REVIEW ARTICLE

Nanocochleate: A Novel Drug Delivery System

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Abstract

Nanocochleate 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 basics of cochleate and nanocochleates, components of nanocochleate drug delivery system, route of administration, dosage form, discovery of nanocochleates, stability of nanocochleate, safety/biocompatibility of the nanocochleate delivery vehicles, advantages of nanocochleates, limitations of nanocochleates, mechanism of action, methods of nanocochleate preparation, and applications of nanocochleate. In a whole nanocochleate represents, a unique novel 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 drug delivery technology.

Key words: Cochleate, drug delivery system, nanocochleate, supramolecular assemblies

INTRODUCTION

Various formulation modifications with liposomes allowed the development of a new class of the drug vehicles called "COCHLEATE."[1] Cochleates are solid particulates which are made up of large continuous, lipid bi-layer sheets rolled up in a spiral structure with no internal aqueous phase between them. 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-shaped, and rigid stable structure is shown in Figure 1.[2]

BASICS OF COCHLEATE AND NANOCOCHLEATES

Cochleate and nanocochleates are novel lipid-based drug delivery system which represents a new approach suitable for the administration of a wide range of therapeutics including drugs, genes, and vaccine antigens. Then anocochleate drug delivery vehicle is based on encapsulating the drug in a multilayered, lipid crystal matrix (a cochleate) to potentially deliver the drug safely and effectively. Nanocochleates are cylindrical (cigar-like) microstructures that consist of series of lipid bilayers as shown in Figure 2.[1] Nanocochleate delivery vehicle are stable phospholipid-cation precipitates composed of simple naturally occurring material like polyphosphotidylserine and calcium as shown in Figure 3. They have unique multilayered structure consisting of a solid, lipid bilayer sheet rolled up in a spiral or in a stacked sheet in order to minimize their interaction with water is shown in Figure 4. They posses little or no aqueous internal space between them. The entire cochleate and nanocochleate structure is a series of solid layers so that, even if the outer layers of cochleate and nanocochleate are exposed to harsh environmental conditions or enzymes, the encapsulated drug molecules will be remain intact within the interior. Nanocochleates contains both hydrophilic and hydrophobic

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Received: 18-07-2016
Revised: 30-07-2016
Accepted: 07-08-2016

surface which makes it more suitable for encapsulation of both hydrophobic drugs like amphotericin B and clofazimine and amphipathic drugs like doxorubicin.[4] It represents the most versatile technology for encapsulating drug within the interior of the nanocochleates tructure. This nanocochleate structure remains intact, even though the outer layers of the nanocochleate may be exposed to harsh environmental conditions or enzymes.[5,6]

Nowadays, oral route remains the alternative way for administrating therapeutic agents. In particular, lipid-based nanocochleate delivery system appears to provide answers to oral delivery challenges by formulating different kinds of biological molecules including genes, proteins and peptides and vaccines, antigens and drugs, especially hydrophobic once that were not having oral bioavailability. Nanocochleates are stable lipid-based delivery formulations whose structures and properties are very different from liposomes. Nanocochleates are unique platform technology for the delivery of with ranges of drugs and molecules such as proteins and peptides, polynucleotides. Thus, it is a potential drug delivery system for the wide class of drugs.

COMPONENTS OF NANO-COCHLEATE DRUG DELIVERY SYSTEM[8]

The three major components used in the preparation of nanocochleates are atmospheric pressure ionization (API), lipids, and cations.

1. Lipids: Phosphatidyl serine (PS), phosphatidic acid (PA), di-oleoyl PS, phosphatidylinositol (PI), phosphatidyl glycerol (PG), phosphatidyl choline (PC), di-myristoyl PS, phosphatidyl ethanolamine (PE), di-phosphatidyl glycerol (DPG), dioleoyl phosphatidic acid, di-stearoyl phosphatidyl serine, di-palmitoyl PG.
2. Cations: Zn^{+2} or Ca^{+2} or Mg^{+2} or Ba^{+2}.

BRIEF DESCRIPTION FOR SOME LIPIDS USED IN NANOCOCHLEATE DELIVERY SYSTEMS[9-11]

A. PC: 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, and essential nutrient[9]
B. PI: It is an important lipid as it is a key membrane constituent. Also, it is a participant in essential metabolic processes in all plants and animals and some bacteria[11]
C. PE: 1,2-diacyl-glycero-3-phospahtidyl 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 membrane bilayers[10]
   - DPG: Also, named as cardiolipin. It is found exclusively in certain membranes of bacteria (plasma membrane and hydrogenosomes) and mitochondria of eukaryotes
   - PG: 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.

DISCOVERY OF NANOCOCHLEATES[12]

Dr. Papahadiopoulos *et al*. discovered the structure of nanocochleates in 1975 and had been used before the 90s. It has been used for the transport of antigens and peptides for the vaccine delivery. Nanocochleates were introduced in 1999, which are having the particle size between 50 and 100 nm. It was demonstrated that by using a hydrogel isolation method, small, and more consistent cochleates can be formed. From these, it is found that cochleates have been an appropriate carrier system for the encapsulation of hydrophobic compounds.

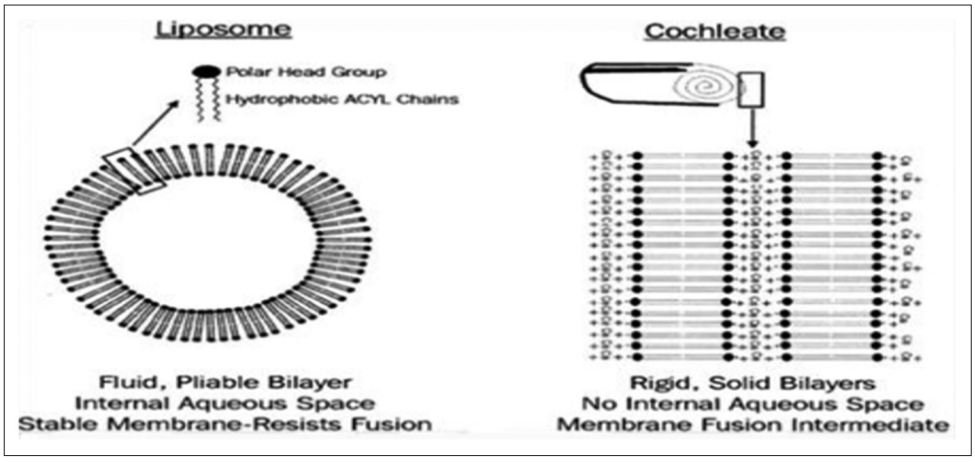

Figure 1: Structural difference between liposomes and cochleates; from Gol and Shah[3]

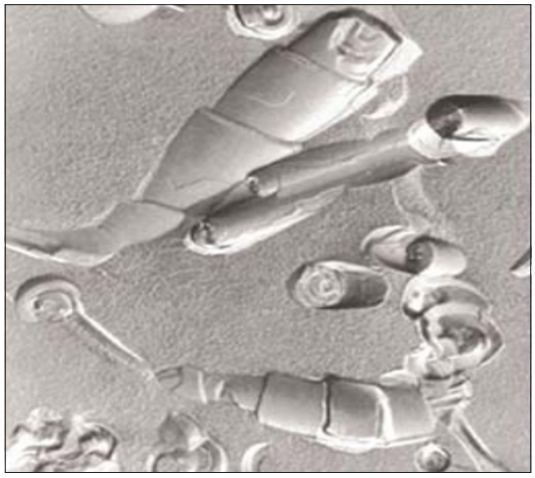

Figure 2: Cigar like structure with its double fold; from Zarif *et al*.[1]

ROUTE OF ADMINISTRATION[13]

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

Dosage forms available for nanocochleate drug delivery[14]

- 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 before use.

Stability of nanocochleate[15]

Encochleation provides protection as well as stability to associated molecules. Because of the entire structure of these cochleate is a series of solid-lipid bilayers, components within the interior of this structure remains intact, even though the outer layers of it may be exposed to harsh external environmental conditions or enzymes. This interior of the structure is essentially free of water and resistant to penetration by oxygen which leads to increase shelf-life of the formulation. Nanocochleates can be stored at room temperature or 4°C, and that may be lyophilized to a powder form. Before *in-vitro* use or *in-vivo* administration, lyophilized cochleates can be reconstituted with liquid. There are no adverse effects on cochleate morphology, structure, or functions on lyophilization.

Safety/biocompatibility of the nanocochleate delivery vehicles[15]

The main two components of nanocochleate are PS and calcium. PS is a natural constituent of all biological membranes and is most concentrated in the brain. The phospholipids used in nanocochleate formulation can be prepared from natural sources or produced synthetically which are composed of anionic lipids are non-inflammatory and biodegradable. Soy PS is available in large quantities and is expensive and suitable for use in humans. These two components which are safe, simple, naturally occurring substances which make nanocochleates safe and biocompatible delivery vehicle. Clinical studies show that PS is very safe which play a role in support of mental functions in the aging brain.

ADVANTAGES OF NANOCOCHLEATES[13,16,17]

1. Because of less oxidation of lipids and water free inner core, the nanocochleate are more stable than liposomes. Nanocochleates maintains the structure after lyophilization, whereas liposomes structures are destroyed by lyophilization. We can lyophilized cochleates to a powder.
2. Lyophilization is a potential method for storing formulations for longer duration of time at room temperature. Before administration of lyophilization, could be an advantageous method for transport and storage.
3. They exhibit the efficient incorporation of hydrophobic drugs into lipid bilayers of nanocochleate structure. They can also exhibit efficient incorporation of antigens with hydrophobic moieties into lipid bilayers of nanocochleate structure.
4. By formulating nanocochleate, intravenous drugs can be administered orally. For example, amphotericin B, a potent antifungal.
5. By encapsulating the drug, it reduces the stomach irritation and other side effects. They protect the encapsulated drug from degradation avoiding by exposure to adverse environmental conditions such as sunlight, oxygen, water, and temperature.
6. The components of lipid bilayer composed of simple lipids, that are naturally occurring and makes nanocochleates a safe and biocompatible delivery vehicles.
7. By encapsulating the drug, they improve the oral

bioavailability of a broad spectrum of compounds, such as those with poor water solubility and proteins and peptides, biopharmaceuticals, which have been difficult to administer. For example, Ibuprofen for arthritis.[17]

8. They are produced easily and safely.[13]

LIMITATIONS OF NANOCOCHLEATES[13]

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 production is high.

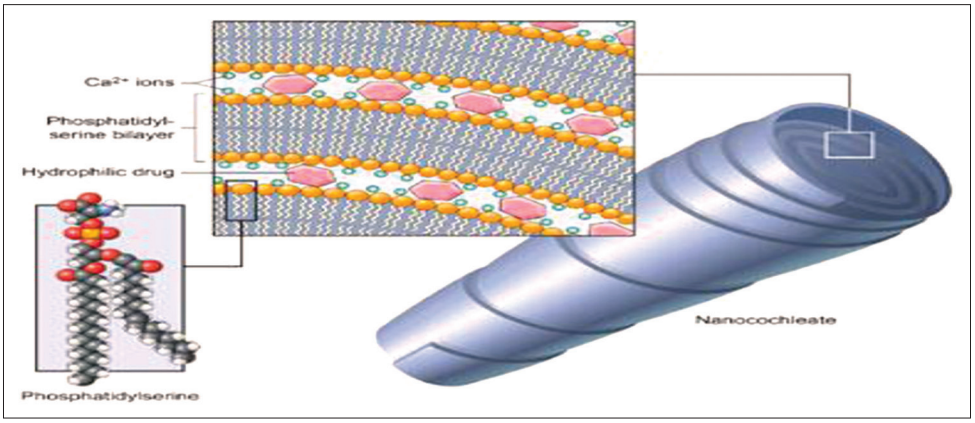

Figure 3: Nanocochleate structure; Vijeta *et al*.[7]

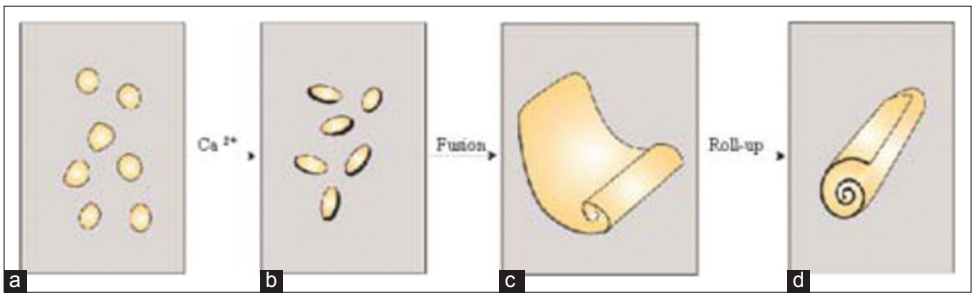

Figure 4: (a-d) Cochleate formation from negatively charged vesicles, which fuse after addition of cation to lead to fused liposomes which roll up to give cochleate cylinders; Sankar and Reddy[1]

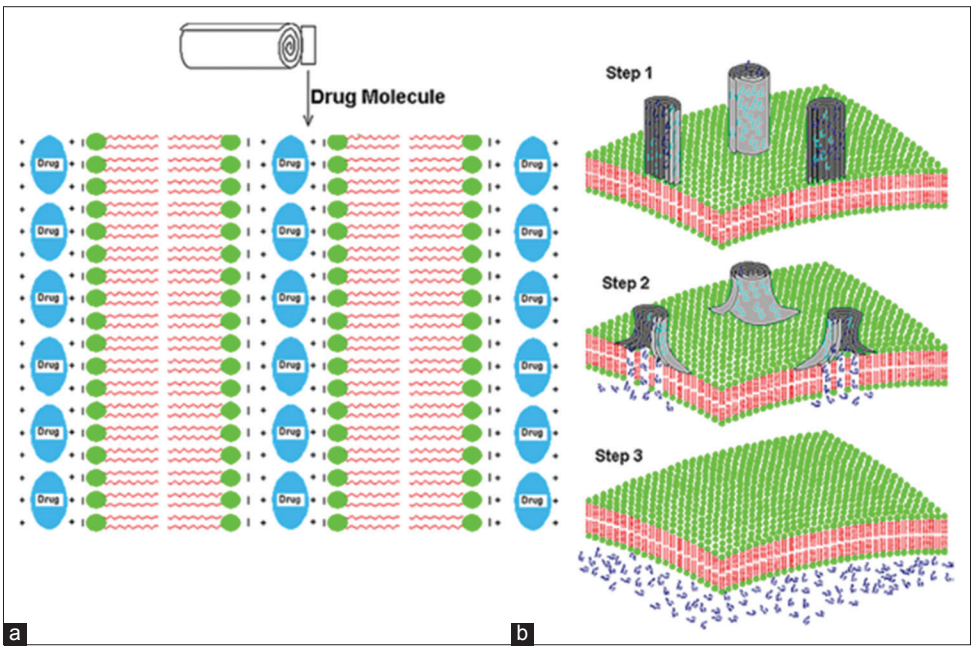

Figure 5: (a and b) Diagrammatic presentation of nanocochleate interaction with the cell membrane; from Yeole *et al*.[18]

MECHANISM OF ACTION[18]

The proposed mechanism of the delivery of hydrophobic drugs loaded in the inter-bi-layer spaces of nanocochleates is shown in Figure 5. The hypothesis states that when lipid bi-layer structure of nanocochleates fuses with the cell membrane then contents of nanocochleates are delivered into cells, thus the release of the drug occurs.

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.[19] There are several methods for cochleate preparation:

1. Hydrogel method[20]

This method comprises of following steps:

Step 1: 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.

Step 2: The liposome suspension is mixed with polymer A such as dextran (molecular weight - 200,000-500,000), polyethylene glycol (PEG) (molecular weight - 3400-8000) or PS.

Step 3: Preferably by injection, the liposome/polymer A suspension is added into polymer B such as polyvinyl pyrrolidone, polyvinyl alcohol, ficoll (molecular weight - 30,000-50,000), and polyvinyl methyl ether (PVMB) (molecular weight - 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 appropriately controlled rate, for example, a rate of 0.1-50 ml/min, and preferably at a rate of 1-10 ml/min.

Step 4: 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.

Step 5: 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. The 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 6].

2. Liposome before cochleates (LC)/dialysis method[20]

In this method, mixture of lipid and detergent are used as the starting material, and the removal of detergent is made by double dialysis.[17] The detergent is added to disrupt the liposomes. The method comprises the following steps:

Step 1: An aqueous suspension containing a detergent-lipid mixture is prepared.

Step 2: The detergent-lipid suspension is mixed with polymer A such as dextran (molecular weight - 200,000 500,000), PEG (molecular weight - 3400-8000) or PS.

Step 3: The detergent-lipid/polymer A suspension is added into a solution comprising polymer B such as polyvinyl pyrolidone, polyvinyl alcohol, ficoll (molecular weight - 30,000-50,000), and PVMB

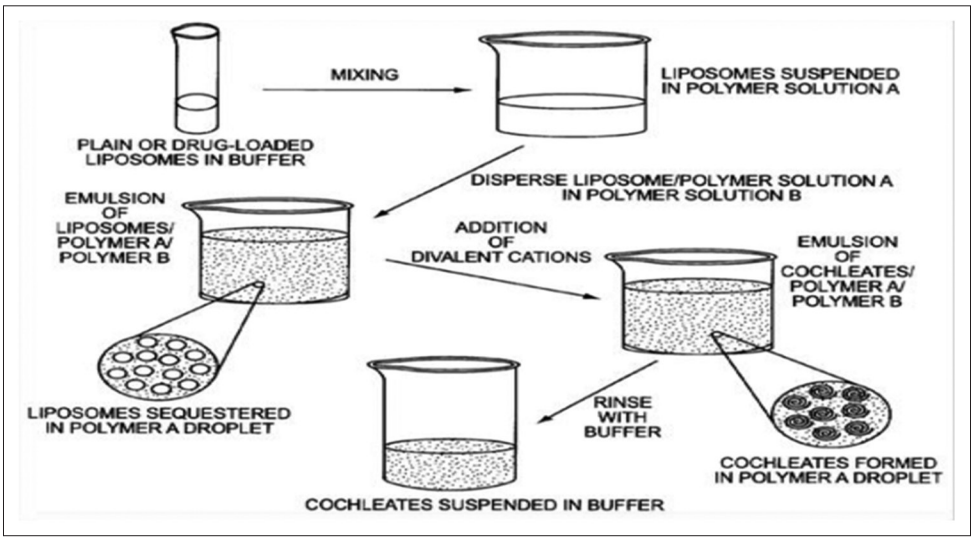

Figure 6: Hydrogel isolation method; from Zarif *et al*.[13]

(molecular weight - 60,000-160,000), wherein polymer A and polymer B are immiscible, thereby creating a two-phase polymer system.

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

Step 5: Washing the two-phase polymer system to remove the polymer.

3. Direct calcium (DC) dialysis method[19]

Unlike LC method, this method does 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.

Step 1: 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 min.

Step 2: The clear, colorless solution was then dialyzed at room temperature against three buffers.

Step 3: 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.

4. Binary aqueous-aqueous emulsion system[19]

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 a 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 <1000 nm.

5. Trapping method[21,22]

This method is useful for the encapsulation of hydrophilic and hydrophobic molecules. 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.

6. Solvent drip method[1,4]

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 dimethyl sulfoxide or dimethylformamide. The solution is then added to liposomal suspension. Since the solvent is miscible in water, a decrease of the solubility of the cargomoiety 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.

APPLICATIONS

1. Development of a nanocochleate based ApoA1 formulation for the treatment of atherosclerosis and other coronary heart diseases hypercholesterolemia, a condition associated with high levels of low-density lipoproteins (LDLs), and low levels of high-density lipoproteins (HDLs), is universally accepted as a major risk factor for atherosclerosis and other cardiovascular

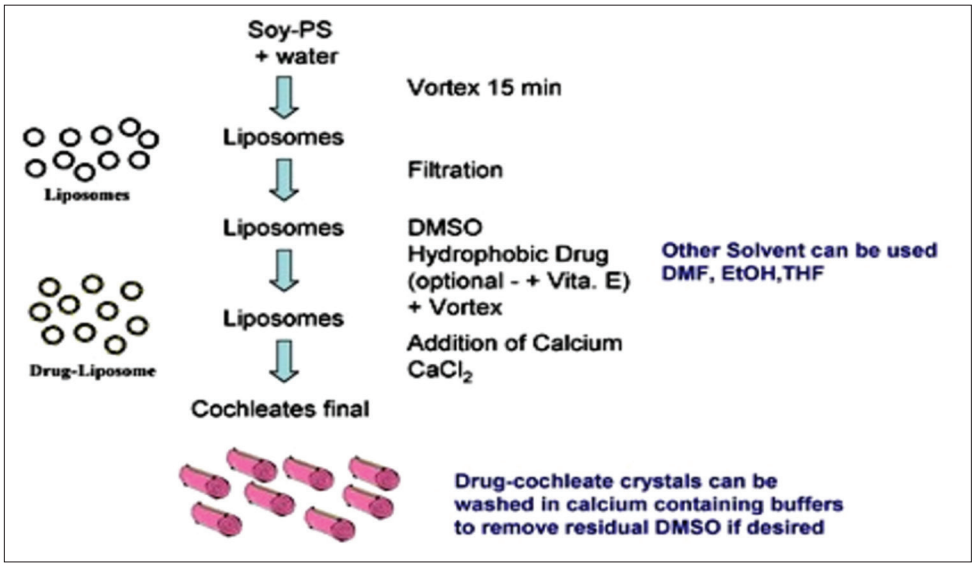

Figure 7: A schematic presentation of the trapping method; from Zarif *et al*.[23]

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 artherosclerosis.

Infusion/intraperitoneal administration of ApoA1 enhances the HDL ability to transport cholesterol to liver 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 gastrointestinal tract enzymes and so it is not delivered to blood as intact molecule.

Hence, 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.
5. Delmas *et al*. investigated benefits of cochleates containing amphotericin 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 Cochleates containing amphotericin B (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.

6. 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 antituberculosis cochleates was achieved using clofazimine as an antibacterial drug model.
7. Nanocochleates can deliver omega-3 fatty acids to cakes, muffins, pasta, soups, and cookies without altering the product's taste or odor.
8. Biodelivery 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 color and taste of food which makes the concept of super foodstuffs a reality, and these are expected to offer many different potential benefits including increased energy, improved cognitive functions, better immune function, and antiaging benefits.[19]

CHARACTERIZATION OF NANOCOCHLEATE FORMULATION[21,24-28]

1. 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[24]
2. Density: The density of nanocochleates is determined with helium or air using a gas pycnometer. The value obtained with air and helium is much more pronounced due to the specific surface area and porosity of the structure[25]
3. Molecular weight measurements: The molecular weight of the polymer and its distribution in the matrix can be evaluated by gel permeation chromatography (GPC) using a refractive index detector. Using GPC, it was shown that polyalkylcynoacrylate nanocochleates are built by an entanglement of numerous small oligomeric subunits rather than by the rolling up of one or a few long polymer chains[25]
4. Drug content: The redispersed nanocochleates suspension is centrifuged at 15,000 rpm for 40 min at 25°C to separate the free drug in the supernatant. Concentration of drug in the supernatant can be then determined by ultraviolet UV-Vis spectrophotometrically after suitable dilution[21]
5. Entrapment efficiency (EE): 100 µl of cochleates is aliquoted into centrifugation tubes. To each tube, 60 µl pH 9.5 ethylenediaminetetraacetic acid and 1 ml of ethanol is added while vortexing. Absorbance of the resulting solution is determined using spectroscopic technique and EE is calculated using below-mentioned equation[24]

$$\text{Entrapment efficiency} = \frac{\text{Amount of drug present in cochleates}}{\text{Total amount present}} \quad (1)$$

6. Stability study: Cochleates dispersions can be kept at 2-8°C and 25 ± 2°C/60% RH for 3-month to check their stability. The stability of the vesicles is determined in terms of change in particle size and percent EE[24]
7. Cochleates-cell interaction: To examine the interaction

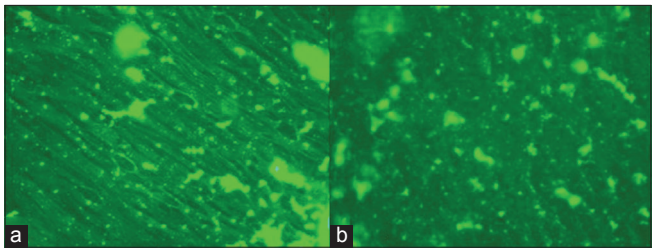

Figure 8: (a and b) Mammalian skin cells exposed to the poly-lysine fluorescent nanocochleate; from Zarif *et al*.[27]

of cochleates with cell membrane, 2% fluorescent lipid in addition to the negatively charged lipid is used to form fluorescent liposomes.[25,26] When cochleates interact with cell membrane involving a fluorescent lipid transfer, cell surfaces become fluorescent under fluorescent microscopes as illustrated in Figure 8a and b

8. 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:

$$A = 6/\rho d \quad (2)$$

Here, A = Specific surface area,
ρ = Density,
d = Diameter of the cochleate[28]

9. Surface charge: The nature and intensity of the surface charge of nanocochleate determine 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 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. In general, 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[25]
10. *In vitro* release study: The *in vitro* release profile of nanocochleates is determined using standard dialysis, diffusion cell, or modified ultrafiltration 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, nanocochleate is added directly into a stirred ultrafiltration cell containing a buffer. At different time intervals, aliquots of the medium are filtered through the ultra-filtration membrane and assayed for the released drug using standard procedures.[29]

CONCLUSION

Nanocochleate delivery vehicles have been shown to be broadly applicable to a wide range of biologically important molecules. Encochleation can improve an end product by enhancing the qualities of the 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 a 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 and desired drug molecule into the body with good potential.

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Source of Support: Nil. **Conflict of Interest:** None declared.