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REVIEW ARTICLE

A Review on Nanocochleate – A Novel Lipid Based Drug Delivery System.

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ABSTRACT

Nanaocochleate represent a new approach for oral and systemic delivery of drugs . It is a novel lipid-based system which is suitable for the oral and systemic administration of a wide variety of molecules with important therapeutic activities, including drugs, genes, and vaccine antigens. This novel formulation approach is applicable to macromolecules as well as small molecule drugs that are hydrophobic and that possess poor oral bioavailability. Studies for oral delivery of clinically important drugs are being carried out in suitable animal models to evaluate their efficacy.

KEYWORDS: Phospholipids, Liposomes, Cochleates.

INTRODUCTION

Now a day, there are number of nontraditional novel dosage forms available in the market. In spite of it; oral route remains the attractive way for administration of therapeutic agents. However many therapeutic agents, especially biological molecules are not taken by the intestine due to their intrinsic impermeability to tissue membranes and enzymatic degradation through the wall of GIT. Carrier or delivery system that facilitates the intestinal uptake of these molecules is of major interests in the drug delivery arena. Moreover, structural modifications of drug molecules are often required to facilitate receptor-mediated drug molecule absorption, which may alter the pharmacological activity of the drug molecule. Therefore, there is an emerging need to develop drug delivery system, which could facilitate diffusion of the drugs across the intestinal membrane[1.] In present scenario, various strategies have been reported to improve intestinal uptake of drug including pro-drug analogue design, application of absorption enhancers and delivery by using lipid-based drug delivery systems[2]. Lipid-based delivery systems including liposomes attracted enormous research efforts as a cross membrane drug delivery vehicle because of their structural resemblance with cell membrane[3,4]. Utilization of liposomes to improve oral absorption of drugs remains unsuccessful mainly due to their poor mechanical stability, low-drug loading capacity and probably the lack of mechanism to facilitate intestinal uptake[5].

In particular, lipid based nanocochleate delivery system appears to provide answers to oral delivery challenges by formulating different kinds of biological molecules, especially hydrophobic ones. Nanocochleates are solid particulates made of large continuous, lipid bi-layer sheets rolled up in a spiral structure with no internal aqueous phase. It is different from liposome in that it has a water-free interior, a rod shape, and a rigid structure. These unique characteristics make nanocochleates a great platform in delivery of drugs that were not having oral bioavailability. These are stable, lipid based delivery formulations whose structure and properties are very different from liposomes. Nanocochleate is most versatile technology for the delivery of a wide range of drugs and molecules such as proteins and peptides, polynucleotide, antiviral agent, anesthetic, 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[6.]

DISCOVERY OF NANOCOCHLEATES [7]:

Dr. D. Papahadjoupoulos and coworkers discovered these structures in 1975, and have been used before 90s for transport of antigens and peptides for vaccine delivery. Nanocochleates were introduced in 1999, which are having particle size less than 100nm. It was demonstrated that by using a hydrogel isolation method, cochleates can be formed in such a way to display small and more consistent particles. These cochleates have been found an appropriate carrier system for the encapsulation of hydrophobic compounds.

INTRODUCTION TO NANOCOCHLEATES:

Nanocochleates are cigar-like structures that consist of a series of lipid bilayers (Figure 1)[8], which are formed as a result of the condensation of small unilamellar negatively charged liposomes. In the presence of calcium, the small phosphatidylserine (PS) liposomes fuse and form large sheets. These sheets have hydrophobic surfaces and, in order to minimize their interactions with water, tend to roll-up into the cigar-like cochleate (Figure 2).

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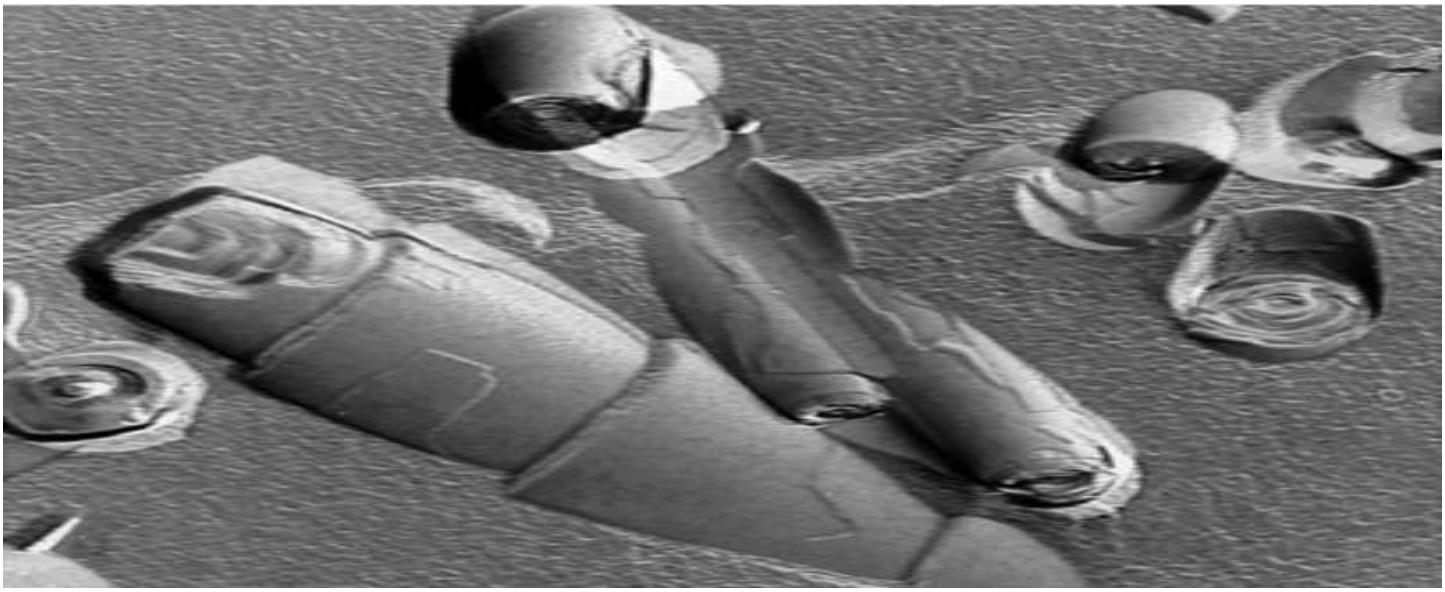

Figure 1: Freeze fractured electron microscopic structure of Nanocochleates; from Zarif L. (8)

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[8,9,10]. 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[8,10,11]. The main components of nanocochleates are phosphatidylserine (PS) and calcium. Phosphatidylserine is a constituent of the brain and is sold in health stores as nutrient supplement.

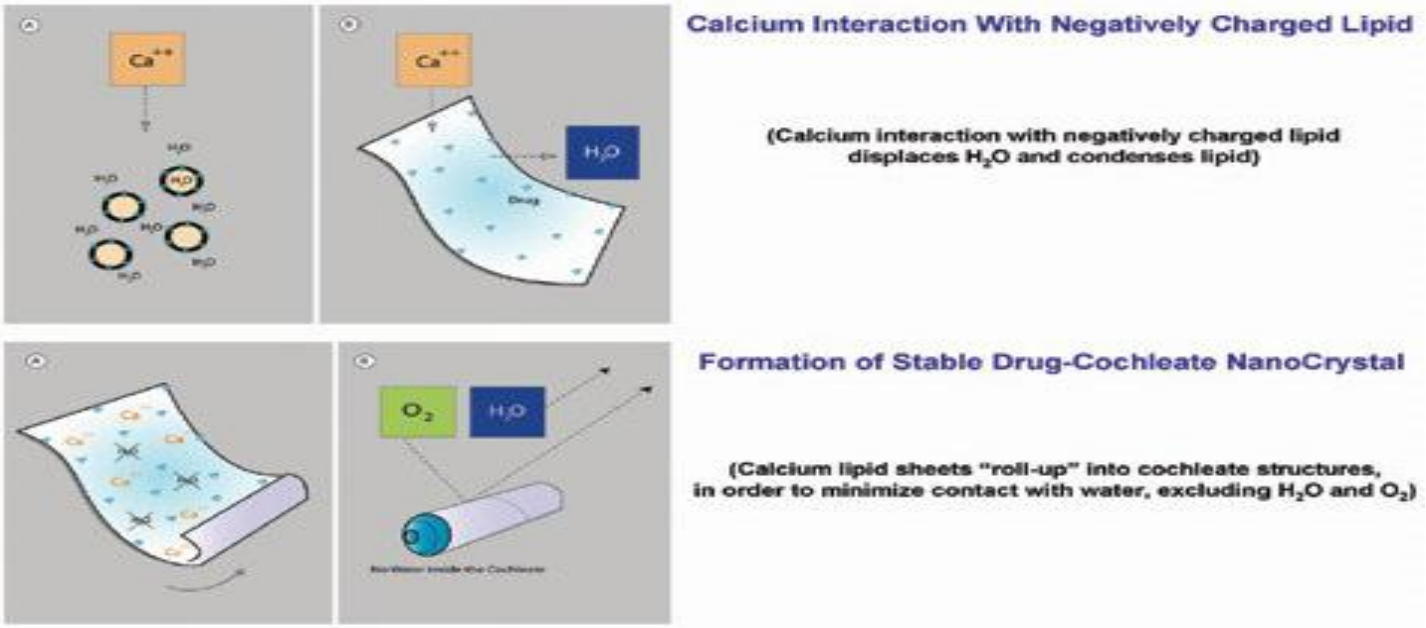

Figure 2: Scheme of formation of Nanocochleates; from Zarif L. et al. (12)

STABILITY OF NANOCOCHLEATE FORMULATIONS:

Encochleation (Figure 2) provides protection and stability to associated molecules. 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 4^0C. Lyophilized cochleates can be reconstituted with liquid

prior to in vitro use or in vivo administration. Lyophilization has no adverse effects on cochleate morphology or functions.[12]

SAFETY/BIOCOMPATIBILITY OF THE NANOCOCHLEATE DELIVERY VEHICLES:

Phosphatidylserine (PS) and calcium which are safe, simple, naturally occurring substances, makes nanocochleates a safe and biocompatible delivery vehicles. Phosphatidylserine is a natural component of all biological membranes and is most concentrated in the brain. The phospholipids used can be produced synthetically, or prepared from natural sources. Soy PS is inexpensive, available in large quantities and suitable for use in humans. Clinical studies show that PS is very safe and may play a role in the support of mental functions in the aging brain. Nanocochleates which are composed of anionic lipids are non-inflammatory and biodegradable. [12]

ADVANTAGES [13, 14, 15]**:**

1. They are more stable because of the less oxidation of lipids and water free inner core.
2. Lyophollization provides the potential method for storing formulations for longer duration of time at room temperatures, which would be advantageous for transport and storage prior to administration.
3. Nanocochleates maintain their structure even after lyophilization, whereas liposome structures are not feasible for lyophilization.
4. They can exhibit efficient incorporation of hydrophobic drugs into the lipid bilayer of the nanocochleate structure.
5. They can exhibit efficient incorporation of antigens with hydrophobic moieties into the lipid bilayer of the nanocochleate structure.
6. Nanocochleates shows potential for controlled release of a drug, antigen or biologically relevant molecule; as cochleates dissociate in vivo.
7. The components of Lipid bilayer, which serves as a carrier and is composed of simple lipids, are naturally occurring and nontoxic.
8. They can be produced as defined formulations composed of predetermined amounts and ratios of drugs or antigens[14, 15].
9. They are produced easily and safely.

LIMITATIONS:

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[16].

MECHANISM OF ACTION:

The proposed mechanism of the delivery of hydrophobic drugs loaded in the inter-bi-layer spaces of nanocochleates is shown in Fig.3. 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 release of the drug occurs.

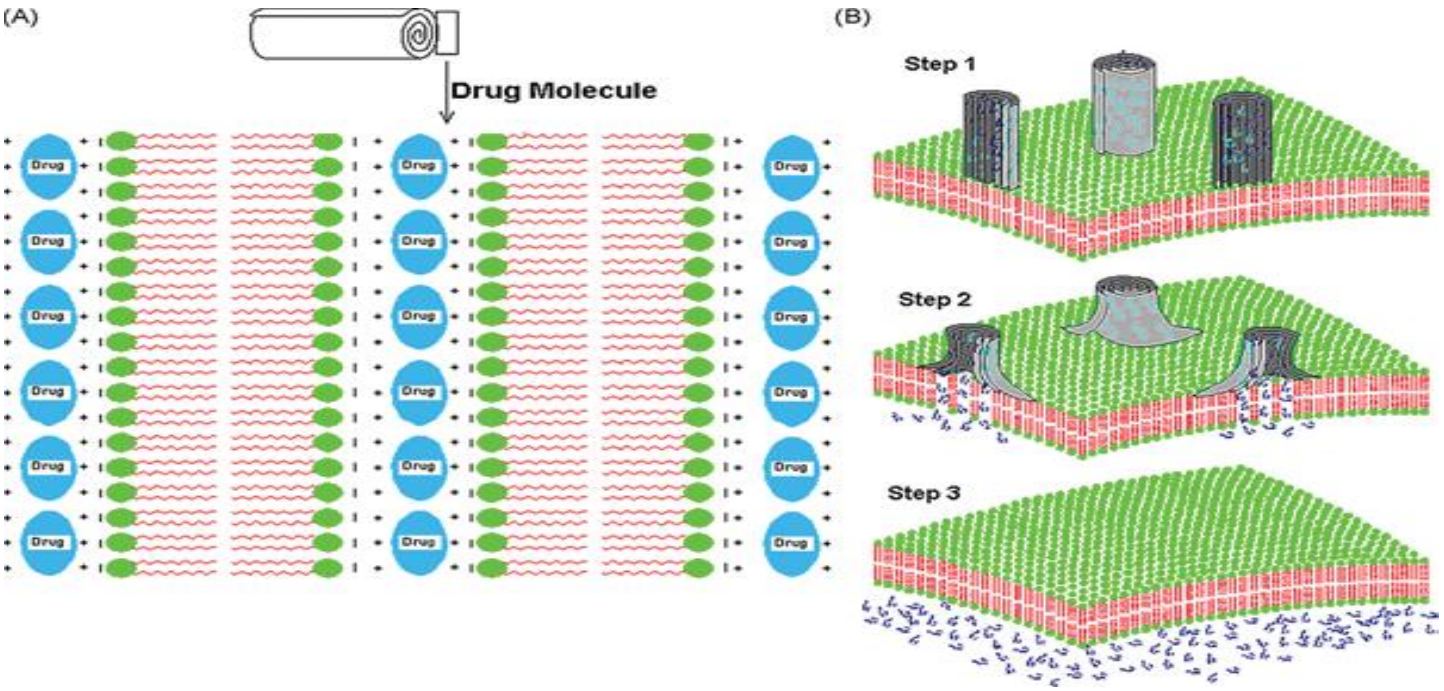

Figure 3: Diagramatic presentation of nanocochleate interaction with the cell membrane.

METHODS OF PREPARATION:

1. HYDROGEL METHOD:

In this method initially the small unilamellar drug loaded liposomes are prepared, which are added to polymer A (Which may be phosphotidyl serine, dextran, polyethylene glycol, etc.). The dispersion of two is then added to another polymer B (which may be polyvinylpyrrolidone, polyvinylalcohol, Ficoll, polyvinyl methyl ether, etc.). The two polymers are immiscible in each other. Immiscibility of the polymers leads to formation of an aqueous two-phase system. The cationic cross-linking of the polymers is achieved by adding a solution of cation salt to the two-phase system, such that the cation diffuses into second polymer, and then into the particles comprised of liposomes/polymer. The formed cochleates aree then washed to remove polymer, which might be resuspended into a physiological buffer or any appropriate pharmaceutical vehicle or lyophilized[17].

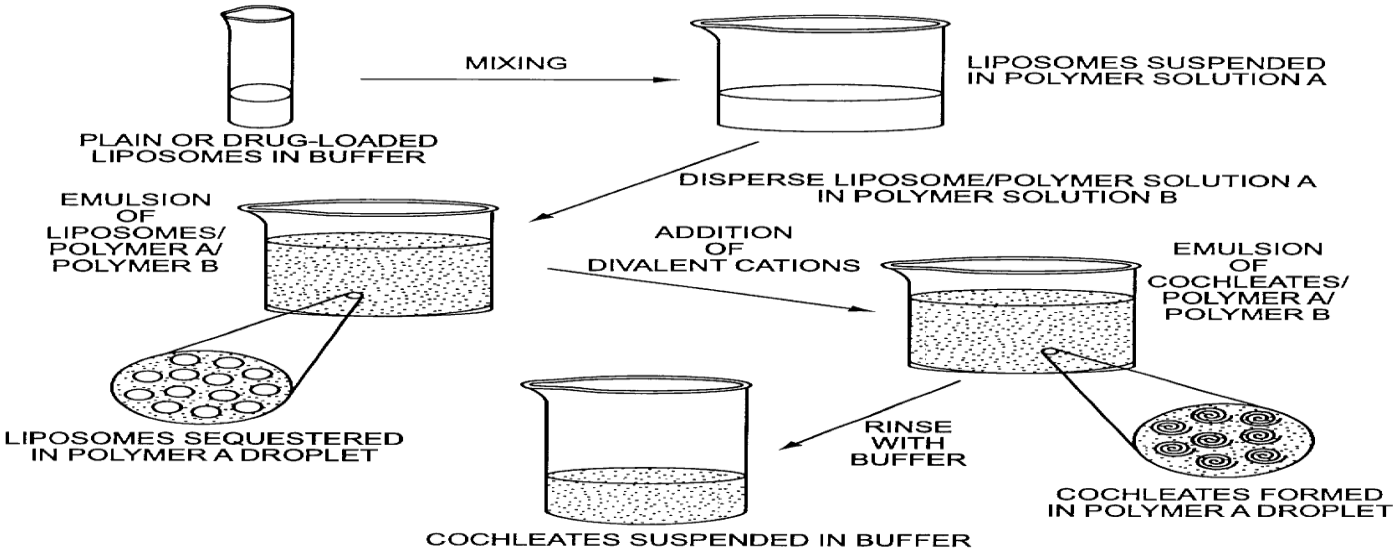

Figure 4: Diagrammatic representation of Hydrogel isolation method; from Jin et al. (17)

2. TRAPPING METHOD:

This method involves the formation of phosphatidylserine liposomes followed by dropwise addition of a solution of CaCl2. Liposomes can be generated by either addition of water to phospholipid powder or by adding the water phase to a phospholipid film.[18].

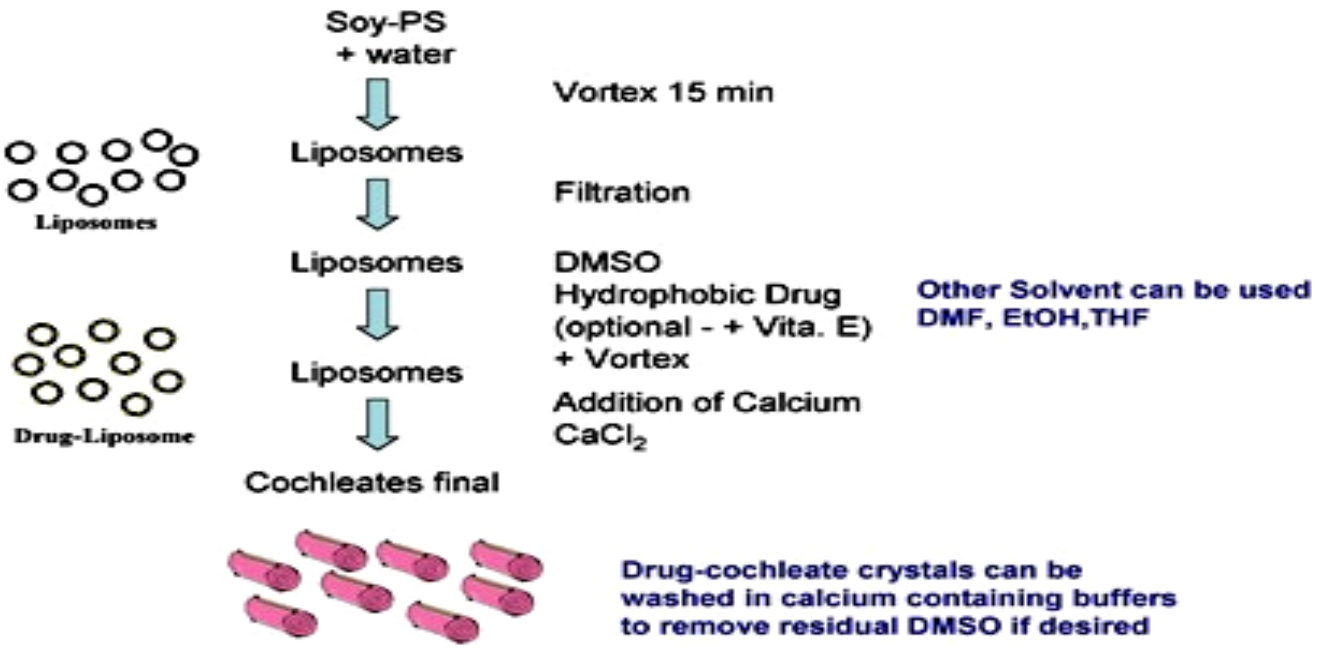

Figure 5: A schematic presentation of the Trapping method; from Zarif L. et al. (12)

3. LIPOSOMES 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. The mixture is dialyzed initially with buffer and followed by calcium chloride solutions leads to formation of cochleates.[18] Mixture of phosphatidylserine and cholesterol (9:1 wt ratio) in extraction buffer and non-ionic detergent is mixed with a pre-selected concentration of polynucleotide. The resulting solution is vortexed for 5 minutes. The solution is dialyzed overnight using a mixture of dialysate and buffer in ratio 1:200 without divalent cations, followed by three additional changes of buffer leads to the formation of small lipid vesicles. The vesicles are converted to a cochleate precipitate, either by the direct addition of Ca^{2+} ions, or by dialysis against two changes of buffer containing 3 mM Ca^{2+} ions, followed by buffer containing 6 mM Ca^{2+}.

4. DIRECT CALCIUM (DC) DIALYSIS METHOD[18]:

Unlike LC method, this method does not involve the intermediate liposome formation and the cochleates formed have been large in size. The mixture of lipid and detergent has been 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. Mixture of phosphatidylserine and cholesterol (9:1 wt ratio) in extraction buffer and non-ionic detergent was mixed with a pre-selected concentration of polynucleotide, and the solution is vortexed for 5 minutes. The clear, colorless solution which resulted was dialyzed at room temperature against three changes (minimum 4 hours per change) of buffer {2 milli Molar (mM) TES N-Tris[hydroxymethyl]-methyl-2aminoethane sulfonic acid, 2 mM L-histidine, 100 mM NaCl, pH 7.4} containing 3 mM $CaCl_2$. The final dialysis routinely used is 6 mM Ca^{2+}, although 3 mM Ca^{2+} is sufficient and other concentrations may be compatible with cochleate formation. The ratio of dialysate to buffer for each change was a minimum of 1:100. The resulting white calcium-phospholipid precipitates have been termed DC cochleates. When examined by light microscopy, the suspension contains numerous particulate structures up to several microns in diameter, as well as needle-like structures.

5. BINARY AQUEOUS-AQUEOUS EMULSION SYSTEM [18]:

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. By this method the cochleates formed are of particle size less than 1000 nm.

APPLICATIONS [19,20,21,22]:

1. Development of a Nanocochleate based Apoprotein (ApoA1) formulation is used for the treatment of atherosclerosis caused due to Hypercholesterolemia. In Hypercholesterolemia, high levels of low-density lipoproteins (LDLs) and low levels of high-density lipoproteins (HDLs), occurs which 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. HDLs facilitate the cholesterol efflux from peripheral cells and, after enzyme-mediated cholesterol esterification, transports cholesterol 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 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. Nanocoochleates can provide a good alternative or the delivery of ApoA1 by oral preparations and can bring a revolution in the treatment of atherosclerosis and other heart diseases.

2. Nanocochleates can be used in the delivery of anti-inflammatory agents. Researchers are currently investigating the potential for using cochleate delivery vehicles to formulate and effectively deliver anti-inflammatory agents, including aspirin, ibuprofen, naproxen, acetaminophen, and COX-2 inhibitors. 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 cochleate containing amphotericin B (CAMB) (20 and 40mg/kg/day) resulted in asurvival 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.

3. Cochleates possess 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. As using Amphotericin B (AmB) as a model, cochleates have been shown to be highly effective at mediating the oral delivery of drugs that are currently only available in injectable formulations.

4. 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.

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

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

7. Bio delivery Sciences International (BDSI), an US based company has developed nanocochleates which can be used to deliver nutrients such as vitamins, omega fatty acids more efficiently to cells, 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.

8. BioDelivery Sciences and collaborators have reported the filing and acceptance by the United states food and drug administration (USFDA) of BDSI's first Innovative new drug application (INDA) for the company's Bioral® Cochleate technology for an enchocleated version of Amphotericin B (CAMB), a potent antifungal agent.

CONCLUSION:

Nanocochleates has shown great potential in oral and systemic administration of a wide range of molecules with important therapeutic activities, including drugs, genes, and vaccine antigens. Encochleation can be helpful in enhancing the qualities of the formulation by increasing shelf-life and thus stability, enhancing bioavailability, reducing dose as well as toxicity, and ultimately efficacy of the end product. In future, this technology can be used as an alternative way to deliver the biological or therapeutic moieties. The exponential increase in patent filing and publications of nanocochleates indicates growing industrial interest as well as academic interest in the area of drug delivery.

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