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Nanocochleates: A novel carrier for drug transfer

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Abstract

Nanocochleates are novel drug delivery system based upon entrapment of desired drug molecule into the multilayered structure containing solid-lipid bilayer in the form of sheet rolled up in spiral shape. The nanocochleate structure provides protection to encapsulated molecule form surrounding harsh environment. Also it has potential to carry both the hydrophilic & lipophilic drug molecule as it is contains both the forms on its surface & its structure. Nanocochleates can be prepared by many methods & can be used to deliver many active agents for many applications. Nanocochleates is having very less limitations than that of other dosage forms & system & hence, it becomes widely applicable & more potential drug delivery system.

Keywords: Nanocochleates, Phagocytosis, Liposomes, Phospholipids.

Introduction

The nanocochleate drug delivery vehicle is based upon encapsulating drugs in multilayered, lipid crystal matrix to potentially deliver the drug safely and effectively. Nanocochleates are cylindrical (cigar-like) microstructures that consist of a series of lipid bilayers.[1] Nanocochleate delivery vehicles are stable phospholipid-cation precipitates composed of simple, naturally occurring materials, generally phosphatidylserine and calcium. They have unique multilayered structure consisting of solid, lipid bilayer sheet rolled up in a spiral or in stacked sheets, with little or no internal aqueous space. This structure provides protection from degradation for associated "encochleated" molecules. Because the entire nanocochleate structure is a series of solid layers & components that are encapsulated within the interior of the nanocochleate structure which remain intact, even though the outer layers of the nanocochleate may expose to harsh environmental conditions or enzymes. Because nanocochleates contain both hydrophobic and hydrophilic surfaces, which are suitable to encapsulate both hydrophobic drugs & hydrophilic drugs.[2]

Routes of administration for nanocochleate drug delivery-

Nanocochleates 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, intra-articular, intra-arterial, bronchial, lymphatic, and intrauterine administration, intra-vaginal or any other mucosal surfaces.[3]

Dosage forms available for nanocochleate drug delivery-

1) For oral administration- Capsules, cachets, pills, tablet, lozenges, powders, granules, or a solution or a suspension or an emulsion.

2) For topical or transdermal administration- Powders, sprays, ointment, pastes, creams, lotions, gels, solutions, patches and inhalants.

3) For parenteral administration- Sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior use.[4]

Advantages of nanocochleate drug delivery system-

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.

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 molecules in vivo as nanocochleates slowly unwinds or otherwise dissociates.

4) They have a lipid-bilayer matrix which serves as a carrier and composed of simple lipids which 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.

6) They improve oral bio-availability of broad spectrum of compounds, such as those with poor water solubility, and protein and peptide biopharmaceuticals, which have been difficult to administer. (e.g. ibuprofen for arthritis).

7) They reduce toxic stomach irritations and other side effect of the encapsulated drug.

8) They encapsulate or entrap the active drug within a crystal matrix rather than chemically bonding with the drug.

9) They provide protection from degradation to the encochleated drug avoiding by exposure to adverse environmental conditions such as sunlight, oxygen, water, and temperature.

10) They can be produced as defined formulations composed of predetermined amounts and ratios of drugs or antigen.[5]

Limitations of nanocochleate drug delivery-

1) They require specific storage conditions.

2) The cost of manufacturing is very high

3) Sometimes aggregation may occur during storage.[6]

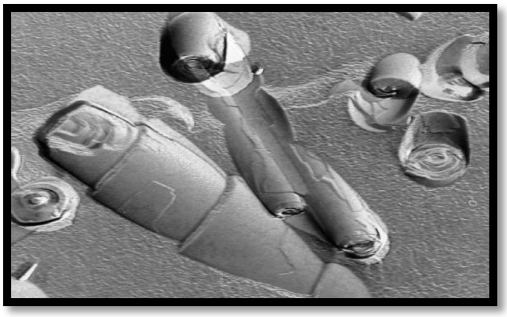

Figure 1: Nanocochleates

Cochleate-cell interaction-

When nanometre sized cochleates and liposomes containing the same fluorescent labelled lipid component are incubated with human fibroblast cells under identical conditions, cell exposed to cochleate shows bright fluorescent cell surfaces, whereas those incubated with liposome cant shows bright fluorescent cell surface. This suggest that cochleates edge can make them fuse with cell surfaces as compare to edge free liposomes. This mechanism of cochleate fusion with cell membrane can be supported by bacterial activity assay using Tobramycin cochleates, which act by inhibiting intracellular ribosomes. Tobramycin bridge cochleate in nanometre size showed improved antibacterial activity than drug's solution which is according to several researchers.[7]

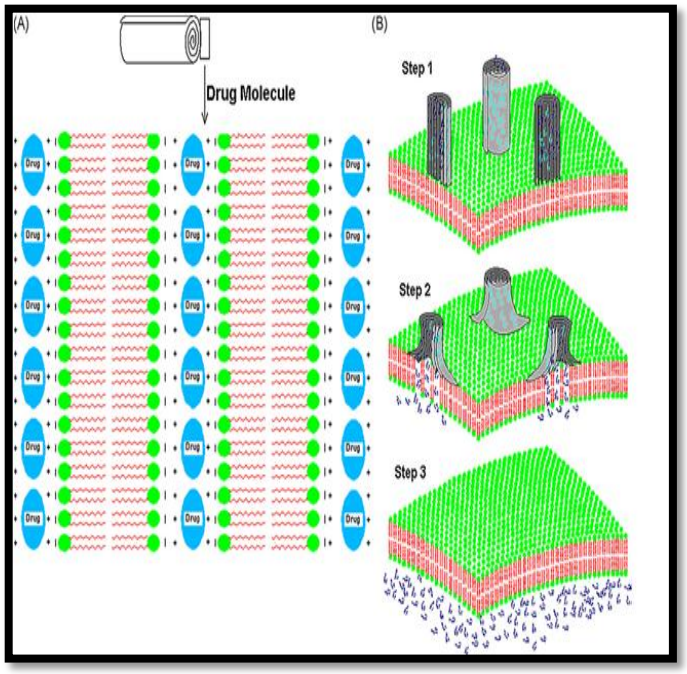

Figure 2: Cochleate-cell interaction

Mechanism of nanocochleate drug delivery-

After oral administration nanocochleates absorption takes place from intestine. Nanocochleates cross across the digestive epithelium and deliver their cargo molecule into blood vessel.[7]

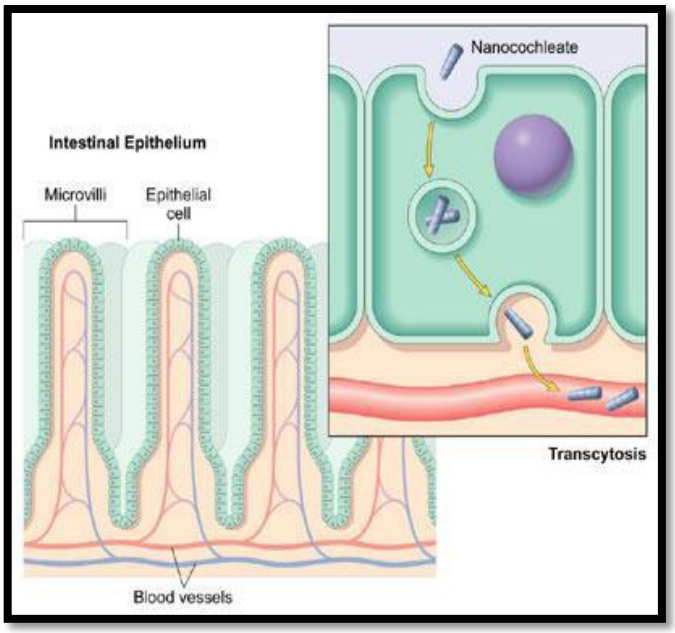

Figure 3: Nanocochleate absorption from intestine

In case of other route except intravenous they cross across the associated cell and reach into circulation. After reaching into circulation they are delivered to targeted cell.[7]

The nanocochleates are usually prepared by following methods-

1) Hydrogel Method.

2) Trapping method.

3) Liposome before cochleates dialysis method.

4) Direct calcium dialysis method.

5) Binary aqueous- aqueous emulsion system.

1) Hydrogel method-

In hydrogel method, the small unilamellar drug loaded liposomes are prepared, which are then added to polymer-A. The dispersion of two is then added to polymer-B. The two polymers are immiscible in each other & so immiscibility of the polymers leads to formation of aqueous two phase system. The cationic cross-linkage of the polymer is achieved by adding a solution of cation salt to two phase system, such that the cation diffuses into second polymer & then into the particles comprised of polymer, allowing the formation of small size cochleate. The formed cochleates are washed to remove polymer, which might be re-suspended into physiological buffer.[8]

2) Trapping method-

This method involves the formation of phosphatidylserine liposomes followed by drop-wise addition of a solution of CaCl2. Liposome can be generated by either addition of water to phospolipid powder or by adding water phase to a phospolipid film.[7]

3) Liposomes before cochleates dialysis method-

In this method mixture of lipid and detergent are used as the starting material and the removal of detergent is done by double dialysis. The mixture is dialysed initially by buffer and followed by calcium chloride solution which leads to formation of cochleates. This method is suitable for encapsulation of hydrophobic material or drug containing hydrophobic region such as membrane proteins.[9]

4) Direct calcium dialysis method-

Unlike liposome before cochleate dialysis method, this method does not involve the intermediate liposome formation and the cochleates are going to be in size. The mixture of lipid and detergent is directly dialysed against calcium chloride solution. In this method the competition

between the removal of detergent from the detergent/lipid/drug micelles and the condensation bi-layer by calcium, results in needle shaped large dimensional structure. Mixture of phosphotidylserine and cholesterol (9:1 wt ratio) in extraction buffer & non-ionic detergent is mixed with a preselected concentration of polynucleotide and the solution is vortex for 5min. The clear, colourless solution which resulted is dialysed at room temperature against three changes of buffer. The final dialysis routinely used is 6mM Ca2+. The ratio of dialysed to buffer for each change is minimum of 1:100. The resulting white calcium-phospholipids precipitates have been termed direct calcium cochleates.[10]

5) Binary aqueous-aqueous emulsion system-

In this method, the small liposomes are formed by either high pH or by film method, and then the liposomes are mixed with polymer, such as dextran. The dextran is then 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.[10]

Evaluation of nanocochleates-

1) Particle Size and Size Distribution

The particle size is one of the most important parameter. Two techniques are used to determine the particle size distribution of which includes photon correlation spectroscopy (PCS) and electron microscopy (EM). The latter includes scanning electron microscopy (SEM), transmission electron microscopy (TEM) and freeze-fracture techniques. The size evaluation of nanocochleate dispersion demonstrates better results, with freeze-fracturing microscopy and photon correlation spectroscopy as quantitative methods. Electron microscopy, however, could be adopted as an alternative option that measures individual particle for size and distribution. It is relatively less time consuming. Additionally, the freeze-fracturing of particles allows for morphological determination of their inner structure. In combination with freeze-fracture procedures, TEM permits differentiation among nanocapsules, nanocochleates and emulsion droplets. Atomic force microscopy (AFM) is an advanced nanoscopic technique applied for the characterization nanocochleates. Atomic force microscopy images can be obtained in an aqueous medium, and for this reason it is an effective means of investigating nanocochleates behavior in a biological environment. Mercury porositometry is an equally suitable technique for the sizing of nanocochleates. The freeze-dried nanocochleates are filled in a dilatometer under vacuum and then measured with the help of a mercury pressure porositometer. This method largely measures particulate agglomerates as mercury fails to penetrate to a greater extent within the primary particles.[11]

2) Specific Surface Area

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

$$A = 6 / \rho d$$

Where A is the specific surface area, ρ is the density and d is the diameter of the cochleate.

In most cases, the measured and calculated specific surface areas fairly comply while in some cases, the residual surfactant could produce a deviation in the measured values.[11]

3) Surface Charge and Electrophoretic Mobility

The nature and intensity of the surface charge of Nanocochleate is very important as it determines their interactions with the biological environment as well as their electrostatic interaction with bioactive compounds. The surface charge of colloidal particles in general and Nanocochleate in particular 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 charge composition critically decides the bio-distribution of drug carrying Nanocochleate. Generally, the Electrophoretic mobility of NP 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. The zeta potential can be obtained by measuring the electrophoretic mobility by applying the Helmholtz-Smoluchowski equation.[12]

4) Surface Hydrophobicity

The surface hydrophobicity of Nanocochleates influences the interaction of colloidal particles with the biological environment. Hydrophobicity and hydrophilicity

collectively determine the bio-fate of Nanocochleates and their contents. Hydrophobicity regulates the extent and type of hydrophobic interactions of Nanocochleates with blood components. Several methods including hydrophobic interaction chromatography, two-phase partition, adsorption of hydrophobic fluorescent or radiolabelled probes, and contact angle measurements have been adopted to evaluate surface hydrophobicity. Recently, several sophisticated methods of surface chemistry analysis have also been used.[7]

5) 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.[12]

6) 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 (PACA) 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.[13]

7) Drug content

The redispersed nanocochleates suspension is centrifuged at 15,000 rpm for 40 min at 25° to separate the free drug in the supernatant. Concentration of drug in the supernatant can be then determined by UV-Vis spectrophotometrically after suitable dilution.[14]

8) Nanocochleate Recovery and Drug Incorporation Efficiency

Nanocochleate recovery also referred to as nanoparticle yield, can be calculated using the following equation:

Nanocochleate recovery (%) = Concentration of drug in nanocochleates / Concentration of nanocochleates recovered X 100.

Drug incorporation efficiency has been expressed both as drug content (% w/w), also referred to as drug loading and drug entrapment (%) represented by the following equation:

Drug incorporation efficiency in nanocochleates = Amount of drug entrapped in nanocochleates / Total amount of added drug.

9) In-vitro Release

The in vitro release profile of nanocochleates can be determined using standard dialysis, diffusion cell or modified ultra-filtration techniques which have been recently introduced and which use phosphate buffer utilizing double chamber diffusion cells on a shake stand. A Millipore, hydrophilic, 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 the Nanocochleate is added directly into a stirred ultra-filtration cell containing buffer. At different time intervals, aliquots of the dissolution medium are filtered through the ultra-filtration membrane using < 2 positive nitrogen pressure and assayed for the released drug using standard procedures.[14]

Applications of Nanocochleate

1) Development of a nanocochleate based Apo-A1 Formulation of the Treatment of Atherosclerosis and other Coronary Heart Diseases.[14]

2) Nanocochleates have been used for delivering proteins, peptides and DNA for vaccine and gene therapy applications.[15]

3) Nanocochleates have the ability to stabilize and protect an extended range of micronutrients and potential to increase the nutritional value of processed foods.[15]

4) Nanocochleates can deliver Omega-3 fatty acids to cakes, muffins, pasta, soups, and cookies without altering the product's taste or odour.[13]

5) Nanocochleates shows 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 shows improved stability and efficacy at low doses. They show improved patient compliance.[14]

6) Cochleates would have the advantage of reducing the toxicity and improving the bactericidal activity.[13]

7) Nanocochleates which can be used to deliver nutrients such as vitamins, omega fatty acids which are more efficient to cell, & also to deliver lycopene without affecting the colour and taste of food which makes the concepts 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.[15]

Conclusion

As nanocochleate have unique multilayered structure, it protects active agents or compounds which are to be carried. It avoids contact of encochleated molecule from harsh environment. Nanocochleates have been widely used for delivery of many active therapeutic agents by defeating over disadvantages associates with other drug delivery systems. & hence, 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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