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Nanocochleates - A Novel Tool For Oral Drug Delivery System.

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ABSTRACT

Nanocochleates are unique novel drug delivery system in which, the desire drug molecule gets encapsulated into the multilayer of lipid crystal matrix. The nanocochleates structure provides protection to encochleated molecules from the degradation. It composed of negatively charged lipid generally phosphatidylserine and calcium. The nanocochleate structure has potential to carry the molecules which are hydrophobic, positively charged, negatively charged and that possess poor oral bioavailability. Nanocochleates is having more advantages than that of other dosage forms and system and hence it represents a new technology for oral and systemic delivery of drugs.

Keywords: Nanocochleates, Encochleated, Oral bioavailability, Systemic delivery.

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INTRODUCTION

Oral drug delivery has been known as most convenient and preferable route amongst all routes of drug administration [1,] and it is more suitable for non-hospitalize, non-acute care patients. Drug delivery system which allows oral delivery, improve patient compliance and also it facilitates treatment outside the hospital [2]. So, the considerable attention has been given on the development of novel oral drug delivery system to protect the drug in GIT and to improve the therapeutic efficacy of newly developed as well as preexisting drug molecule, and it also provides controlled and sustain release of drug to the targeted site [1]. The oral bioavailability is the highly desirable property for the molecules which are under investigation in the drug discovery process [3]. Near about 40% of newly developed drug having poor solubility thus shows bioavailability problems. Hence, since a no. of years the pharmaceutical development is found on formulation approaches to overcome solubility and related bioavailability problems [4]. The two important parameters that is solubility and permeability strongly influences oral bioavailability of drugs. Low solubility of drugs limits the drug dissolution rate and results in low oral bioavailability [5]. So, to overcome these problems researcher's focuses on the nanotechnology.

Nanotechnology is characterized as the science, engineering and technology carried out at the nano scale. The idea of nanotechnology was initially proposed by Feynman in 1959 and the term nanotechnology was first devised by Norio Taniguchi in the year 1974 [6].whereas the term nanomedicine was coined by Drexler and colleagues in 1980's [7].The drugs which exposed to nanotechnology exhibits superior characteristics including resistance to settling, high solubility, protection from degradation, enhance drug release increase adhesion to biological membranes, improved absorption and therapeutic effect [8].

Various approaches are present for the oral delivery of drugs. For example- 1) Converting drug to lipophilic prodrug 2) Conjugating a drug with lipophilic moieties and 3) Encapsulating a drug into particulate systems [9.]

Initially lipid based drug delivery system gaining more interest by researchers to improve oral bioavailability. One of them called liposome, It is more favourable due to its resemblance with the cell membrane and having various advantages like it provides selective passive targeting to tumor tissues, increase efficacy and therapeutic index of drug molecule, reduce toxicity, increase circulation lifetime e.g. stealth liposomes [10,11].

But the liposomes are failed to improve the oral absorption of drug because of their poor mechanical stability, low drug loading capacity, cost of production is high, phospholipids

sometimes undergoes oxidation and hydrolysis like reaction, short half-life[11].So, there is need to develop such a drug delivery system to solve the above problems. Cochleates and Nanocochleates are novel vesicular system which could satisfy the present needs.

Background of the Cochleate and Nanocochleate System

Cochleae were first described by papahadjopoulos and coworkers in 1975; they reported that these crystalline structure were formed as intermediates in the preparation of large unilamellar vesicles [12].He named these cylindrical structure "cochleates''. The term "cochleates" was coined due to the resemblance of the structure to a Snell with spiral shell [11].Hey proposed that fusion of unilamellar phosphatidylserine vesicles In the presence of ca^{++}creates large planar lamellae which roll up to form cylinder(figure .1)[13]. These cochleates have been used before 90s for transport of antigens and peptides for vaccine delivery. Nanocochleates where introduced in1999, which having particle size less than1000 nm [14]. It was demonstrated that by using binary phase system, such as two non-miscible hydrogels; cochleates can be form which having mean particle size of less than500 nm. These cochleates were highly suitable for the encapsulation of hydrophobic drugs [11].

Structure and Compositions

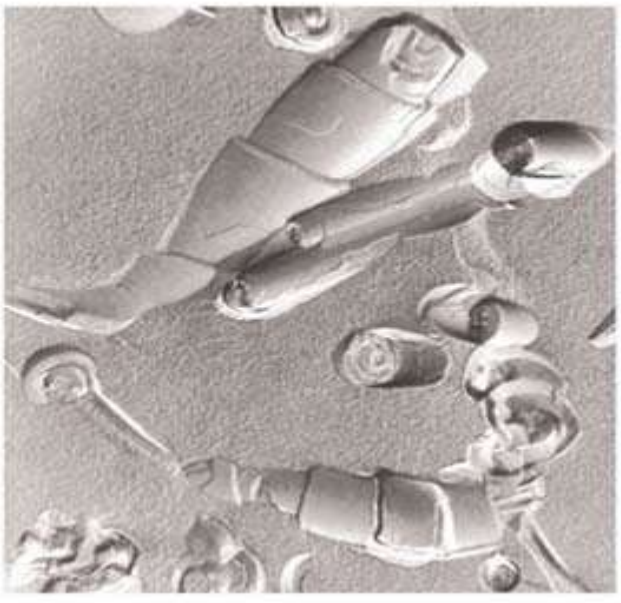

Figure 1: Cylindrical shape nanocochleates [2].

Nanocochleates are stable, negatively charged phospholipids cation precipitates composed of naturally occurring materials, eg. Phosphatidylserine and calcium .Nanocochleates are cigar like structures which are consist of series of lipid bilayers, which are formed as a result of the condensation of small unilamellar negatively charged liposomes [14]. In the presence of divalent cations causes a profound structural change in the lipids. The divalent cations like calcium

organize the negatively charged lipid into solid a sheet that rolled up on themselves, excluding water and forms a cigar like cochleates (figure.2)[15, 16].

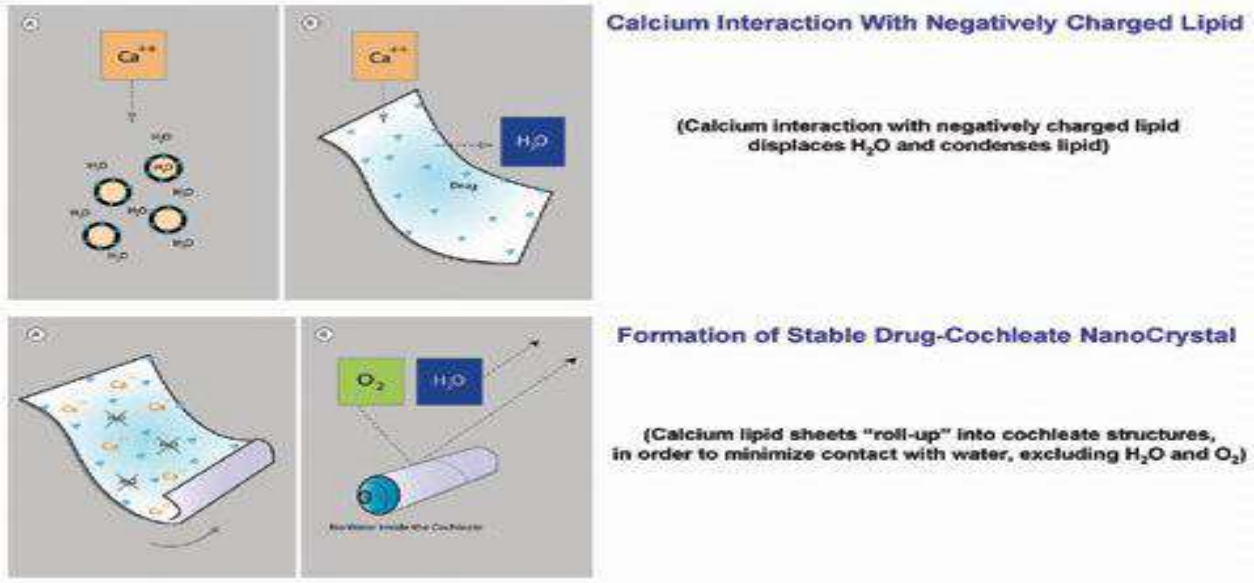

Figure 2: Schematic representation of formation of nanocochleates [14].

Nanocochleates consist of at least 75% of lipid,

1. A purified soy based phospholipids that contains phosphatidylserine (PS), dioleoylphosphatidylserine (DOPS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylglycerol (DPG), dioleoylphosphatidic acid (DOPA), disteroylphosphatidylserine (DSPS) and dimyristoylphosphatidylserine (DMPS), dipalmitoylphosphotidylglycerol (DPPG).
2. Multivalent cations which can be Zn^{++}or Ca^{++} or Mg^{++}.
3. A drug : Nanocochleate formulation technology is particularly applicable to Macromolecules and small molecules, drugs that are hydrophobic, amphiphilic, negatively or positively charged and also applicable for the drugs that are possess poor oral bioavailability [2,7,15].

Advantages of Nanocochleates As A Drug Delivery System [9, 18, 19]

The nanocochleates having water free interior so the advantages are numerous like,

1. They are more stable than the liposomes, because of less oxidation of lipids.
2. The lipids used in the preparation of the nanocochleates are found in animal and plant cell so they are non-toxic, non-immunogenic and non-inflammatory.
3. They are produced easily and safely.
4. The nanocochleates maintain their structure even after liophilization, whereas liposomes structure gets destroyed by liophilization.
5. They exhibit efficient incorporation of biological molecule in to the lipid-bilayer of the nanocochleates, especially hydrophobic one.

6. They improve oral bioavailability of broad spectrum compounds, such as those drugs which having poor water solubility, and also useful for the delivery of dedicated drugs like proteins and peptides.
7. They encapsulate or entrap the drug within a lipid bilayer rather than chemical bonding with the drug.
8. They reduce the stomach irritation & other side effects of the encapsulated drug.
9. They protect the drug from degradation from the harsh environment condition in the stomach.
10. They have potential for the slow or timed release of the drug in vivo as nanocochleates slowly unwinds or otherwise dissociate.

Limitations [9]

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.

Routes of Administration [18, 20]

Nanocochleates drug delivery vehicle allows an efficient oral delivery of drug. But with the help of this drug delivery, we can deliver the drug by parenteral, rectal, topical, sublingual, powders, granules, or in the form of solution, suspension or emulsion.

Dosage Form [21]

The nanocochleate formulation can be administered by the different routes which are listed in the table 1 below,

Table 1: Nanocochleate formulation administered by the different routes

Oral	Topical or Transdermal	Parenteral
Tablets, pills, capsules, lozenges, Powders, granules, solution, suspension, or emulsion**.**	Powder, sprays, ointments, pastes, creams, lotions, patches and inhalents.	Sterile isotonic aqueous or nonaqueous solution, dispersion, suspension or emulsion or sterile powder which may be reconstituted in to sterile injectable solutions or dispersion just prior to use.

Mechanism of Drug Delivery through the Nanocochleates [22, 9]

The proposed mechanism of the cross membrane delivery of lipophilic drugs encochleated in to the lipid-bilayer spaces of cochleates is shown in (Figure.3) .When lipid bi-layer structure of nanocochleates fuses with the cell membrane and releases the drug.

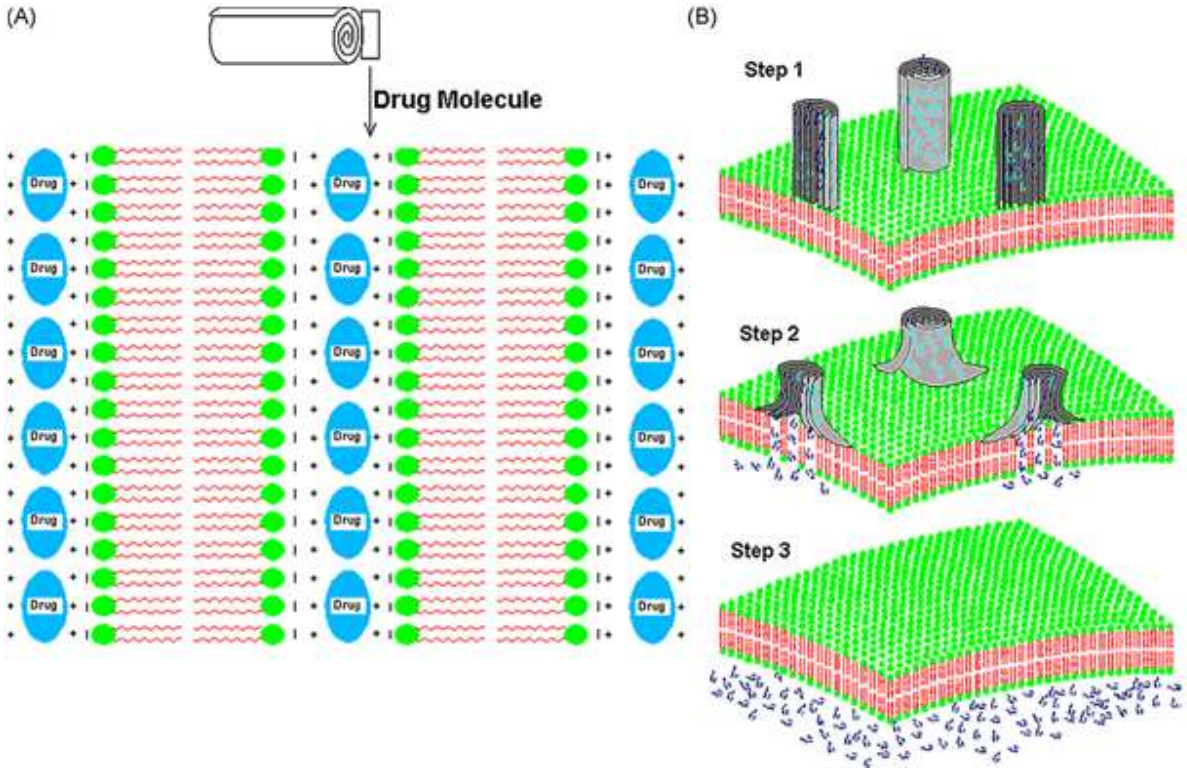

Figure 3: Schematic representation of nanocochleate interaction with the cell membrane [2].

Absorption after oral administration

After oral administration nanocochleates absorption takes place mainly from intestine. Nanocochleates cross the digestive epithelium and deliver their cargo molecules into blood vessel (Figure.4). In case of other routes except intravenous one they cross the associated cell (in similar manner) reach into circulation and deliver to targeted cells.

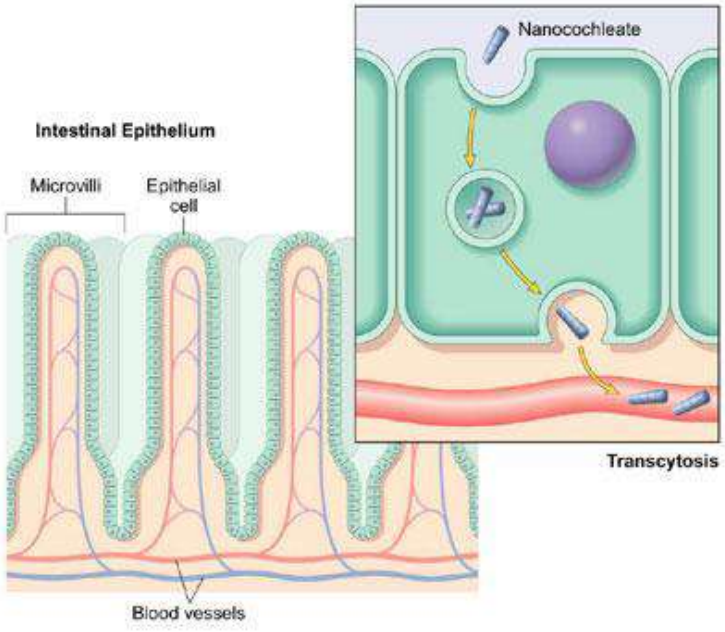

Figure 4: Nanocochleate absorption from intestine [19].

Delivery to targeted cells

The interaction of calcium with negatively charged lipids has been studied extensively. Many naturally occurring membrane fusion events involve the interaction of calcium with negatively charged phospholipids (generally phosphatidylserine or phosphatidylglycerol). Calcium-induced perturbations of membranes containing negatively charged lipids, and the subsequent membrane fusion events, are important mechanisms in many natural membrane-fusion processes. Hence, cochleates can be envisioned as membrane-fusion intermediates.

Delivery after Phagocytosis

The membrane phosphatidylserine receptors are present in macrophases and neutrophills which phagocytose nanocochleate. Nanocochleate then comes into close approximation to a liposome membrane results in perturbation and reordering of the liposome membrane, this fusion events between the outer layer of the nanocochleate and the liposome membrane. This fusion results in the delivery of a small amount of the encochleated material into the cytoplasm of the target cell (figure.5).

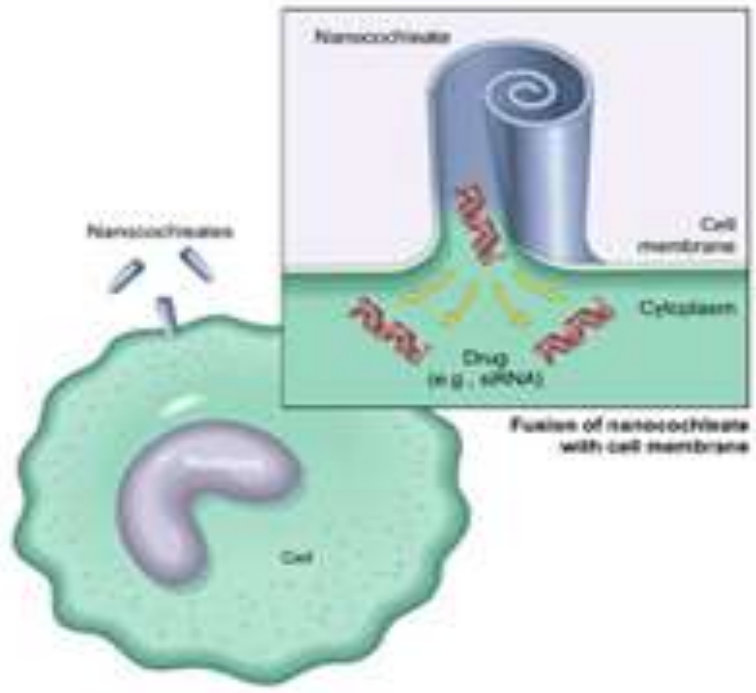

Figure 5: Nanocochleate Delivery to Macrophage [19].

Delivery by cell membrane fusion

In nanocochleate first comes into close approximation to a natural membrane, a perturbation and reordering of the cell membrane is induced, results in a fusion event between the outer layer of the

nanocochleate and the cell membrane. This fusion results in the delivery of encochleated drug molecule into the cytoplasm of the target cell (figure.6).

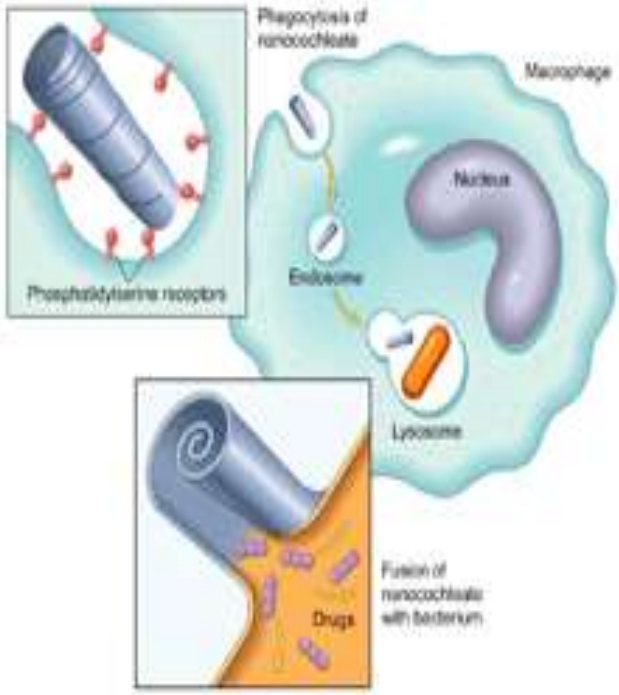

Figure 6: Nanocochleate Delivery by Direct Membrane Fusion [19].

Preparation methods for nanocochleates:

The nanocochleates are usually prepared by following methods,

1. Trapping method
2. Hydrogel Method
3. Binary aqueous- aqueous emulsion system
4. Liposome before cochleates dialysis method
5. Direct calcium dialysis method

Trapping method[18,19]

This method involves the formation of phosphatidylserine liposomes followed by drop wise addition of a solution of $CaCl_2$. Liposomes can be generated by either addition of water to phospholipid powder or by adding the water phase to a phospholipid film. Noteworthy due to miscibility of solvent in water, a decrease of the solubility of the cargo moiety is observed after addition of the solution to liposomal suspension. It includes the following steps:

Step-1: Prepare the liposomes form Phosphatidylserine by vortexing the solution for 15 minutes.

Step-2: Separate the liposomes from above solution by filtration.

Step-3: Add the trapping solvent and hydrophobic Drug to above separated liposomes (DMSO, DMF, EtOH or THF)

Step-4: Add the Calcium chloride solution drop wise to solution of step-3 the crystalline cochleates get appear in the resulting solution.

Step-5: The resulting cochleates are washed with calcium containing buffer to remove the residual solvent.

The trapping method schematically represented in (figure 7).

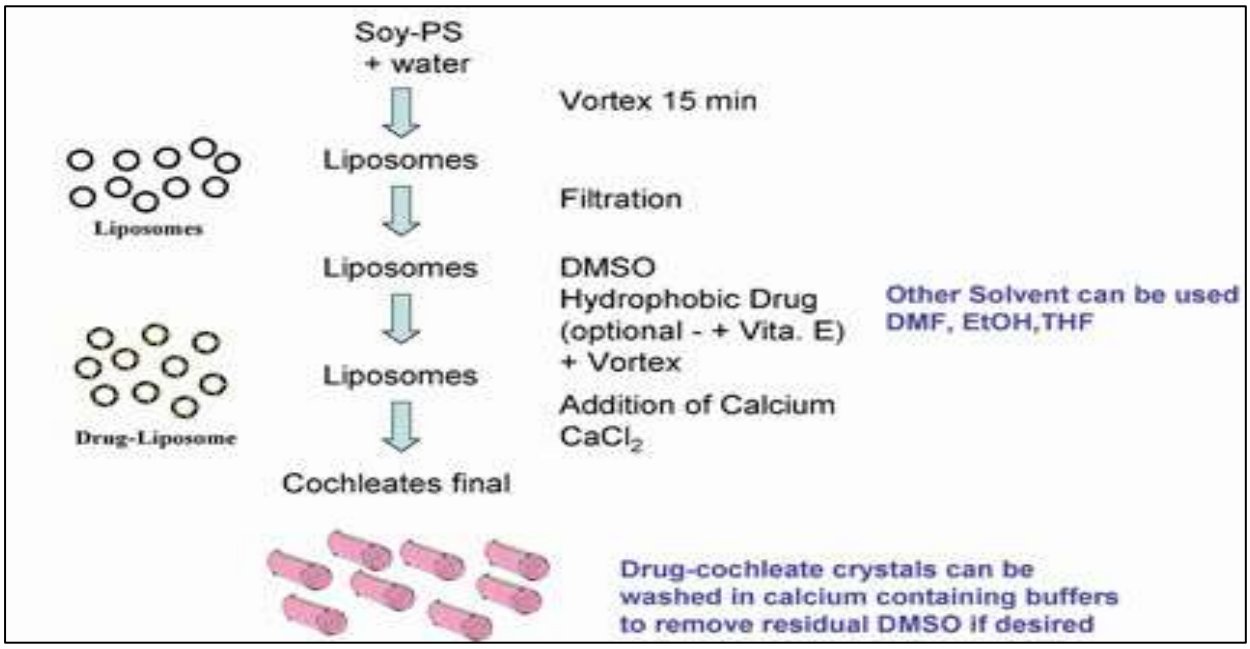

Figure 7: Process Flow of Trapping Method [24].

Hydrogel Method[20, 22]

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 microfluidization or other related methods.

Step 2- The liposome suspension is mix with polymer A 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 pyrolidone, 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 cochleates.

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 cochleae structures are maintained throughout the wash step, and that they remain as precipitates. This method is represented in (figure 8).

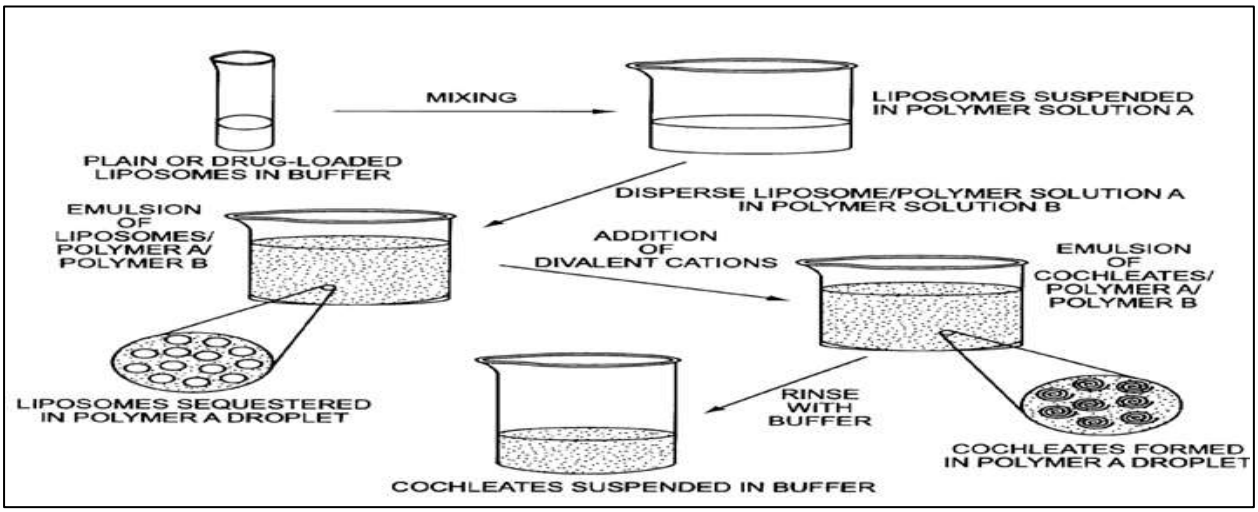

Figure 8: Process flow of Hydrogel Method [20].

Binary Aqueous–Aqueous Emulsion System Method[18,19]

This method is based on the incompatibility between two-phase systems of polymers solutions, both of which are aqueous and immiscible with each other. This method does not require organic solvents. This method involves the following steps:

Step-1: Preparation of liposomes.

Step-2: Mixing liposomal suspension into polymer solution A.

Step-3: Addition of this polymeric liposomal suspension by injection into the polymer solution B; thus creating a two-phase aqueous system.

Step-4: This is followed by addition of a solution of cation salt to form small sized cochleates, washing of the cochleates and re-suspension of cochleates in physiological buffer.

Step-5: Further the cochleates can be lyophilized and filled into soft or hard gelatin capsules, made into tablets or other dosage forms.

Advantages:

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

2. Better process recovery.
3. Economic processing.

Liposomes Before Cochleates (LC) Dialysis Method[27,18]

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.

This method comprises of following steps:

Step-1: 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.

Step-2: The resulting solution is vortexed for 5 minutes.

Step-3: 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.

Step-4: The vesicles are converted to a cochleate precipitate, either by the direct addition of Ca2+ ions, or by dialysis against two changes of buffer containing 3 mM Ca2+ ions, followed by buffer containing 6 mM Ca2+.

Direct Calcium (DC) Dialysis Method[19,11]

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 baitlayers 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 pre-selected concentration of polynucleotide, and the solution is vortexed for 5 minutes.

Step-2: 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-2-aminoethane sulfonic acid, 2 mM L-histidine, 100 Mm NaCl, pH 7.4} containing 3 mM CaCl2.

Step-3: The final dialysis routinely used is 6 mM Ca2+, (although 3 mM Ca2+ is sufficient and other concentrations may be compatible with cochleate formation.)

Step-4: The resulting white calcium-phospholipid precipitates have been termed DC cochleates.

The ratio of dialysate to buffer for each change was a minimum of 1:100. When examined by light microscopy, the suspension contains numerous particulate structures up to several microns in diameter, as well as needle-like structures. This method is schematically represented in (figure 9).

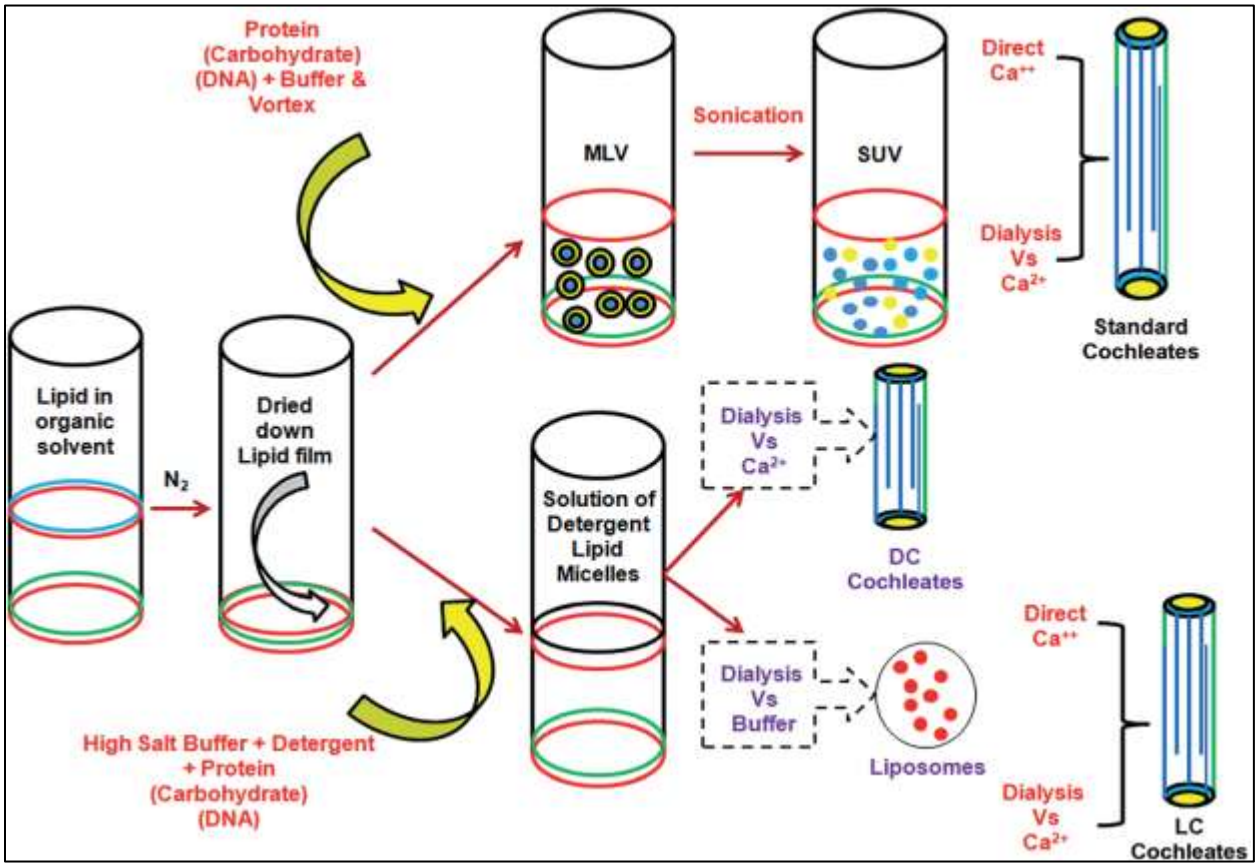

Figure 9: Cochleates preparation by liposomes before cochleates dialysis and direct cochleates dialysis method [11].

Properties of Nanocochleates

The cochleates are mainly prepared from phosphatidylserine (PS) and calcium. The PS shows considerable binding affinity for calcium due to the tendency of calcium to lose part of its hydration shell and to displace water upon complex formation [13].as a result of neutralization of the electrostatic charge, calcium causes liposomes composed PS to aggregate and fuse with each other [12]. It has been reported that ca^{++} forms a more tightly packed highly ordered and less hydrated structure than doe's mg^{++} with phosphatidylserine, also it is required in much lower concentration than mg^{++}. It is well documented that ca^{++} plays a vital role in natural membrane fusion phenomena while other cations listed above are ineffective in most such systems [11].Nanocochleate facilitates cross membrane diffusion for charged and impermeable molecules finds wide application n drug delivery. The therapeutic agents like peptides are soluble but impermeable to tissue membranes. The agents who have binding site inside the cell rather than cell surface receptor for that the cross

membrane permeation is important. The nanocochleate drug delivery system facilitates oral absorption for peptide drug that possess a net positive charge [24].

The nanocochleates can be stored by freeze drying which provides potential to be stored for longer periods of time at room temperature which would be the extra advantage for worldwide distribution and storage prior to administration. Also, the nanocochleates keep their structure even after lyophilization. The nanocochleates have potential for slow release of drug molecule in vivo as the nanocochleate slowly dissociates with time. They are produce easily and safely and do not have any negative impact on health [25]. It has been reported that the high tension at the lipid bilayer edges of nanocochleate caused the creation of kind of driving force for drug molecule to penetrate faster and the nanocochleates can also be targeted. The nanocochleate have ability to encapsulate materials of all shape and size and the percent of encapsulation depends on the nature of drug [29].When the nanocochleates containing active molecules are given orally, they are preferentially taken up by the macrophages in gastro intestinal track and go through lymphatic vessels or they can even deliver drug molecules to the site of action. Therefore, this system effectively developed for oral administration [26, 27].

In the presence of clustering agent the nanocochleate formation depend on the gel crystalline phase transition temperature of the anionic lipid mixture. When the different mixtures of lipid hydrated at room temperature produced different sized cochleates depending on their transition temperature and presence or absence of clustering agent [11].

APPLICATIONS

1. Development of Apo A1 formulation of the treatment of Atherosclerosis and other Caronary Heart Diseases [28].
2. Nanocochleates have been used for delivering proteins, peptides and DNA for vaccine and gene therapy applications [29].
3. Nanocochleates have the ability to stabilize and protect an extended range of micronutrients and potential to increase the nutritional value of processed foods [29].
4. 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 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[30].

5. Nanocochleates can deliver omega-3 fatty acid through cakes, muffins, pasta, soups, and cookies without altering the products taste or odour [9].

CONCLUSION

Nanocochleates are a lipid based drug delivery system that shows potential in the systemic as well as oral delivery of wide range of molecules with important therapeutic activities, including drugs, genes and vaccine antigens. The nanocochleate have unique multilayer structure which can be helpful in enhancing the qualities of the formulation by protecting the drug, enhancing bioavailability, reducing dose as well as toxicity, increasing shelf life thus stability, and ultimately efficacy of the product. 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. Hence, 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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