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Review Article

Nanocochleate: A review

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ABSTRACT

There are various based nanocaries system are available in current scenario**.** The nanocochleate is a novel drug delivery system which has ability to encapsulate the water soluble cationic drugs hydrophilic and hydrophobic drugs. The nanotechnologies used to encapsulate the poorly water soluble drugs for increase their bioavailability. The main purpose of development of the nanocochleate drug delivery to improve bioavailability of therapeutic active agents.

Keywords: Cochleate Nanocochleates, Hydrogel method, Trapping method

INTRODUCTION [5,16,17]

With the liposome formulation various modification is done which develop a new class of drug delivery called cochleate. Nanocochleate delivery vehicles are stable phospholipid-cation precipitates composed of simple, naturally occurring and synthetic materials, generally phosphatidylserine and calcium The nanocochleate drug delivery deliver the drug safely and effectively. The vehicle is based upon encapsulating drugs in multilayered, lipid crystal matrix to potentially. Nanocochleates are cylindrical (cigar-like) microstructures that consist of a series of lipid bilayers. 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 hydrophilic and hydrophobic surfaces, which are suitable to encapsulate both hydrophobic drugs & hydrophilic drugs.

HISTORY [15]

The cochleates was discovered by Dr. Dimitrious Papahadjoupoulos and his coworkers in 1975 as precipitates formed by the interaction of negatively charged phosphatidylserine and calcium. He named this cylindrical structure as cochleates. The structure was resembled to a snail with a spiral shell therefore this called cochleates. The cochleates structure is formed either aggregates of stacked sheets formed by trapping method or large cell like structure formed by dialysis method. In 1999, cochleates were introduced to develop smaller, but rather more consistent particles. It was demonstrated that by using a binary phase system,

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such as two non-miscible hydrogels; cochleates can be formed that display a small mean particle of less than 500 nm. These cochleates were highly suitable for the encapsulation of hydrophobic drugs.

Composition of Cochleates [5,9]

The cochleates essential consist of drug, lipid and cations. The different kinds of drug used in formulation of cochleate which was protein, peptide, polynucleotide, antiviralgent, aneaesthetic agent, anticancer agent, immunosupprescent, anti-inflammatory agent, tranquilizer and nutritional supplement. The lipids used for preparation of cochleates are phosphotidylserine, Phosphatidic acid, Phosphatidylinosotol, Phosphotidyl glycerol , Phosphotidylcholine, Phosphotidyl ethalonamine, Diphosphotidyl- glycerol, Dioleoyl phosphatidic acid, Dipalmitoylphosphatidyl glycerol. The divalent cation are used in preparation of cochleates which is Zn^{++}, Ca^{++} , Mg^{++} , $Ba^{++,}$ Fe^{++}

Routes of administration for nanocochleate drug delivery [17]

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.

Dosage forms available for nanocochleate drug delivery [15]

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.

Advantages of nanocochleate drug delivery system [13,15,17]

1) Lipids in nanocochleates are less susceptible to oxidation therefore they are more stable than liposome. In liposomes structure are destroyed by lyophilization but nanococholeates maintain structure even after lyophilization.
2) Nanocochleates exhibits effective incorporation of biological molecule, specifically 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 reduce toxic stomach irritations and other side effect of the encapsulated drug.
7) They encapsulate or entrap the active drug within a crystal matrix rather than chemically bonding with the drug.
8) They provide protection from degradation to the encochleated drug avoiding by exposure to adverse environmental conditions such as sunlight, oxygen, water, and temperature.
9) They can be produced as defined formulations composed of predetermined amounts and ratios of drugs or antigen.
10) they improve oral bioavailability of poorly water soluble drugs, protein and peptides which is difficult to administer.

Limitations of nanocochleate drug delivery [13,17]

1) Nanocochleates require specific storage conditions.
2) The cost of manufacturing is very high
3) Sometimes aggregation may occur during storage

Cochleate-cell interaction [18]

When nanometer 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 can't shows bright fluorescent cell surface. This suggest that cochleates edge can make them fuse with cell surfaces as compare to edge free liposomes. For examine the interaction of cochleate with the cell membrane, 2% fluorescent lipid was mixed with polymer (phosphatidyl serine) to form a fluorescent liposome 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 nanometer size showed improved antibacterial activity than drug's solution which is according to several researchers.

Mechanism of Nanocochleate drug delivery [5,17]

After oral administration nanocochleates absorption takes place from intestine.
The cell membrane contained lipid in large amount. When another lipid molecule come in contact with cell membrane the lipid molecules fused to each other and distributed into the cell. The lipid

Nanocochleates cross across the digestive epithelium and deliver their cargo molecule into blood vessel.

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.

Method of preparation:

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[11,12]

In hydrogel method, the small unilamellar drug loaded liposomes are prepared, which are then added to polymer- A like dextran, phosphatidylserine or polyethylene glycol. The dispersion of two is then added to polymer-B solution such as polyvinyl alcohol, polyvinyl pyrolidone, polyvinyl methyl ether and ficoli. 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. By using a syringe pump at a regulated pace of 1 ml/min to 50 ml/min, this can be accomplished mechanically. The formed cochleates are washed to remove polymer, which might be re-suspended into physiological buffer.

2) Trapping method [1,6,7,13,14]

This method involves the formation of phosphatidylserine liposomes followed by drop-wise addition of a solution of calcium chloride. Liposome can be generated by either addition of water to phospholipid powder or by adding water phase to a phospholipid film. In continuous vortexing, calcium chloride solution (3Mmol) is added to 1 ml of liposomal small unilamellar vesicle dispersion to form cochleates. A precipitate that is cooled at 2 to 8 C is created.

3) Liposomes before cochleates dialysis method [3]

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 of lipid detergent is blend with polymer A like phoshpothidylserine, polyethylene glycol or dextran. The solution A introduced into solution consisting of polymer B e.g, polyvinyl alcohol, polyvinyl pyrolidone, polyvinyl methyl ether and ficoll) in which both polymers are immiscible, producing a polymer system of two phases. A cation moiety solution is introduced to the polymer structure in two phases. 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.

4) Direct calcium dialysis method [3,4]

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 dialyzed 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, colorless solution which resulted is dialyzed at room temperature against three changes of buffer. The final dialysis routinely used is Ca^{+} $^{+}$(6Mmol). The ratio of dialyzed to buffer for each change is minimum of 1:100. The resulting white calcium-phospholipids precipitates have been termed direct calcium cochleates.

5) Binary aqueous-aqueous emulsion system [3]

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.

Evaluation of nanocochleates

1) Particle Size and Size Distribution [2]

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.

2) Specific Surface Area [2]

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

$$A = \frac{6}{\rho d}$$

Where A is the specific surface area

ρ is the density and

d is the diameter of the cochleate.

3) Surface Charge and Electrophoretic Mobility [5]

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.

4) Surface Hydrophobicity [15]

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. The methods used for determination of surface hydrophobicity are 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.

5) Density [2]

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.

6) Molecular Weight Measurements [10]

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.

7) Drug content [2, 10]

The re-dispersed nanocochleates suspension is centrifuged at 15,000 rpm for 40 min at 25°. After the centrifugation is done separate the free drug in the supernatant. Concentration of drug in the supernatant can be then determined by UV-Vis spectrophotometer after suitable dilution.

8) Entrapment Efficiency [4,5,.10]

Drug entrapment efficiency is determine by separating non-encapsulated drug from the nancochleate suspension centrifugation at 5000 rpm for 30 min at 27 ^{0}C. the sediment vesicles were disrupted with solution of disodium EDTA to break nanocochleates structure into Nanoliposome then add ethanol to release the entrapped drug. Then the resulting solution subjected to UV Visible Spectrophotometer for determination of absorption. The entrapment efficiency is calculated by following equation:

Entrapment Efficiency :

$$\frac{\text{Total amount of drug} - \text{Free ammount of drug}}{\text{Total amount of added drug}} * 100$$

9) In-vitro Release [1,5,10]

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.

Applications of Nanocochleate [2,8]

1) Development of a nanocochleate based Apo-A1 Formulation of the Treatment of Atherosclerosis and other Coronary Heart Diseases.
2) Nanocochleates have been used for delivering proteins, peptides and DNA for vaccine and gene therapy application.
3) Nanocochleates have the ability to stabilize and protect an extended range of micronutrients and potential to increase the nutritional value of processed foods.
4) Nanocochleates can deliver Omega-3 fatty acids to cakes, muffins, pasta, soups, and cookies without altering the product's taste or odour.
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.
6) Cochleates would have the advantage of reducing the toxicity and improving the bactericidal activity.
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.
8) Nanocochleates are used to deliver anti-inflammatory agents.

Conclusion

The nanocochleate had unique multilayered structure, it protects active pharmaceutical ingredient 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.

Table 1 : Composition of Cochleate

Drug	**Lipid**	Divalent Cations
Protein Peptide Polynucleotide Antiviralagent Anaesthetic agent Anticancer agent Immunosuppressant Anti-inflammatory agent Tranquilizer Nutritional supplement	Phosphatidylserine Phosphatidic acid Phosphatidylinosotol Phosphotidyl glycerol Phosphotidylcholine Phosphotidylethalonamine Diphosphotidylglycerol Dioleoyl phosphatidic acid Dipalmitoylphosphatidylglycerol	Zn^{++}, Ca^{++} , Mg^{++} , Ba^{++} , Fe^{++}

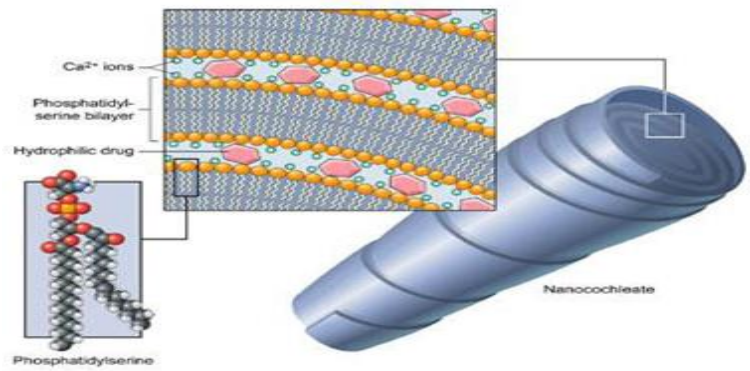

Fig 1: Structure of Nanocochleates

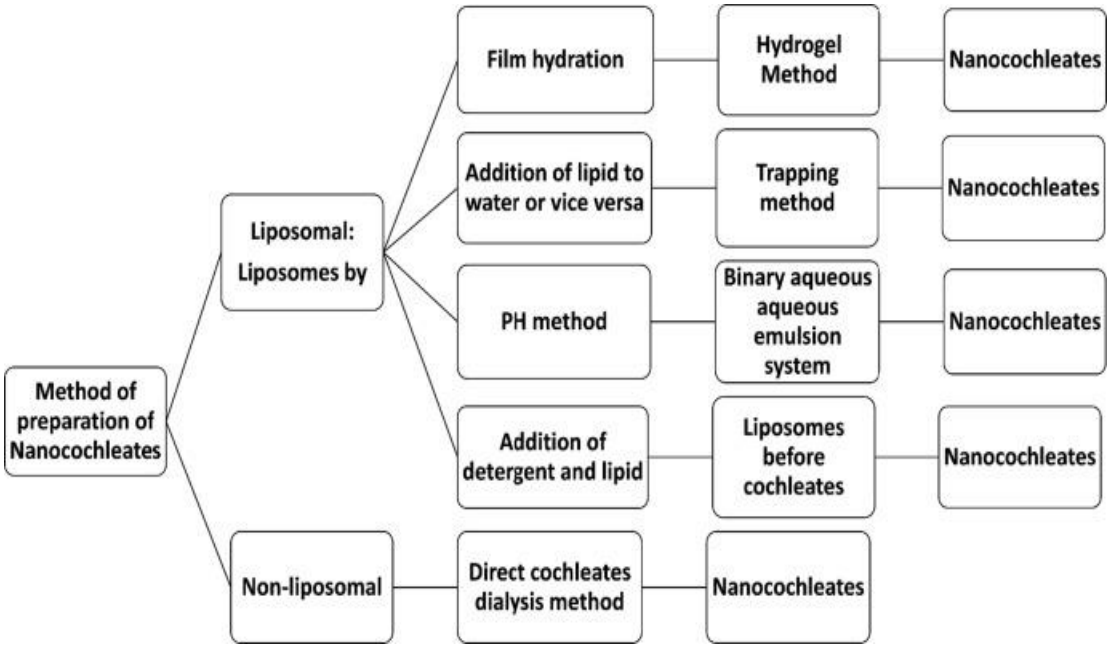

Fig : 2 Method of Preparation

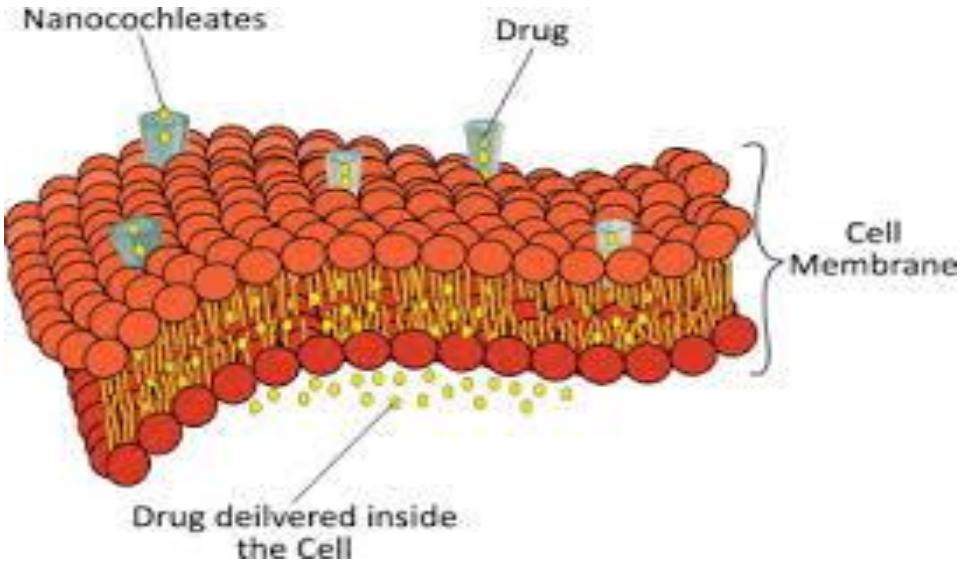

Fig 3 : Cochleate Cell Interaction

Drug loaded liposome was prepared

↓

Add this liposome to polymer A (Dextran, Phosphatidylserin, Polyethylene Glycol)

↓

The two dispersion added to polymer b solution (Polyvinyl Alcohol, Polyvinyl Pyrolidone, Polyvinyl Methyl Ether and Ficoli)

↓

Formation of two aqueous phase

↓ addition of solution of cation salt

Formation of cochleate

Fig 4 : Hydrogel Method

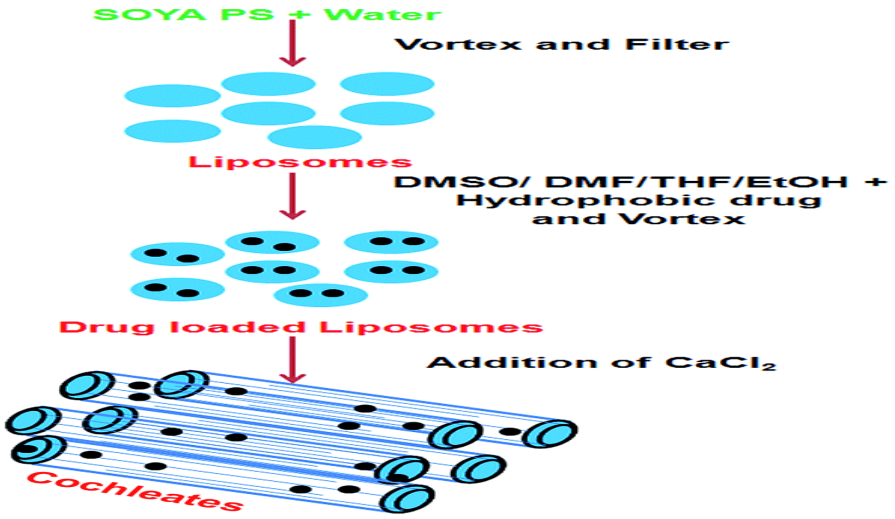

Fig 5: Trapping Method

Mixture of lipid and detergent is blend with polymer A (Phoshpothidylserine, Polyethylene Glycol or Dextran)
↓
Add above dispersion to polymer B (polyvinyl alcohol, polyvinyl pyrolidone, polyvinyl methyl ether)
↓ add cation salt solution
The mixture dialyzed by buffer
↓
Formation of cochleate

Fig 6: Liposomes before cochleates dialysis method

REFERENCES

1. Mali A J, et al. Fabrication And Application Of Dimyristoyl Phoshatidylcholine Biomaterial- Based Nanocochleates Dry Powder Inhaler For Controlled Release Resveratrol Delivery Future Journal of Pharmaceutical Sciences 2021; 7 (47): 1-12
2. Pawar S F, et al. Nanocochleate- An Important Drug Delivery System Offering Unique Features World Journal of Pharmaceutical Research 2020 ; 9(9) :621-637
3. Nayak S, et al. Recent Nanocochleate Drug Delivery System For Cancer Treatment : A Review International Journal of Current Pharmaceutical Research 2019; 11(6): 28-32
4. Olem Çoban Zelihagül De Gim S¸ et al. Efficacy of Targeted Liposomes And Nanocochleates Containing Imatinib Plus Dexketoprofen Against Fibrosarcoma Drug Delivery Res 2019; 80(5): 556-565
5. Talke S , et al. A Review On Nanocochleates Novel Approach For Drug Delivery World Journal of Pharmacy And Pharmaceutical Sciences 2018; 7(7): 284-294
6. Bothiraja C, et al. Development Of Novel Biofunctionalized Chitosan Decorated Nanocochleates As A Cancer Targeted Drug Delivery Platform Artificial Cells, Nanomedicine, And Biotechnology Taylor And Francis 2018 ; 1-16
7. Ishwor Poudel, Raj Ahiwale. Development Of Novel Biotinylated Chitosan-Decorated Docetaxel-Loaded Nanocochleates For Breast Cancer Targeting Artificial Cells, Nanomedicine, And Biotechnology, 2018 ; 1-13
8. Khairnar S B, Saudagar R B Nanococleates : An Overview International Journal of Pharma And Chemical Research 2017; 3(1): 17- 24
9. Sgudhodana, Nagarsekar K Judeh Zma. Recent Advances And Developments In Cochleate Technology, Nanomedicine And Nanotechnology 2017; 2(2)
10. Pawar A Y, et al. Nanocochleate : A Novel Drug Delivery System Asian Journal of Pharmaceutics 2016; 10(3): 234- 242
11. Vyas H, et al. Nanocochleates- A Novel Bypass of Conventional Drug Delivery System 2015
12. Yadav A, Chaudhary S. Formulation Development And Characterization Of Nanocochleates For The Improvement Of Permeability Of Drug Journal of Advanced Pharmacy Education And Research 2016; 6(3): 14-21
13. Pawar A, et al. An Insight Into Cochleates, A potential Drug Delivery System The Royal Society of Chemistry 2015: 28-51

14. Bothiraja C, et al. Fisetin- Loaded Nanocochleates : Formulation, Characterization, In Vitro Anticancer Testing, Bioavailability And Bio-distribution Study Expert Opinion Drug Delivery 2014; 11(1) Page No : 17-29
15. Bhosale R R, et al, Nanocochleates: A Novel Carrier For Drug Transfer Journal of Scientific And Innovative Research 2013; 2(5): 964-969
16. Wasankar S R, et al. Nanocochleate : A Review Research J Pharma Dosage Forms And Tech 2012;4(3): 153-159
17. Panwar V, et al. Nanocochleates : As Drug Delivery Vehicle International Journal of Pharmacy And Biological Science 2011; 1 (1): 31- 38
18. Shankar V R, Reddy Y D. Nanochochleates – A New Approach In Lipid Drug Delivery International Journal of Pharmacy And Pharmaceutical Sciences 2010; 2(4): 220-223
19. Thiruganesh Ramasamy, et al. Nanocochleate – A New Drug Delivery System Fabad J. Pharm. Sci. 2009; 34: 91–101,