JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

NANOCOCHLEATES: NANOLIPID-CARRIER SYSTEM FOR DRUG DELIVERY

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ABSTRACT:-

Different systems of lipid-based nanocarriers are available in which Multi-layered cochleates stand out among these lipid-based nanocarriers as a unique nanocarrier system for hydrophilic and hydrophobic drugs with superior and increased stability, effectiveness, and enhanced drug permeability and reduced doses. This nanotechnology is used for drugs with problems such as low solubility and low permeability. Calcium divalent cation is added to liposomal vesicles to transform them into nanocochleate, a new lipid-based drug delivery method. This innovative lipid-based formulation technique can be used with drugs in the BCS class, such as BCS class IV and III. This article focuses on the history and structure Of Nanocochleates, Role Of Cation, Advantages, Disadvantages, Components Of Nanocochleates, Stability Of Nanocochleate Formulation, Mechanism Of Nanocochleate Drug Delivery, Routes Of Administration For Nanocochleate Drug Delivery, Method Of Preparation, Evaluation/Characterization Of Nanocochleates.

History

Cochleates are used to transfer antigens and peptides for vaccine delivery. They were discovered in 1975 by Dr. Dimitrious Papahadjoupoulos and his colleagues as precipitate generated by the interaction of negatively charged phosphatidylserine with calcium. Because of their rolled-up shape, he gave these cylindrical formations the Greek term "COCHLEATE," which means "SHELL." Cochleate structures are not uniformly created; instead, they can result in enormous needle-like structures from the dialysis approach or aggregation of stacked sheets formed by the trapping method. In 1999, cochleates were added to the hydrogel separation process to produce smaller, more uniform particles. One way to create cochleates with a mean particle size of less than 500 nm is to use a binary phase system, like non-miscible hydrogels. These nanocochleates were ideal for encapsulating hydrophobic drugs.

INTRODUCTION

Enhancement of bioavailability and formulation technique is always a challenging area in creating novel nanotechnology-based formulations, where researchers concentrate on changing the drug delivery mechanism. A liposome is essentially a vesicle with at least one phospholipid and cholesterol lipid bilayer that is used to encapsulate nutrients or drugs and appropriately distribute them. As one of these lipid-based nanocarriers, cochleate, along with increased oral bioavailability and site-specific drug delivery that results in fewer side effects, has emerged as a novel nanocarrier multi-layered system for hydrophilic and hydrophobic drugs with better and improved stability, efficacy, improved drug permeability, and reduction in drug dose. Since hydrophilic and hydrophobic medicines have higher oral bioavailability, site-specific medication delivery reduces negative effects. Specifically, it has been demonstrated that cochleate technology works well for the therapeutic oral administration of hydrophobic drugs, composed of a

bilayer of negatively charged phospholipids rolled up into a rigid spiral rod by contact with multi-cationic metal ions. It differs from liposomes in that it is rod-shaped, stiff, and has a water-free interior. Because of these special qualities, cochleates are an excellent delivery system for drugs that were not previously orally accessible. Rather than using lipid-based drug delivery systems, cochleates are being developed. Due to their inherent impermeability to tissue membranes and their enzymatic breakdown via the GIT wall, many therapeutic agents especially biological molecules are not absorbed by the gut. Bioral^{TM} Amphotericin B was the first product to encapsulate a lipophilic drug. This 50 nm-diameter nanocochleate is made up of crystalline forms that possess anhydrous interiors that enclose the drug molecule and shield it from the gastrointestinal tract's breakdown. Due to their restricted mechanical stability, low drug loading capacity, high production costs, and occasional phospholipid oxidation and hydrolysis such reactions' short half-life, liposomes are unable to increase drug absorption by oral administration. To address the aforementioned issue, a medication delivery system of this kind must be developed. Cochleates and Nanocochleates are new vesicular systems that may be able to meet the needs of the current. Nanocochleates are made the pure form of a phospholipid derived from soy that has a minimum of 75% lipid by weight.

STRUCTURE OF NANOCOCHLEATES

The stable, negatively charged phospholipid cations precipitate known as nanocochleates are made of elements that are found naturally including calcium and phosphatidylserine. Nanocochleates are structures that resemble cigars and are made of a continuous lipid bilayer. A structure resembling a cigar is created when tiny unilamellar anionic liposomes condense. Cations that are divalent, like calcium, arrange negatively charged lipids into solid sheets that roll up on themselves and resemble cigars when they are free of water. Both hydrophobic and hydrophilic surfaces may be found on nanocochleates, making them appropriate for encasing hydrophobic and hydrophilic medications.

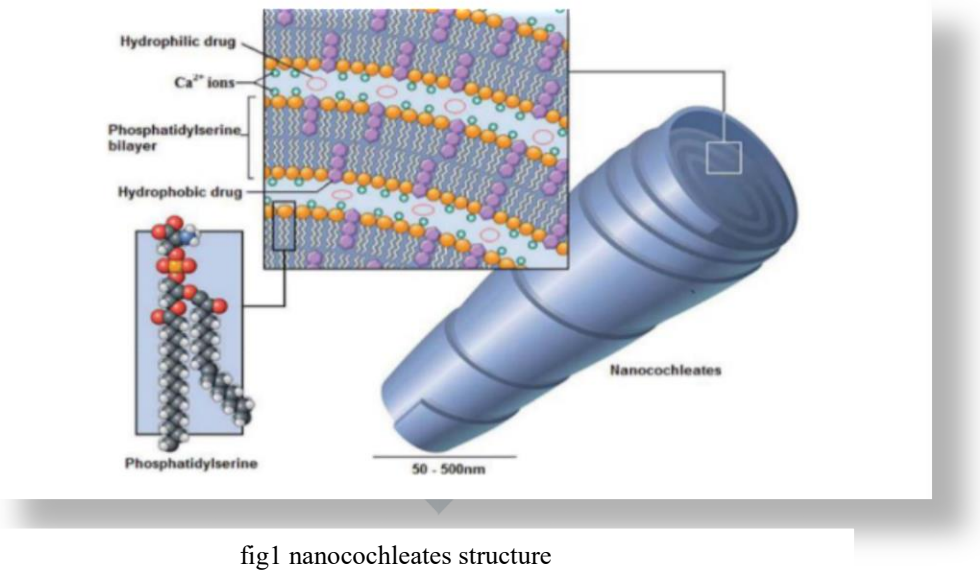

fig1 nanocochleates structure

ROLE OF CATION

Employed in the formation of nanocochleates, usually divalent cations. Ca++, Mg++, Ba++, and Zn++ are examples of cations that help the lipid sheet roll in a cochleate structure. Multivalent cations cause phospholipid that is anionic in the liposome's outer bilayer to become unstable, causing the bilayered structure to collapse. The divalent cations Zn++, Ba++, Mg++, and Ca++ can be included in nanocochleate formulations. According to reports, when calcium divalent cation is combined with phospholipids, it generates a structure that is less hydrated, more densely packed, and highly organized. Compared to Mg++, it is needed at far lower concentrations. The natural membrane fusion phenomenon is known to be significantly influenced by Ca++, whereas other multivalent cations are often ineffectual in these systems.

ADVANTAGES

1. Because their lipids are less prone to oxidation and have a non-aqueous inner core, cochleates surpass liposomes in stability.
2. The lyophilization procedure extends the formulation's shelf life when stored at room temperature.
3. Encochleated medications are shielded from environmental variables including sunshine, water, air, heat, or digestive enzymes that might break them down.
4. The nanocochleate's lipid bilayer is made up of naturally occurring simple lipids making it a safer and more biocompatible delivery system since lipids are non-inflammatory, non-immunogenic, and non-toxic.
5. Drugs that are required to be administered parenterally can be taken orally as cochleates e.g. Amphotericin B
6. They raise a variety of drugs' oral bioavailability, including high lipophilicity chemicals, genes, vaccines, proteins, and peptides, as well as biopharmaceuticals that are challenging to give, such as ibuprofen and artemisinin.

DISADVANTAGE

1. Aggregation may happen at times during storage
2. High production costs
3. Stability problem during storage

COMPONENTS OF NANOCOCHLEATES

Lipid:- This lipid can be a combination of one or more of these fats or lipid combined with additional fats or lipids, or it can be phosphatidic acid (PA) , phosphatidylinositol (PI),phosphatidyl serine (PS), dioleoylphosphatidylserine (DOPS), or phosphatidyl glycerol (PG). The lipid can also include dimyristoyl phosphatidylserine (DMPS), dipalmitoyl phosphatidylglycerol (DPPG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphotidylglycerol (DPG), and disearoyl phosphatidylserine (DSPS).

Drug:- A drug, which can be any of the following: polynucleotide, peptide, antiviral, protein , anaesthetic, immunosuppressive, steroidal anti-inflammatory agent, non-steroidal anti-inflammatory agent, vitamin, nutritional supplement, vasodilatory agent, tranquillizer, and/or herbal product.

Cation:- An example of a multivalent cation is Zn+2, Ca+2, Mg+2, or Ba+2. As a result, it is showing promise as a carrier for a broad variety of drugs for medicinal purposes.

STABILITY OF NANOCOCHLEATE FORMULATION

The entire formulation is protected and stabilized by the drug molecules' internal encapsulation. Components inside this structure stay intact even if its exterior layers may be damaged because the entire structure consists of solid lipid bilayers. Exposed to enzymes or severe external environmental conditions. The formulation's shelf life has been extended since the inside is virtually airtight and impervious to oxygen infiltration. Lyophilized nanocochleates can be kept at ambient temperature or 40°C in a powdered form. Lyophilized cochleates reconstituted using a liquid before being administered in vivo or used in vitro. The morphology or functions of the cochleate are not negatively impacted by lyophilization.

MECHANISM OF NANOCOCHLEATE DRUG DELIVERY

A substantial amount of the cell membrane is composed of lipids. When a cell membrane comes into touch with another lipid molecule. The substance is distributed throughout the cell by the lipid molecules that have bonded to one another. This method makes use of the medication delivery system for nanocochleates. The medicine is released gradually when the target cell's membrane fuses with the structure of the lipid bilayer of the nanocochleate.

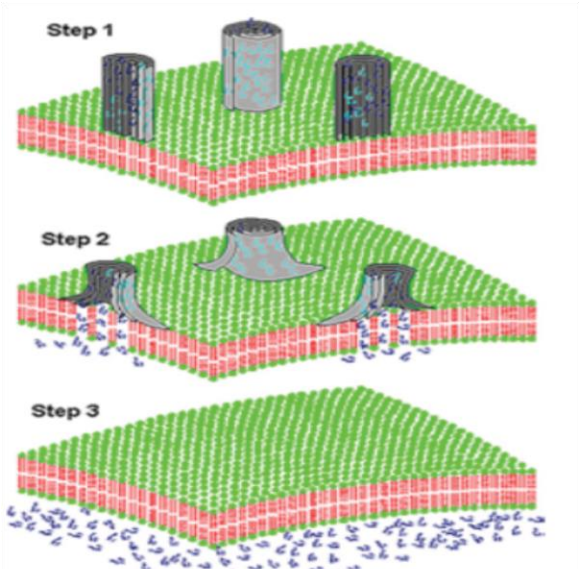

fig 2 mechanism of nanocochleate drug delivery

ROUTES OF ADMINISTRATION

Drug delivery vehicles made of nanocochleates enable effective oral administration of drugs. administered via parenteral, transdermal, intramuscular, sublingual(under the tongue), mucosal, lymphatic, ocular, subcutaneous (underneath the skin), intravenous (through an IV), mucosal, spinal, intra-articular, intra-arterial, bronchial(respiratory), nasal, and intrauterine, rectal as well as intra-vaginal or any other mucosal surfaces, can be alternative routes of administration. Oral delivery forms include tablets, granules, lozenges, capsules, cachets, powders, granules, pills and suspensions or emulsions.

METHOD OF PREPARATION

1. Trapping method
2. Hydrogel method
3. Aqueous-aqueous emulsion system
4. Dialysis method
5. Direct cochleates dialysis method

1. Trapping

Using this procedure, phosphatidylserine liposomes are formed and then a calcium chloride solution is added dropwise. Water may be added to phospholipid power or the water phase can be added to phospholipid film to create liposomes. Notably, after adding the liposomal suspension's solution, there is a drop in the solubility of the cargo moiety due to the solvent's miscibility in water.

After 15 minutes of vortexing the solution, phosphatidylserine is ready to create liposomes. Use filtering to separate the liposome from the fluid above. To the above-separated liposomes, add the hydrophobic medication and the trapping solvent (ethanol, dimethyl sulfoxide). Drop by drop, add the calcium chloride solution to the step 3 solution until crystalline cochleates develop in the final solution. The resultant cochleates are cleaned of any remaining solvent by washing them in a buffer containing calcium.

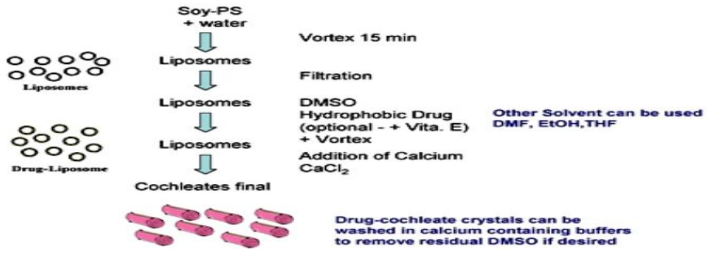

fig 3 trapping method

2. Hydrogel

The small unilamellar drug-loaded liposomes can be produced using techniques like the film hydration process and sonication. Then, drug-loaded liposomes are introduced to Polymer-A, such as polyethylene glycol (MW 3400–8000) and dextran (molecular weight (MW) 200,000–500,000). Polymer B solution, such as polyvinyl alcohol (PVA), polyvinyl methyl ether (PVME), and polyvinylpyrrolidone (PVP), is created. The liposome Using injection, polymer-A suspension and Polymer-B solution are combined. Due to their immiscibility, the two polymers combine to create an aqueous two-phase polymer system. This can be done manually with a needle pump at a rate that is appropriately monitored—ideally, between 1 and 10 ml/min. A divalent cation salt solution is included in the polymers to cause cationic cross-linking. It aids in producing cochleate at the nanoscale. The produced cochleates are submerged in a buffer that has a positively charged molecule in it to aid in the removal of the polymer. To ensure that the cochleate structures hold together when being washed stage and persist as precipitates, a calcium cation is added to the buffer for bathing.

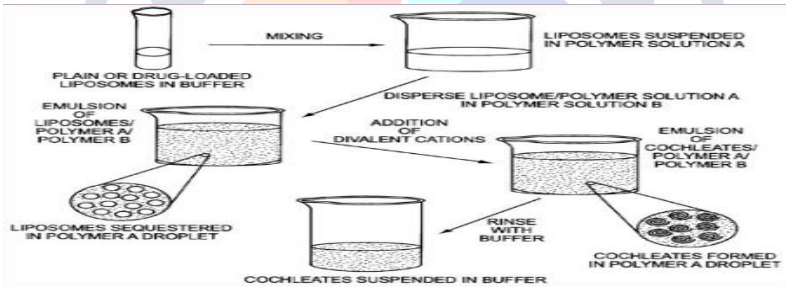

fig 3 hydrogel method

3. Aqueous-Aqueous Emulsion System

The technique is based on the fact that two-phase systems of aqueous, immiscible polymer solutions are incompatible. An organic solvent is not needed for this procedure. The film technique or a high pH is used to create liposomes. A polymer -A is combined with liposomes. e.g. Dextran. Subsequently, the liposome/dextran stage is incorporated into another non-miscible polymer, such as PEG. The third stage solution then contains calcium, which gradually diffuses from one stage to the next to produce nanocochleates. Afterwards, the gel is cleaned with physiological buffer 37. It is employed in the creation of cochleates smaller than 1000 nm.

4. Dialysis Method

Small-sized cochleates made of lipids, detergent, a physiologically significant chemical, and a cation are produced using this technique. The purpose of the detergent addition is to disturb the liposomes. First, a buffer is used to dialyze the mixture. The cochleates are then created by adding calcium chloride. Double dialysis is used to eliminate the detergent. The lipid-detergent combination is used to create an aqueous suspension. The suspension made in step 1 is combined Using polymer A, for example polyethylene glycol (PEG), dextran, or phosphatidylserine. The polymer-lipid-detergent A suspension is introduced to a solution containing polymer B, such as polyvinyl alcohol (PVA),

polyvinyl methyl ether (PVME), and polyvinylpyrrolidone (PVP). A two-stage polymer system is formed by the immiscible polymers A and B. A cationic moiety solution is introduced into the two-stage system of polymers. To get rid of the polymer the two-stage polymer system is cleaned. The medications cyclosporine and griseofulvin were tested to create cochleate using the procedure. The process yielded cochleates in size from 50 to 100 nm.

5. Direct Cochleates Dialysis Method

When comparing the Direct Calcium (DC) dialysis technique with the Liposome before Cochleates dialysis method, there is no intermediate liposome generation. Using this technique, large cochleates are produced. The lipid and detergent combination is dialyzed against a calcium chloride solution directly. Using this process, needle-shaped large-dimensional structures are produced by a struggle between calcium-induced bilayer condensation and detergent extraction from detergent, lipid, and drug micelles.
Step 1: In an extraction buffer, phospholipid and cholesterol are combined in a 9:1 weight ratio.
Step 2: After adding non-ionic detergent and a certain concentration of API, the mixture is vortexed for five minutes.
Step 3: At room temperature, the clear solution produced in Step 2 is dialyzed against three different buffer changes.

Step 4: Although 3 mM Ca2+ is enough, the last dialysis is completed in a solution containing 6 mM Ca2+. DC cochleate 36 is the resulting whitish calcium phospholipid.

EVALUATION/CHARACTERIZATION OF NANOCOCHLEATES:-

1. Particle size determination
2. Entrapment efficiency (EE):
3. Drug content
4. Density
5. Stability
6. Specific Surface Area
7. Surface Charge
8. In-Vitro

Determination of Particle Size Distribution: The mean particle size of dispersed cochleates is measured using the laser diffraction method using a Malvern 2000SM (Malvern, UK). The analysis is conducted at a temp. of 30±2°C and a detection angle of 90°. The volume mean diameter D, or average diameter of a sphere with the same volume as the particle under observation is used to indicate the mean vesicle size. Transmission Electron Microscopy (TEM)were used to examine the Cochleates' structure and morphology. To create a thin liquid film, a drop of diluted material is applied on a copper grid covered with carbon. After eliminating the surplus solution, the sample is inspected and captured on camera using a Zeiss EM 109 transmission electron microscope(TEM) at an 80 Kv accelerating voltage.

Entrapment Efficiency (EE) of Nanocochleate: Centrifugation tubes are filled with an aliquot containing 100 µl of cochleates. While vortexing, 1 ml of ethanol and 60 µl of pH 9.5 EDTA added to each tube. The end product is a colourless, transparent solution. Equations were utilized to determine the entrapment efficiency by measuring absorbance after the samples were appropriately diluted. Entrapment efficiency is calculated as follows: total API / quantity of API present in.

Drug Content: The dispersed nanocochleate suspension is centrifuged for 40 minutes at 25°C at 15,000 rpm. The complementary medication After an appropriate dilution, the concentration in the supernatant can be measured using a technique such as UV-Vis spectrophotometry.

Stability Study: Cochleates dispersions can be kept for three months at a temp. of 2 to 8 °C and 25±2 °C/60% RH. The cochleate particle size and change in entrapment efficiency (%EE) are used to assess the formulation's stability.

Density: Using a gas pycnometer, one may ascertain the density of nanocochleate in either air or helium. When using air and helium, the estimate obtained is much greater unique as a result of the structure's porosity and particular surface area.

Specific Surface Area: A sorptometer is used to measure the specific surface area of lyophilized nanocochleate.

$$A = 6/\rho d$$

Is the equation. where d is the cochleate's diameter, ρ is its density, and A is its specific surface area. Occasionally, there is a little difference in the observed values due to residual structure, and other times the measured and computed specific surface areas substantially match.

Surface Charge Determination: The nature and strength of a nanocochleate surface charge dictate how it interacts with its biological surroundings and how it forms an electrostatic bond with bioactive substances. By measuring the particle velocity in an electrical field, the surface charge may be approximated. Nanocochleate velocities are measured by laser diffractometry, such as velocimetry or laser Doppler anemometry.

Drug Release Study ln Vitro: Diffusion Cell Approach Double chamber diffusion cells on a shaking stand are typically employed in the diffusion cell technique. The two chambers are separated by the Millipore low protein binding membrane. Phosphate buffer is present in the receiver chamber, and the formulation is present in the donor chamber. The released drug is measured in the receptor compartment using conventional analytical techniques at various time intervals.

CONCLUSION:- Nanocochleates are Innovative drug delivery technology that can be applied to encapsulate genes, vaccines, and antigens. It also helps to protect the active agent because of its distinctive multi-layered structure, which improves the formulation of nanocochleates by lowering dosage and toxicity, increasing shelf-life stability, and improving bioavailability. It is utilized to encapsulate the medication molecules, giving them better stability and shelf life while shielding them from hostile environmental circumstances. Peptide medication encapsulation in vesicular systems has several benefits, including longer half-lives, higher stability, and decreased toxicity. Regarding the delivery of vaccinations, antigens, proteins, peptides, and API genes, it is a potential approach.

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