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A REVIEW ON NANOCOCHLEATE

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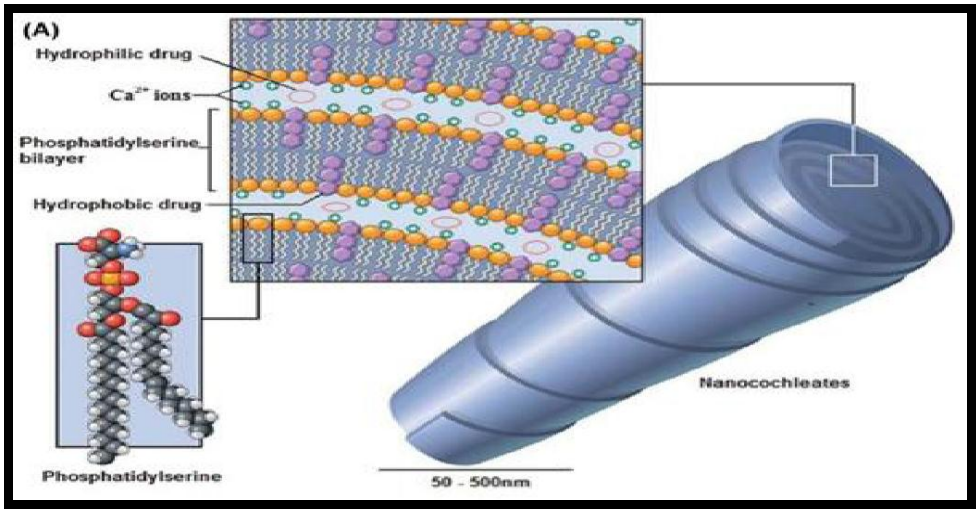
ABSTRACT
Nanaocochleate represent a new technology for oral and systemic delivery of drugs. It is a novel lipid-based system which represent a unique technology platform suitable for the oral and systemic administration of a wide variety of molecules with important therapeutic biological activities, including drugs, genes, and vaccine antigens. Nanocochleate formulation technology is particularly applicable to macromolecules as well as small molecule drugs that are hydrophobic, positively charged, negatively charged, and that possess poor oral bioavailability. Proof-of-principle studies for cochleate-mediated oral delivery of macromolecules as well as small molecule drugs is being carried out in appropriate animal models with well established, clinically important drugs, which currently can only be effectively delivered by injection.

KEYWORDS: Phospholipid, Liposomes, Phagocytosis.

INTRODUCTION
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 are cylindrical (cigar-like) microstructures that consist of a series of lipid bilayers (Fig.1, 2).[1] Nanocochleate delivery vehicles are stable phospholipid-cation precipitates composed of simple, naturally occurring materials, generally phosphatidylserine and calcium. They have a unique multilayered structure consisting of a 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 encapsulate within the interior of the nanocochleate structure remain intact, even though the outer layers of the nanocochleate may be exposed to harsh environmental conditions or enzymes.[2,3] Because nanocochleates contain both hydrophobic and hydrophilic surfaces, they are suitable to encapsulate both hydrophobic drugs like amphotericin B and clofazimine and amphipathic drugs like doxorubicin.



DEFINATION

"Nanocochleates is a unique tailor-based system used for micro-encapsulation entrapping them in supramolecular assemblies composed of negatively charged phospholipids and a divalent cation.

ADVANTAGES

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 molecule in vivo as nanocochleates slowly unwind or otherwise dissociate.
4. They have a lipid bilayer matrix 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, non-immunogenic and non-inflammatory.
5. They are produced easily and safely.
6. They improve oral bioavailability of a 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 toxicity stomach irritation and other side effects of the encapsulated drug.
8. They encapsulate or entrap the subject drug within a crystal matrix rather than chemically bonding with the drug.
9. They provide protection from degradation to the encochleated drug caused by exposure to adverse environmental conditions such as sunlight, oxygen, water and temperature.

STRUCTURE AND FUNCTION

Cochleate and nanocochleate are cigar like spiral rolls(Figure 2) formed of negatively charged phospholipid bilayers, which are rolled up through the interaction with multivalent counter ions (Ca2+ orZn2+) as bridging agents between the bilayers. As a particulate system, cochleates possess unique properties like superior mechanical stability and better protection for encapsulated drugs compared with liposomes due to their solid matrix. Cochleates also maintain their phospholipid bilayer structures.These solid particles are so flexible that they can readily convert to liposomes by extracting the bridging counter ions out of the inter bilayer spaces. Such unique properties have made cochleates an ideal system for delivering insoluble ingredients which can be loaded in the matrix of a phospholipid bilayer while avoiding the instability problem of liposomes.[2, 3, 16-18]

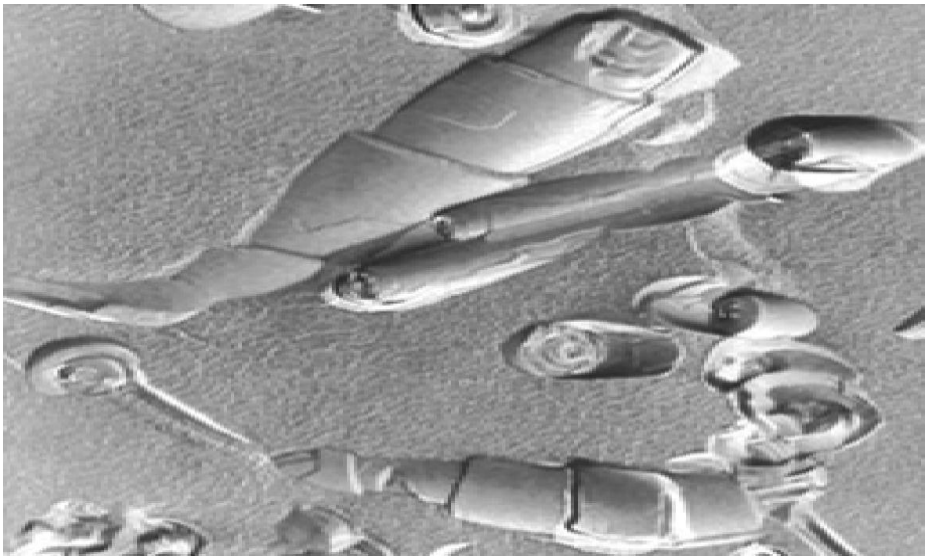


Figure 1: Cigar like structure of nanocochleates.

Cochleate delivery vehicles are stable phospholipidcation precipitates composed of simple, naturally occurring materials (phosphatidylserine and calcium). They have a unique multilayered structure consisting of alternating layers of calcium and phospholipid in large, continuous, solid, lipid bilayer sheets rolled up in a spiral or stacked, with little or no internal aqueous space. This structure provides protection from degradation for associated "encochleated" molecules. Divalent cation concentrations in-vivo in serum and mucosal secretions are such that the cochleate structure is maintained. Hence, the majority of cochleateassociated molecules are present in the inner layers of a solid, non-aqueous, stable, impermeable structure.[3, 19, 20] A transverse section of rolled cochleate.

COMPONENTS OF NANO-COCHLEATE DRUG DELIVERY SYSTEM

The three major components used in the preparation of nanocochleates are atmospheric pressure ionization (API), lipids, and cations.

1. Lipids: Phosphatidyl serine (PS), phosphatidic acid (PA), di-oleoyl PS, phosphatidylinositol (PI), phosphatidyl glycerol (PG), phosphatidyl choline (PC),

di-myristoyl PS, phosphatidyl ethanolamine (PE), di-phosphatidyl glycerol (DPG), dioleoyl phosphatidic acid, di-stearoyl phosphatidyl serine, di-palmitoyl PG.
2. Cations: Zn+2 or Ca+2 or Mg+2 or Ba+2.

DOSAGE FORMS AVAILABLE FOR NANOCOCHLEATE DRUG DELIVERY

• 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 before use.

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.
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.

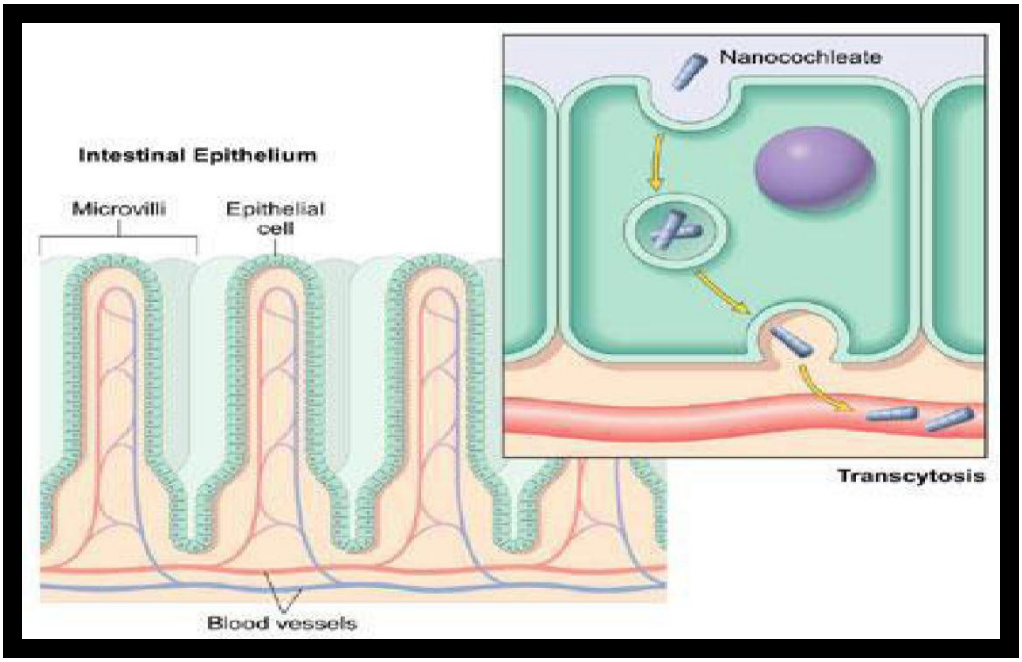


Figure 2: Nanocochleate absorption from intestine.

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.

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.

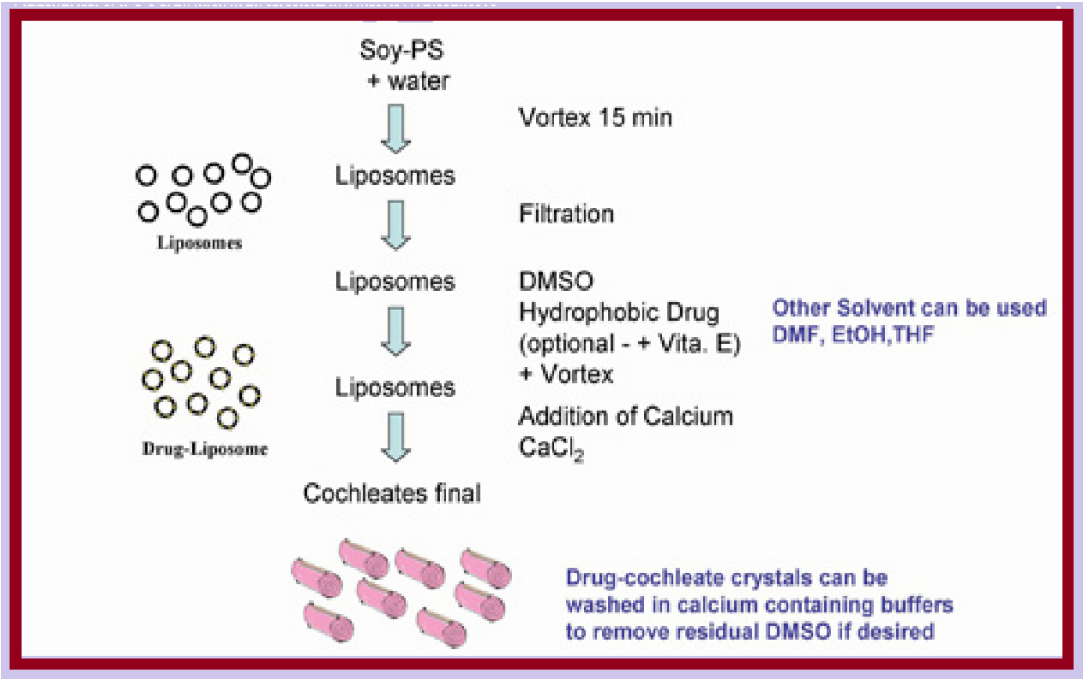


Figure 3: Schematic presentation of trapping method.

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.

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.

Evaluation and characterization of cochleates

1)Particle size determination

The mean particle size of the liposomal dispersion and cochleates dispersion was determined by laser diffraction technique using Malvern 2000SM (Malvern, UK). Analysis was carried out at 30±2°C temperature keeping angle of detection 90°. The mean vesicle size was expressed in terms of volume mean diameter D [4, 3] which is the average diameter of a sphere having volume same as that of the particle under measurement.

2)Determination of entrapment efficiency (EE) of cochleates

One hundred micro liters of cochleates was aliquoted into centrifugation tubes. To each tube 60 µl pH 9.5 EDTA and 1ml of ethanol were added while vortexing. The resulting solution is clear and colorless.[37] The samples were suitably diluted and absorbance determined at 289 nm to calculate entrapment efficiency as per equation 3.

***Entrapment Efficiency*= amount of drug present in cochleates /total amount of present*100.**

3)Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR measurements of KCZ, DOPS-Na and drug loaded freeze dried cochleates were obtained on JASCO FTIR 4100 (Japan) equipped with Spectra manager version 2. Samples were prepared by mixing with KBr and placing in the sample holder. The spectra were scanned over the wave number range of 3600–400 cm−1 at ambient temperature.

STABILITY OF NANOCOCHLEATE

These are stable, lipid based delivery formulations whose structure and properties are very different from liposomes. These unique structure provides protection from degradation for encochleated, namely, encapsulated molecules. Because the entire cochleate structure is a series of solid layers, components within the interior of the cochleate structure remain intact, even though its outer layers may be exposed to harsh environmental conditions or enzymes, such as in the stomach. Animal studies demonstrated that nanocochleates cross the digestive epithelium and deliver their cargo molecules into target cells (2223). These unique properties of nanocochleates were used to mediate and enhance the oral bioavailability of a broad spectrum of biopharmaceuticals, including compounds with poor water solubility, such as amphotericin B (24, 25).. They are stable to lyophilization and can be reconstituted with liquid prior to administration. Cochleate is most versatile technology for the delivery of a wide range of drugs and fragile molecules such as proteins and peptides.

SAFETY/BIOCOMPATIBILITY OF THE NANOCOCHLEATE DELIVERY VEHICLES-

The main two components of nanocochleate are PS and calcium. PS is a natural constituent of all biological membranes and is most concentrated in the brain. The phospholipids used in nanocochleate formulation can be prepared from natural sources or produced synthetically which are composed of anionic lipids are non-inflammatory and biodegradable. Soy PS is available in large quantities and is expensive and suitable for use in humans. These two components which are safe, simple, naturally occurring substances which make nanocochleates safe and biocompatible delivery vehicle. Clinical studies show that PS is very safe which play a role in support of mental functions in the aging brain.

LIMITATIONS

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.

APPLICATIONS OF NANOCOCHLEATE

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 applications.
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.

RESULTS AND DISCUSSION

1.Characterization of vesicles formed

The vesicles formed were spherical in shape and unilamellar in nature. However, the vesicle size of the formed vesicle tends to vary as the molar ratio of the structural lipids is changed. The vesicles also show variable entrapment efficiency with the maximum percent entrapment of 39.86 ± 0.38 %. The results are complied under table 1.

Table 1: Characterization of small unilamellar vesicles.

S. No.	Composition (molar ratio)	Vesicle size (nm)	Entrapment (%)
1.	Soya lecithin: Cholesterol (5:5)	401	27.63 ± 0.15
2.	Soya lecithin: Cholesterol (6:4)	435	34.73 ± 0.52
3.	Soya lecithin: Cholesterol (7:3)	431	39.86 ± 0.38
4.	Soya lecithin: Cholesterol (8:2)	451	26.95 ± 0.19
5.	Soya lecithin: Cholesterol (9:1)	506	25.29 ± 0.44

2.Size and size distribution of nanocochleates formed

The nanocochleates were subjected to particle size analysis and particle size distribution and average diameter (Z- Average) was found out to be 1005 nm with peak obtained at 522.6 nm, following results were obtained.

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	1005	Peak 1:	525.6	100.0	56.10
PdI:	0.192	Peak 2:	0.000	0.0	0.000
Intercept:	0.948	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

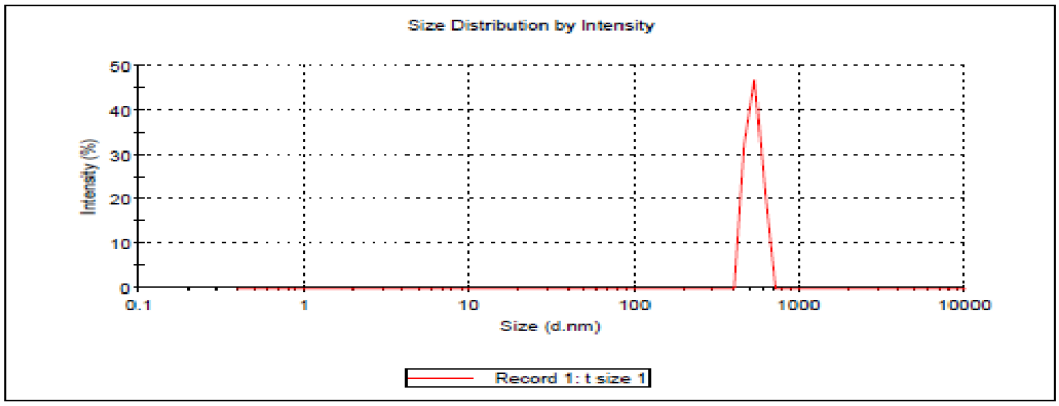


Figure 1: Particle size of cochletaes by Malvern zetasizer.

3. Surface morphology of cochleate particles (SEM studies)

The images are shown in Figure 3. The micro size rods and clusters of such elongated rod shaped cochleates were observed.

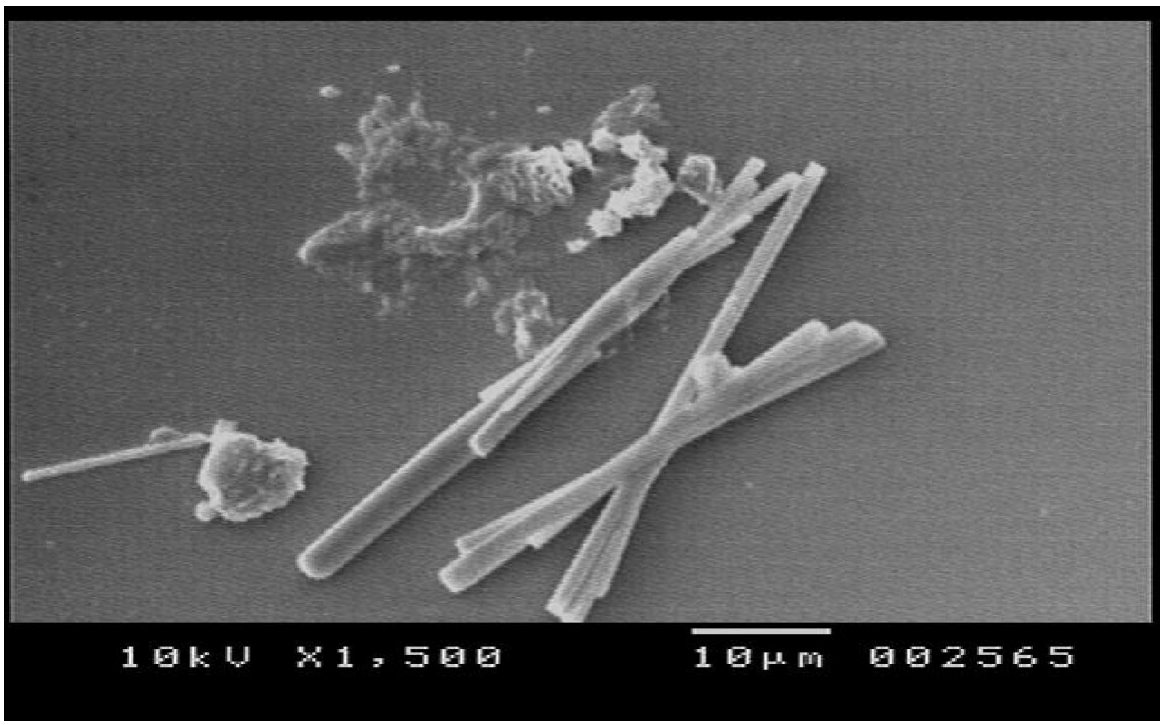


Figure 3: SEM images of cochleate particles.

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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