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Review Article

Nanococleates: An Overview

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ABSTRACT

Nanocochleates are novel drug delivery system offering systemic and oral delivery of various charged drug molecules 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. Nanocochleate can encapsulate drugs which are hydrophobic, positively charged, negatively charged and poor orally bioavailable. 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: Cochleate, Bilayers, Phosphatidylserine, Liposomes.

1. INTRODUCTION

Recently, many drug delivery platforms have emerged and are present in either in preclinical stage or in an advanced clinical trial with the intent of trying to demonstrate efficient oral absorption. Reported strategies to improve drug absorption through cross membrane diffusion included pro-drug analogue design, application of absorption enhancers and enzyme inhibitors and delivery by using lipid based systems. Various approaches have been reported for oral delivery of tissue impermeable drugs .for example i) converting a drug to lipophilic pro-drug, ii) conjugating a drug with lipophilic moieties, and iii) encapsulating a drug into particulate systems. Particulate systems may offer good protection of delicate biological agents with no need for chemical modification of the molecules themselves[2]. Now-a-days vesicles as a carrier system have become the vehicle of choice in drug delivery. Encapsulation of the drug in these vesicular structures is an system which predicts to prolong the existence of drug in the systemic circulation[3] .The nanocochleate drug delivery vehicle is based upon encapsulating drugs in a multilayered, lipid crystal matrix (a cochleate) to potentially deliver the drug safely and effectively. Nanocochleates contain both hydrophobic and hydrophilic surfaces, which are suitable to encapsulate both hydrophobic drugs & hydrophilic drugs[5.] Nanocochleates are cylindrical (cigar-like) microstructures that consist of a series of lipid bilayers[1] Nanocochleates are 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.[4]

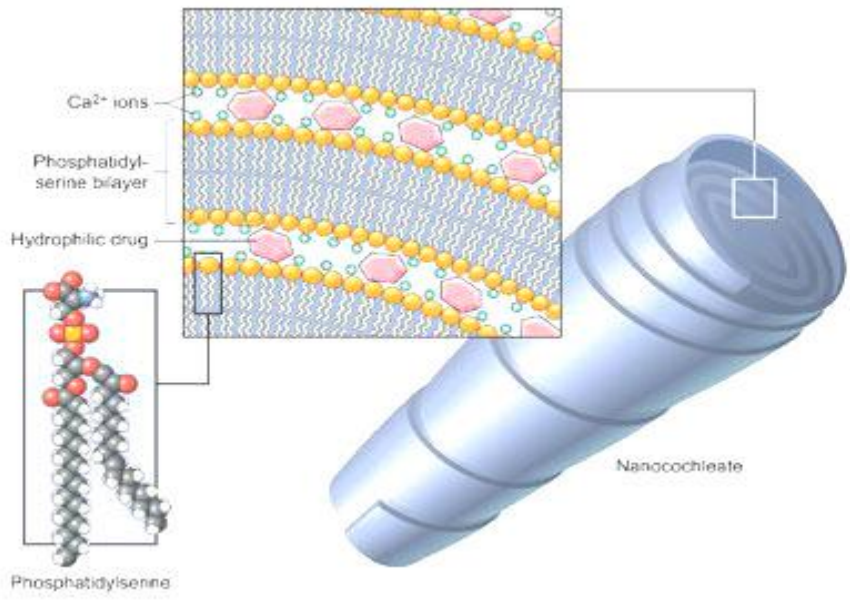

Fig. 1: Nanocochleate Structure

2. DISCOVERY OF NANO COCHLEATES

Cochleates were discovered in 1975 by Dr. D. Papahadjoupoulos and co-workers, and have been used in the 80s and 90s to transport antigens and peptides for vaccine delivery. Cochleate structures reported in the literature have not always been uniform, resulting either in aggregates of stacked sheets and cochleates by the trapping method or large size needles-like structures by the Dialysis method. Nanocochleates were introduced in 1999 to develop smaller but more consistent particles. It was demonstrated that by using a binary phase system, such as two non-miscible hydrogels, cochleates can be formed that display a small mean particle of less.[10]

3. 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.[6]

4. DOSAGE FORM[5]

• **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 prior to use.

5. ADVANTAGES[7]

a) They are more stable because of the less oxidation of lipids.
b) They can be stored by freeze drying, which provides the potential to be stored for long periods of time at room temperatures, which would be advantageous for worldwide shipping and storage prior to administration.
c) They can maintain their structure even after lyophilization, whereas liposome structures are destroyed by lyophilization.
d) They can exhibit efficient incorporation of hydrophobic drugs into the lipid bilayer of the cochleate structure.
e) They can exhibit efficient incorporation of antigens with hydrophobic moieties into the lipid bilayer of the cochleate structure.
f) They have the potential for slow release of a drug, antigen or biologically relevant molecule in vivo as cochleates dissociate.

g) They have a lipid bilayer, which serves as a carrier and is composed of simple lipids which are found in animal and plant cell membranes-, so that the lipids are non-toxic.
h) They are produced easily and safely.

6. LIMITATIONS[8]

a) They require specific storage condition.
b) Sometimes aggregation may occur during storage; this can be avoided by the use of aggregation inhibitor.
c) The cost of production is high.

7. STABILITY OF NANOCOCHLEATE FORMULATIONS

Encapsulation of the drug molecules in the interior provides protection and stability to the entire formulation. 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 40°C. Lyophilized cochleates can be reconstituted with liquid prior to in-vitro use or in-vivo administration. Lyophilization has no adverse effects on cochleate's morphology or functions.[11]

8. MECHANISM OF NANOCOCHLEATE DRUG DELIVERY[4]

The proposed mechanism of the delivery of hydrophobic drugs loaded in the inter-bi-layer spaces of nanocochleates. 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. The schematic diagram is shown in Fig. 2.

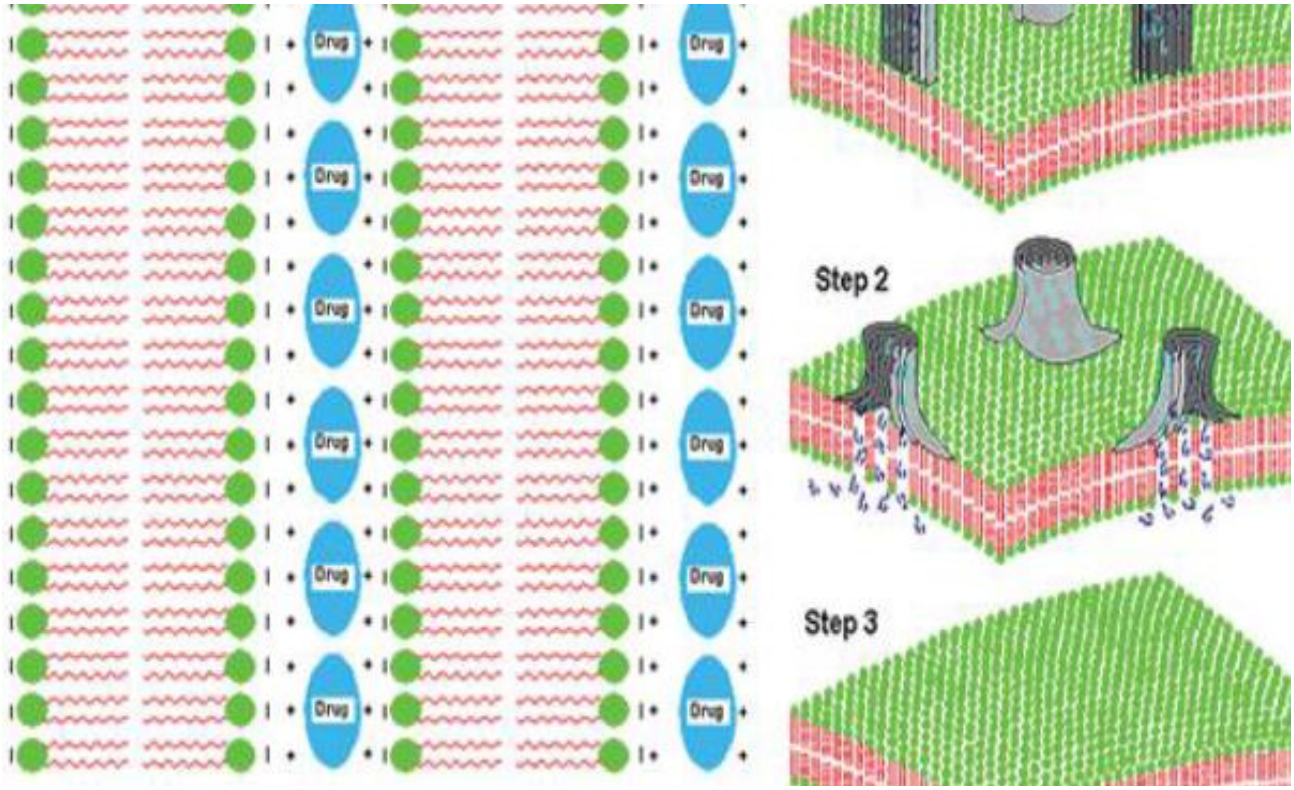

Fig. 2: Diagramatic presentation of nanocochleate interaction with the cell membrane

9. FORMULATION METHODS

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.

Hydrogel method[10]

Formation of the cochleates is multi-step process. In hydrogel method initially the small unilamellar drug loaded liposomes were prepared, which were added to polymer A (Which may be phosphotidylserine, dextran, polyethylene glycol, etc.). The dispersion of two was then added to another polymer B (which may be polyvinylpyrrolidone, polyvinyl alcohol, Ficoll, polyvinyl methyl ether, etc.). The two polymers were immiscible in each other. Immiscibility of the polymers leads to formation of an aqueous two phase system.

The cationic cross-linking of the polymers was 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. A allowing the formation of small-sized cochleates. The formed cochleates were then washed to remove polymer, which might be re-suspended into a physiological buffer or any appropriate pharmaceutical vehicle or lyophilized. The schematic diagram is shown in Fig. 3.

Trapping method[7]

This method involves the formation of phosphatidylserine liposomes followed by drop wise 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. A schematic presentation of the preparation methodology is given in Fig.4.

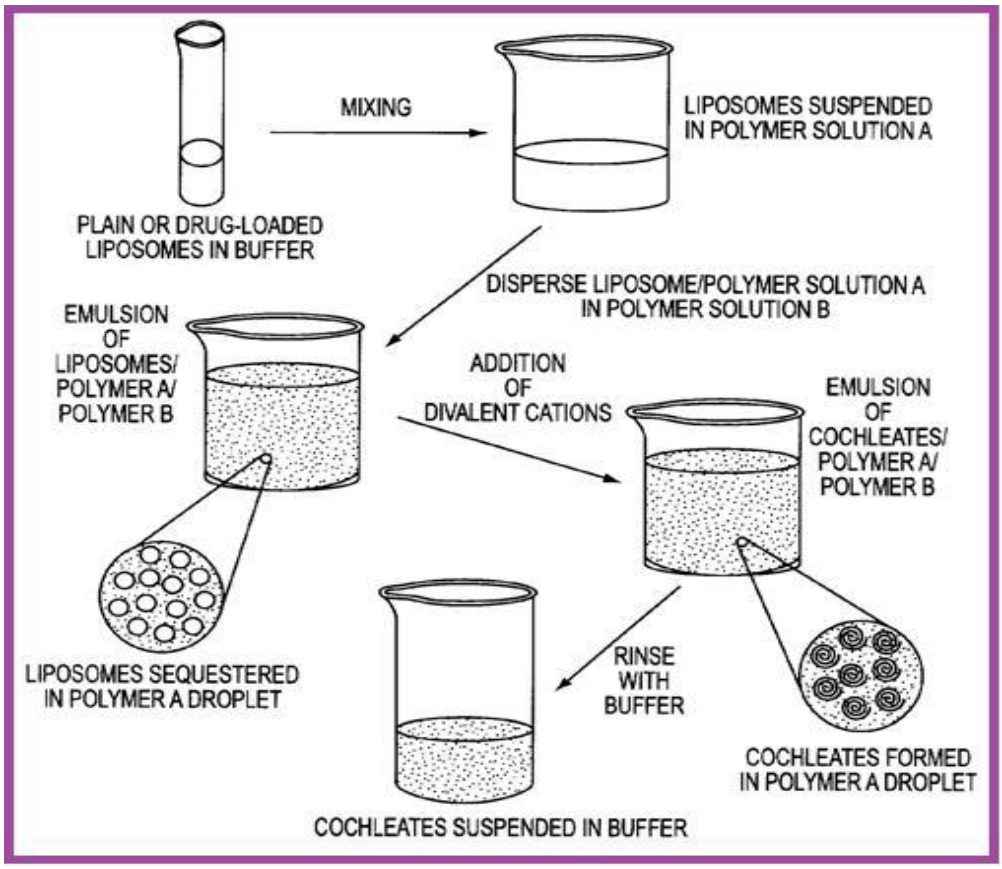

Fig. 3: Hydrogel method

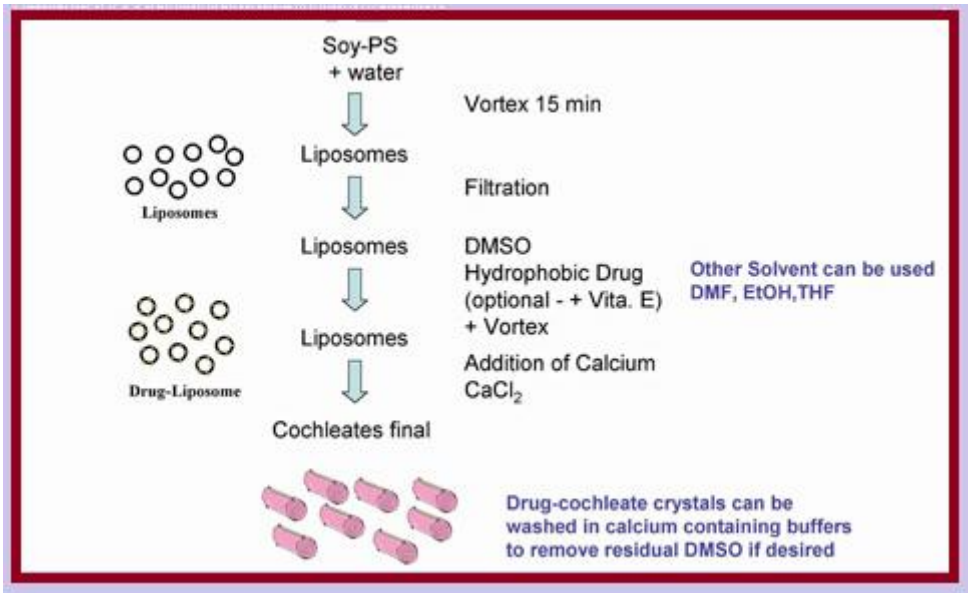

Fig. 4: Schematic presentation of trapping method

Liposome before cochleates dialysis method[6]

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 dialyzed 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.

Direct calcium dialysis method[8]

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 have directly been 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 was 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 The final dialysis routinely used is 6 mM Ca2+, although 3 mM Ca2+ 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.

Binary aqueous- aqueous emulsion system[2]

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. 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. CHARACTERIZATION OF NANO-COCHLEATE FORMULATION

- **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[12.]
- **Entrapment efficiency (EE):** One hundred micro liters of cochleates is aliquoted into centrifugation tubes. To each tube 60 µl pH 9.5 EDTA and 1ml of

ethanol is added while vortexing. Absorbance of the resulting solution is determined using spectroscopic technique and entrapment efficiency is calculated using below mentioned equation.[12]

$$\text{ENTRAPMENT EFFICIENCY} = \frac{\text{AMOUNT OF DRUG PRESENT IN COCHLEATES}}{\text{TOTAL AMOUNT PRESENT}}$$

- **Stability study:** Cochleates dispersions can be kept at 2-8°C and 25±2°C/60% RH for a period of 3 months to check their stability. The stability of the vesicles is determined in terms of change in particle size and percent entrapment efficiency.[12]

- **Cochleates-cell interaction:** To examine the interaction of cochleates with cell membrane, 2% fluorescent lipid in addition to the negatively charged lipid is used to form fluorescent liposomes. When cochleates interact with cell membrane involving a fluorescent lipid transfer, cell surfaces become fluorescent under fluorescent microscopes as illustrated in fig.5[3]

- **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

$$A = 6 / \rho d$$

Here A is the specific surface area, ρ is the density & d is the diameter of the cochleate.[3]

- **Surface charge:** The nature and intensity of the surface charge of nanocochleate determines 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 (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 composition of charge decides the bio-distribution of drug carrying nanocochleate. Generally, 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.[3]

- ***In vitro* release study:** The *in vitro* release profile of nanocochleates is determined using standard dialysis, diffusion cell or modified ultra-filtration 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 ultra-filtration cell containing buffer. At different time intervals, aliquots of the medium are filtered through the ultra-filtration membrane & assayed for the released drug using standard procedures.[6]

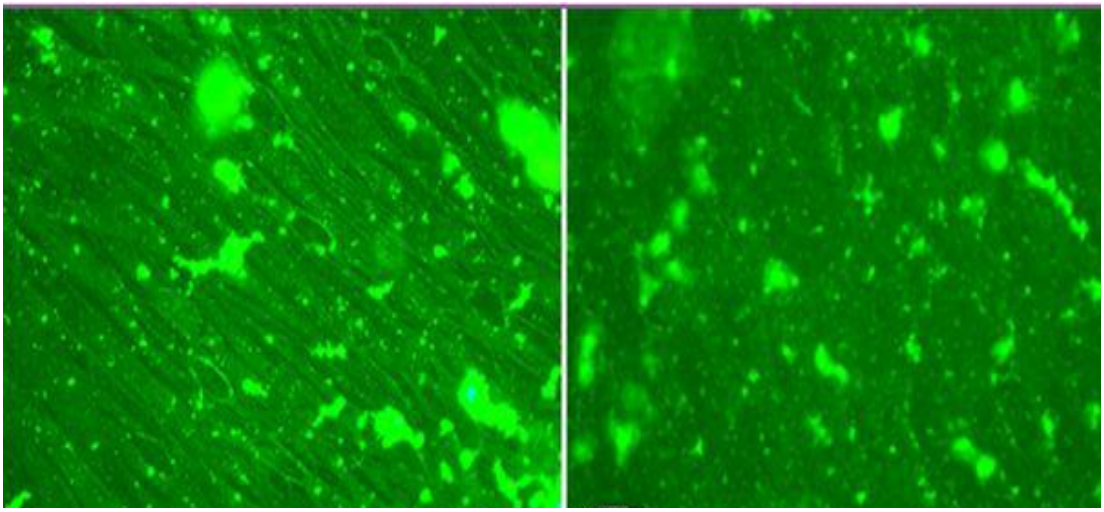

Fig. 5: Mammalian skin cells exposed to the poly-lysine fluorescent nanocochleate

11. APPLICATION[5]

1) Development of a Nanocochleate based ApoA1 Formulation for the Treatment of Atherosclerosis and other Coronary Heart Diseases.
2) Cochleates for the Delivery of Anti-inflammatory Agents.
3) Cochleates for the Delivery of Antimicrobial Agents.
4) Bio geode 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.

12. CONCLUSION

Nanococleates have been widely used for delivery of many active therapeutic agents as the hydrophilic and hydrophobic drug delivery can be possible by this system due to bilayer structure of lipids. Encocleation can be useful in enhancing the quality of formulation by upgrading shelf life, stability, bioavailability, reducing toxicity. Thus in future this drug delivery system can be used as an alternative to deliver the biological or therapeutic agents.so this review article focus on the therapeutic potential of new class of drug carrier i.e. nanococleates which definitely can drive the pharmaceutical world to the new era of drug delivery of highly challenging drugs.

13. REFERENCES

1. Zarif, L, and Perlin, D. Amphotericin B Nanocochleates: From Formulation to Oral Efficacy, Drug Delivery Technology, volume 2(4): 34-37.
2. Vyas H, Upadhyay T, Thakkar N, Patel K, U Upadhyay, Nanocochleate: Novel Bypass of Conventional Drug Delivery System, PharmaTutor, 2014,volume 2(2): 90-97.
3. Gol D, Shah V. Nanocochleates: a novel approach for drug delivery, World Journal of Pharmaceutical Research,2014, volume 3(2): 1920-1944**.**
4. Yeole S, Pimple S, Chaudhari P. A Review on Nanocochleate – A Novel Lipid Based Drug Delivery System, Journal of Biomedical and Pharmaceutical Research, 2013, volume 2 (1): 01-07.
5. Wasankar S, Makeshwar K, Deshmukh A, Burghate R. Nanocochleate: A Review, Research Journal of Pharmaceutical Dosage Forms and Technology. 2012, volume 4(3): 153-159.
6. Bhosale R, Ghodake P, Mane A, Ghadge. Nanocochleates: A novel carrier for drug transfer, Journal of Scientific and Innovative Research 2013; volume 2 (5): 964-969.
7. Ramasamy T, Khandasamy U, Hinabindhu R, Kona K. Nanocochleate –

A New Drug Delivery System, FABAD journal of Pharmaceutical Science.,2009, volume 3(4): 91–101.
8. Zarif L, Jin T, Segarra I, Mannino R. Novel hydrogel isolated cochleate formulations, process of preparation and their use for the delivery of pharmaceutical agents. PCT Application WO 01/52817 A2. Filed 1/22/2000 .US patent 6,153,217.
9. Panwar V, Mahajan V, Panwar A, Darwhekar G, Jain D. Nanocochleate: as drug delivery vehicle, International Journal of Pharmacy and Biological Sciences, 2011, volume 1(1), Volume 1(1): 31-38.
10. Ravi Sankar V, Reddy Y. Nanocochleate – a new approch in lipid drug delivery, International Journal of Pharmacy and Pharmaceutical Sciences, 2010, volume 2(4): 220-223.
11. Delmarre D, Lu R, Tatton N, Krause-Elsmore S, Gould-Fogerite S, Mannino R. Formulation of Hydrophobic Drugs Into Cochleate Delivery Vehicles: A Simplified Protocol & Formulation Kit. Drug Delivery Technology. 2004, 4(1):64-69.
12. Landge A, Pawar A, Shaikh K. Investigation of cochleates as carriers for topical drug delivery. International Journal of Pharmacy and Pharmaceutical Sciences. 2013, volume 5(2): 314-20.