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# NANOCOCHLEATE – AN IMPORTANT DRUG DELIVERY SYSTEM OFFERING UNIQUE FEATURES

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## ABSTRACT

There are various lipid-based nanocarriers systems are available in current scenario. Among these lipid-based nanocarriers multi-layered cochleates emerge as a novel nanocarrier system for hydrophilic and hydrophobic drugs with better and improved stability, efficacy, improved drug permeability and reduction in drug dose. It also helps to increase oral bioavailability of hydrophilic and hydrophobic drugs and helps for site specific drug delivery with less side effects. This article focuses on structure and composition of cochleates, various method of preparation of cochleates, mechanism of drug delivery, applications, evaluation and limitations of nano cochleates.

**KEYWORDS:** Liposomes,Lipid-based nanocarrier, Nanocochleate.

## HISTORY

Cochleates were discovered in 1975 by Dr. Dimitrious Papahadjoupoulos and his coworkers as precipitates formed by the interaction of negatively charged phosphatidylserine and calcium and have been used to transfer of antigen and peptides for vaccine delivery.[1] He named these cylindrical structures "COCHLEATE" which in the Greek, means “SHELL” because of their rolled-up form. Cochleate structures are not uniformly formed,they cause

either aggregates of stacked sheets formed by trapping method or large size needles like structures formed by the dialysis method.[2] In 1999, cochleates were introduced to develop smaller, but rather more consistent particles by hydrogel isolation method.[3] The cochleates can be formed that are small mean particle of less than 500 nm by using a binary phase system, such as non miscible hydrogels.These nano cochleates were highly suitable for the encapsulation of hydrophobic drug.[4]

## INTRODUCTION

Improvement of bioavailability and formations technique is always a thurst area in development of new formulation using nanotechnology where researchers focus on modification in drug delivery system . Basically liposome is a vesicle having at least one lipid bilayer made up of phospholipids and cholesterol in which nutrients or drugs are encarporated to deliver in desired manner.[5] Among these lipid -based nanocarriers liposomes, cochleates, 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 because of increased oral bioavailability of hydrophilic and hydrophobic drugs in addition to site specific drug delivery causing less side effects.[6] The nanocochleate drug delivery is based upon encapsulating drugs in a multi-layered, lipid crystal matrix which potentially deliver the drug safely and effectively to the target site.[7] Various lipid-based nano-carrier systems available such as lipoproteins, lipid nanoparticles, lipid nanocapsules and liposomes but they show limitations due to stability, oxidation and incompatibility for delivery of protein and peptide. Cochleates are developed as an alternative for a lipid-based drug delivery system. Many therapeutic agents, especially biological molecules are not absorbed through the intestine due to their intrinsic impermeability to tissue membranes and their enzymatic degradation through the wall of GIT.[8]Cochleate is a spiral structure consist of phospholipid bilayer to which hydrophobic and hydrophilic drugs are incorporated to prevent the oxidation, to improve the permeability and to reduce the dose of drugs.[9] Thus, it provides a potential delivery system for the wide class of drugs. This novel nanocarrier system approach is applicable to macromolecules as well as small molecule drugs that are hydrophobic and drugs with poor oral bioavailability.[8,10]

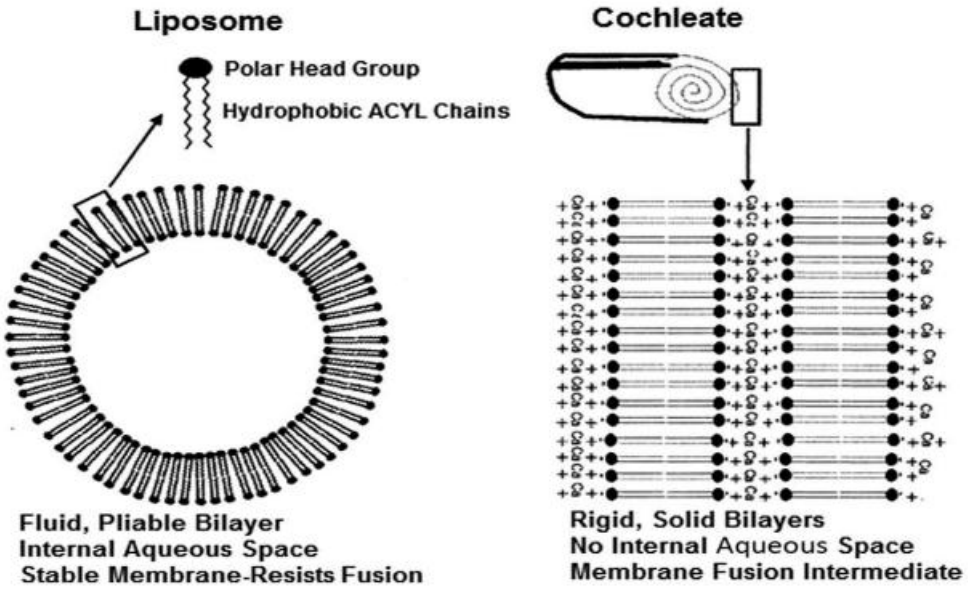


**Fig. 1: Difference between liposome and nanocochleate structure[3].( Reproduced with permission from ref.3.RSC Adv 2015.).**

**Route of Administration[9,11,12,13]**

Nanocochleate drug delivery system suitable for an efficient oral delivery of drugs, it can also be used for all other kind of formulations to be administered by all most all routes.

1) Topical or transdermal administration: powders, creams, paste, ointment, patches, gel, lotions are used. Eg. ketoconazole prepared a formulations for topical administration based on nanocochleates.
2) Oral administration : tablets, capsules, solutions, suspensions, emulsion, pills, cachets. Eg. Amphotericin B and Raloxifene are used for fungal infection and in cancer treatment respectively.
3) Parentral administration: sterile isotonic solutions, dispersions, emulsions, suspensions, sterile reconstituted powders into injectable solutions just prior to use. Eg. Amphotericin B (slow infusion only) and paclitaxel are prepared a formulations for Intravenous administration based on nanocochleates formulations.

**Structure and composition**

Cochleate and Nanocochleate are cigar like spiral rolls structure formed by addition of negatively charged phospholipid bilayers (liposomes), which are rolled up through the interaction with multivalent counter ions of metal (Ca2+ or Zn2+) as bridging agents between the phospholipid bilayers.[4,14]

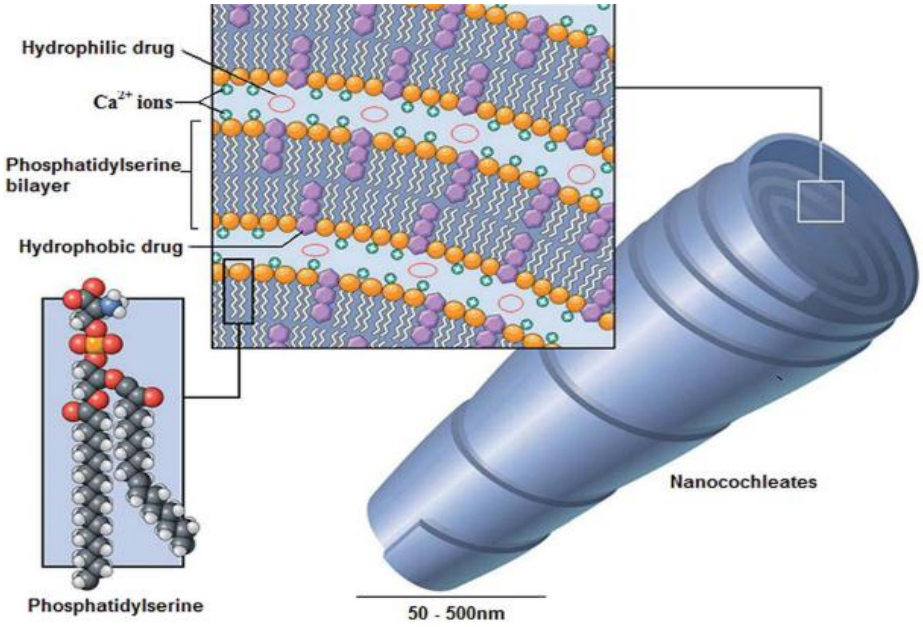


**Fig. 2: Structure of Nanocochleates[3,15] (Adopted with permission from ref 3.RSC Adv 2015).**

During this process, the close approach of bilayers is dependent on dehydration of the head group of the phospholipid. They roll-up in order to minimize their interaction with water. They possess little or no aqueous phase. The bilayers in a cochleate are arranged very neatly at a very close repeating distance of 54 Angstrom. Small liposomes rather than the larger ones yield cochleates.[3,14]

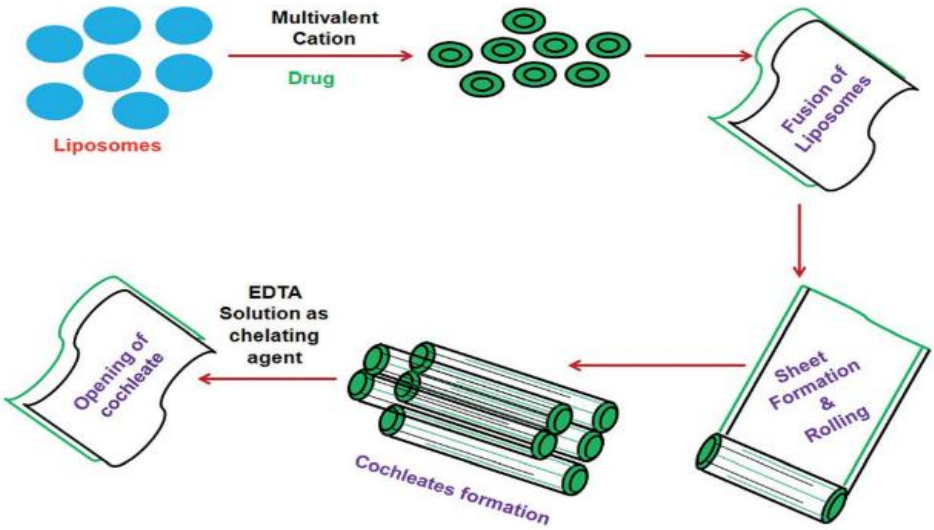


**Fig. 3: Process of Cochleates formation.[3] (Firstly prepare liposome then add multivalent cation like Ca++ ,form fusion of liposome after that rolling and cochleates formation).**

**The various constituents of a cochleate**

**There are three basic requirements to formulate the cochleates**

a) Lipid bilayers,

b) Cations

c) Drug which is to be delivered.

| Requirements | Role in cochleates | List of content | Specific characteristic/ function | Reference |
|---|---|---|---|---|
| Cations | To faciliatates the rolling or stacked up lipid sheets | $Zn^{2+}$, $Mg^{2+}$, $Ba^{2+}$, $Ca^{2+}$. | The stacked is formed by interacting cations with lipid bilayer | [9,16,17] |
| Lipids | Encapsulating the drug in lipid crystal matrix to potentially deliver the safely and effectively | Phosphatidylserine<br>Phosphatidic Acid<br>Phosphatidylinositol<br>Phosphatidyl glycerol<br>Phosphatidyl choline<br>Phosphatidylethanolamine<br>Diphosphatidyl glycerol<br>Dioleoyl phosphatidic acid<br>Distearoyl phosphatidyl serine<br>Dipalmitoyl phosphatidyl glycerol | It used as a vehicle for administration of nutrients and beneficial for pharmaceutical drugs. | [12,11] |
| Drugs | Entrapment of drug into multi-layered structure containing lipid bilayer to form cochleates | Proteins<br>Peptides<br>Polynucleotides<br>Antiviral agents<br>Anaesthetics agents<br>Anticancer agents<br>Immunosuppressant<br>Tranquilizer<br>Anti-inflammatory agents<br>Nutritional supplements, etc. | Wide class of drug used for therapeutic action | [3,15] |

**List of lipids used in nanocochleates drug delivary system are tabulated below with brief information about it**

| Lipids | Information |
|---|---|
| Phosphatidyl choline(PC) | It is the major component of lecithin. It is the main functional constituent of the natural surfactants and the body's foremost reservoir of choline, an essential nutrient.[4] |
| Phosphatidylethanolamine (PE) | 1,2-Diacyl-glycero-3-phospahtidyl ethanolamine is the second most abundant phospholipid in animal and plant lipids. It is the main lipid component of microbial membranes. It is a key building block of membrane bilayers.[18] |
| Phosphatidyl inositol (PI) | It is an important lipid as it is a key membrane constituent. Also it is an participant in essential metabolic processes in all plants |

| | |
|---|---|
| | and animals and in some bacteria.[9] |
| Di-phosphatidyl glycerol(DPG) | Also named as Cardiolipin. It is found exclusively in certain membranes of bacteria(plasma membrane & hydrogenosomes) and mitochondria of eukaryotes.[19] |
| Phosphatidylserine (PS) | PS is required for targeting and functioning of intracellular several singaling.[20] |
| Phosphatidic Acid (PA) | This are used for singling pathway of cell growth, reproduction and proliferation.[21] |

**Advantages[14,22]**

The cochleates have a non-aqueous (lipid) structure, and therefore they have various advantages associated with their use which are as follows;

1) They are more stable than other lipid based nanocarrier system because they cause less oxidation of lipids.
2) They can be stored by freeze drying, which provides ability to be stored them for long periods of time at room temperature, which would be advantageous for shipping and storage prior to administration.
3) They are more stable than liposome as they have potential to maintain their structure after the process of lyophilization, because liposomes structure can get destroyed or altered after lyophilization.
4) Exhibit efficient incorporation of hydrophobic drugs into the lipid bilayer of the cochleate structure.
5) Exhibit efficient incorporation of antigens with hydrophobic moieties.
6) Have the potential for sustain release of a drugs and molecules.
7) Have a lipid bilayer rolled in their structure, which serves as a carrier and is composed of simple lipids which are found in animal and plant cell membranes, so the lipids are biocompatible.
8) They are safe to use and easy to formulate.
9) They can be produced as defined formulations composed of predetermined amounts and ratios of drugs or antigens.

**Limitations[2,14]**

1. Proper attention required during storage condition, because sometimes aggregation may occours during storage which may leads to decrease in efficacy as formulation and stability.

2. second most important disadvantage associated with the nanocochleates is its cost of production is high.

**Table: Shows commercial available nanocochleates formulation.**

| Sr.No | Drug | Method of preparation | Uses | Reference |
|---|---|---|---|---|
| 1 | Imatinib mesylate | High pH/ film hydration method | In treatment of fibrosarcoma and other cancer | [10] |
| 2 | Amphotericin B | Trapping/High pH method | Antifungal activity | [23] |
| 3 | Artemisia | Dehydration/hydration process | Leishmaniasis | [24] |
| 4 | Paclitaxel(mol.wt.120000) | Trapping method | Anticancer activity | [25] |
| 5 | Erlotinib HCL | Trapping method | Anticancer activity | [26] |
| 6 | Thyme essential oil | Film hydration method | Antioxidant | [27] |
| 7 | Resveratrol | Trapping method | In the treatment diabetes | [28] |
| 8 | Rifampicin | Film hydration method | Treatment of tuberculosis | [29] |
| 9 | hydroxycamptotecin | Film hydration method | Broad spectrum cancer including leukemia, breast cancer | [30] |
| 10 | Fisetin | Trapping method | Anticancer agent | [31] |

**Methods of preparation**

**1. Trapping method[6,12,32]**

This method involves the following steps In first step the drug loaded liposome by addition of phosphatidylserine or any other phospholipids are prepared and then above mixture is dropwise added in calcium chloride solution and it form the cochleates as shown in fig.2.

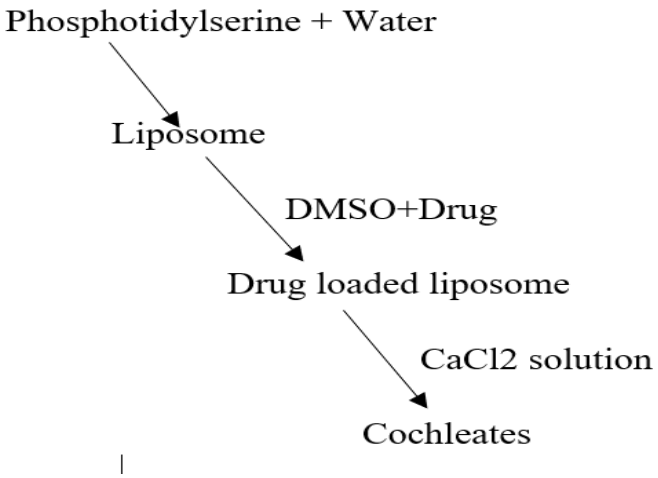


**Fig. 4: Preparation of nanocochleates by Trapping method.[9]**

**2. Hydrogel method**[7,8,11]

**This method involves the following steps**

I. Firstly prepare small unilamellar drug loaded liposomes, then which are added to polymer- A. The dispersion of liposome and polymer A is then added to polymer-B. The two polymers are immiscible with each other ,leads to formation of aqueous two phase system.

II. The cationic cross-linkage of the polymer is form due to addition of solution of cationic salt to two phase system, such that the cation diffuses into polymer-B & then into the particles comprised of polymer, allowing the formation of small size cochleate.

III. The formed cochleates are washed to remove polymer, which might be re-suspended into physiological buffer.

IV. they remain as precipitates as shown in fig.3

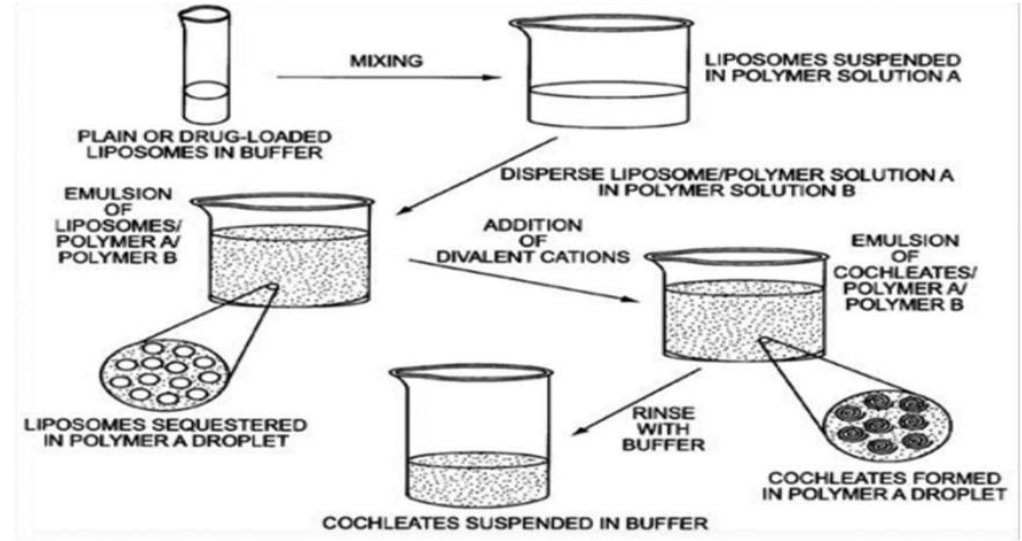


**Fig. 5: Preparation of Nanocochleate by Hydrogel method.**[2,33]

**3. Aqueous-aqueous emulsion system**[2,7,11]

This method involves the formation of small unicellular vesicle liposomes by using 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. By using this method the cochleates formed are of particle size less than 1000 nm.

**4. Dialysis method[16,11]**

- **Liposomes before cochleates (LC) dialysis method**

A second method for preparing the small-sized cochleates consist of detergent and a biologically relevant molecule and cation. The detergent is added to disrupt the liposomes. The method made up of the following steps:

I. An aqueous suspension containing a detergent-lipid mixture is prepared.
II. The detergent-lipid suspension is mixed with polymer A such as dextran, Polyethylene glycol or Phosphatidylserine.
III. The detergent-lipid/polymer A suspension is added into a solution comprising polymer B such as poly vinyl pyrolidone, poly vinyl alcohol and poly vinyl methyl ether (PVMB), the polymer A and polymer B are immiscible with each other to form a two-phase polymer system.
IV. A solution of a cationic moiety is added to the two-phase polymer system.
V. Now wash the two-phase polymer system to remove the polymer and cochleates are formed.

- **Direct calcium (DC) dialysis method[11]**

This method does not involves the intermediate liposome formation, involves the removal of detergent directly by dialysis against a 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. It is result in needle shaped structures with large length and diameter of cochleates are formed.

**Mechanism of drug delivery**

Liposome containing Medium unilamellar vesicles(MUVs) contains 2-4 layers of lipid which surround the core and small unilamellar vesicles (SUVs) contain a single layer of lipid . liposomes are starting material for preparation of nanocochleates within size range of 20-100nm (SUVs) and 100-1000nm(MUVs).[9]

**Formulation of cochleates involve two process**

1. Nucleation and
2. Aggregation process

In the nucleation step, phosphatidylserine molecule of lipid binds with Ca+2 ion and forms a longitudinal cochleate cylinder which eventually rolls up to form cochleates as well as addition of ethanol, forms cylindrical nanocochleates.

In second step, a microfluidic device is used to form of the spherical cochleate. Addition of ethanolic solution initiates nucleation process and then addition of a mixture of ethanol and aqueous calcium chloride (50:50) form long cylindrical cochleates.[9]

In Nanocochleates system drug are loaded in lipid bilayer, which are hypothetically prove that when the lipid bilayer of nanocochleates are fused with the cell membrane then content of nanocochleates are transferred into cells , thus the release of drug occurs.[8,11]

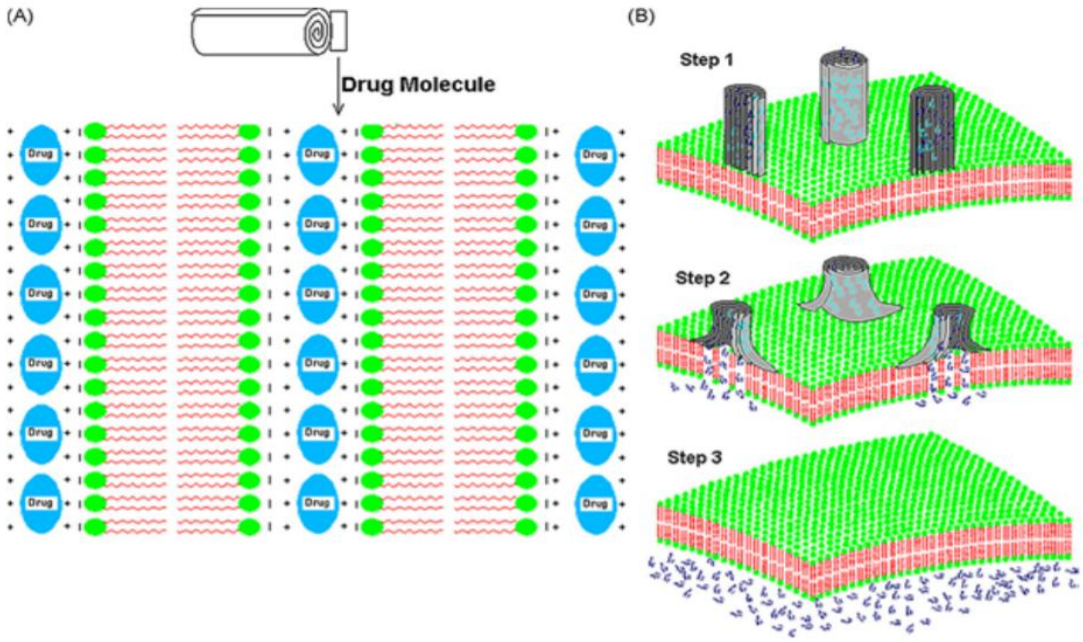


**Fig. 6: Nanocochleate interaction with cell membrane(adapted from yeole etal).[8,11]**

**Evaluation of Nanocochleate**

- **Particle size determination**

The particle size and size distributions are most important parameters in novel drug delivary system by using scanning electron microscopy(SEM) and transmission electron microscopy(TEM) can be determined. The size evaluation of nanocochleates for better results, freeze fracturing microscopy and photon correlation spectroscopy are used for quantitative determination. Atomic force microscopy is a advanced nano technique used for

charecterization of nanocochleates. and also use of laser diffraction technique for mean particle size of the liposomal dispersion and cochleates dispersion can be determined.[12,34,35]

- **Density**

The density of nanocochleates is determined by helium or air using a gas pycnometer.[12]

- **Molecular weight measurements**

The evaluation molecular weight of the polymer and its distribution in the matrix can be measured by gel permeation chromatography (GPC) using a refractive index detector. Using GPC, it was shown that polyalkylcynoacrylate nanocochleates are built by an entanglement of the rolling up of one or a few long polymer chains.[10,35]

- **Drug content**

At 15000 rpm the redispersed nanocochleates suspension is centrifuged for 40 min at $25^0$C to separate the free drug content in the supernatant. In the supernatant present of free drug to make suitable dilution of drug concentration, they can be determined by ultraviolet (UV-Visible ) spectrophotometer.[6,11]

- **Entrapment efficiency (EE)**

Entrapment efficiency of nanocochleate suspension determined by using centrifugation at 5000 rpm for 30 min at $27^0$C. To make some dilution of nancochleates suspension by addition of EDTA and ethanol then take absorbance of resulting solution is determined using spectroscopic technique and calculate the entrapment efficiency of nanocochleates.[6,11]

$$\text{Entrapment efficiency} = \frac{\text{Amount of drug present in cochleates}}{\text{Total amount present}}$$

- **Stability study**

Stability study of nanocochleates dispersions can be perform for 3 month at 2-8°C and 25 ± 2°C/60% RH to check their stability. To the check the particle size, percent entrapment efficiency and drug release of formulation.[11,34]

- **Specific surface area**

By using sorptometer to determine the specific surface area of freeze dried nanocochleates.

The following equation are use to calculate specific surface area of nanocochleates.[11,36]

$$A = 6/\rho d$$

Here, A = Specific surface area,

ρ = Density,

d = Diameter of the cochleate

- **Surface charge**

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 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 nature and intensity of the surface charge of nanocochleate determine their interactions with the biological environment as well as their electrostatic interaction with bioactive compounds.[11]

- **In vitro release study**

Nowdays, In vitro release profile of nanocochleates is determine by using standard dialysis, diffusion cell or modified ultrafiltration techniques. Phosphate buffer with double chamber diffusion cells on a shake stand is generally used for In vitro release study.[12,11]

**Applications**

i. Nanocochleates have been used to deliver proteins, peptides, and DNA for vaccine and gene therapy application.[37,38]
ii. Nanocochleates are used to deliver amphotericin B, as a antifungal agent, This prepared cochleates of amphotericin B improve the stability and efficacy at low doses.[37,39,40]
iii. Antifungal drug delivery eg. Ketoconazole (KCZ) is an antifungal drug and usually prescribed for fungal infections such as athlete's foot, candidiasis and ringworm.[6]
iv. Delivery of vaccines eg. vaccines contain usually live attenuated or inactivated pathogens for prevention of disease like measles, mumps, polio, rubella, tetanus etc.For the protection of antigens, vaccine adjuvant delivery system (VADS) was used with carriers such as liposomes, cochleates, virosomes etc.[6,9]
v. Delivery of anti-inflammatory compounds.[11]
vi. Delivery of volatile oils which is obtained from Artemisia absinthium L. shows poor solubility and poor stability. So essential oil (EO) is encapsulated in nanocochleates to improve the stability and solubility by showing better therapeutic efficacy. In vivo studies were performed to show EO-Aa-NC-based formulation for reducing the lesion size and showing better results than standard drug glucantime.[9,6]

vii. Delivery of antibacterial agents and combating bacterial multidrug resistance.[14,23]

viii. Delivery of insulin and encapsulated insulin with magnatocochleates.[41,42]

ix. Delivery of anticancer agents and delivary of nanoliposomes for oral use of paclitaxel.[43,44,23,26]

x. Nanocochleates used as carrier for topical drug delivery.[34]

xi. Nanocochleates have been also used to deliver nutrients such as vitamin, omega fatty acids.[4,45,15]

xii. Delivery of recombinant factor VIII.[9,46]

xiii. Nanocochleates are immunomodulator for nasal vaccine.[47]

## CONCLUSION

Nanocochleates are novel drug delivary system approach which is brodly applicable to encapsulation of genes,vaccines ,antigens also it helps to protect active agent because have unique multi-layered structure helpful in enhancing the qualities of formulation of nanocochleates by increasing shelf life stability, bio availability,reducing the dose as well as toxicity.

In future this,technology can be used as an alternative way to deliver biological moiety or therapeutic agents.

This review article specially focus on therapeutic potential of new class of drug nano carrior i.e. nanocochleates.Nano cochleates are promising for oral, transdermal and dermal delivery and also gaining more importance in pharmaceutical development for transfer of suitable and desired drug molecules effectively.

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