | Menopausal therapy  |

### CONCLUSION AND FUTURE ASPECTS:

Nanocochleate, a lipid based drug delivery system has been shown to be broadly applicable to a wide range of biologically important molecules. This new type of cochleate is inherent in the physico-chemical properties of metal ion-bridged cochleates in terms of their conversion back to liposomes upon the extraction of cation bridging agent and their ability to fuse with the cell membrane. So due to this property it will be useful to deliver charged hydrophilic drugs across the tissue membrane. This looks promising for a safe and sure delivery of various drugs for passive and active targeting. However require more research. They represent a new technology platform for oral and systemic delivery of drugs and even to genes, and vaccine antigens which shows a new path for treatment of many difficult diseases ensuring the better drug delivery system nanocochleates still requires further study and work on it. Still nanocochleate possesses strong therapeutic potential in the area of drug delivery.

So this review article enlighten the therapeutic potential of a new class of drug carrier i.e. nanocochleates which definitely can drive the pharmaceutical world to the new era of drug delivery of highly challenging drugs

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