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Review Article

Insights of Nanocochleates in Conventional Drug Delivery System.

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ABSTRACT

Nanocochleates are a novel drug delivery device in which different charged drug molecules are orally delivered into a multilayered structure comprising a solid-lipid bilayer in the shape of a spiral-rolled sheet. The nanocochleate structure offers protection from the harsh world around it. All the preparation process involves using the charge ratio to monitor the particle size between the bridging agents and lipids. This nanocochleate was formed by the interaction between negatively charged lipids and drugs or peptides acting as inter-bi-layer bridges instead of multi-cationic metal ions, capable of microencapsulating water-soluble cationic drugs or peptides into its inter-lipid bi-layer space. A process known as "hydrogel co-chleation" or simply by increasing the proportion of multivalent cationic peptides over negatively charged liposomes can be generated in submicron size cochleates. Nanocochleates have very less limitations and dosage types than other lipid drug delivery systems; it is thus generally applicable and more likely to be a drug delivery system.

KEYWORDS

Cochleates, peptides, encapsulate, liposomes.

1. INTRODUCTION

The nanocochleate drug delivery vehicle is focused on the multilayered, lipid crystal matrix encapsulation of drugs to potentially deliver the drug safely and effectively[1]. Cylindrical (cigar-like) microstructures that consist of a series of bilayers of lipids are nanocochleates. The delivery vehicles of nanocochleate are stable phospholipid-cation precipitates usually made of calcium and phosphatidylserine[2,3]. Drug delivery systems allowing oral delivery increase patient compliance and promote out-of-hospital treatment, which has a major effect on healthcare economics[4,5]. For oral delivery of tissue impermeable drugs, various approaches have been documented, such as I converting a drug into a lipophilic pro-drug, ii) conjugating a drug with lipophilic moieties, and iii) encapsulating a drug into particulate systems. Due to their structural similarity to cell membrane, lipid-based delivery mechanisms like liposomes have attracted enormous research efforts as a cross-membrane drug delivery vehicle[6,7]. The use of liposomes to enhance oral absorption of hydrophilic drugs remains ineffective primarily because of their poor mechanical stability, low loading capacity of the drug and possibly lack of mechanism to promote cross-membrane diffusion in the intestine. Cochleate technology has been shown in particular to be successful in the therapeutic oral delivery of hydrophobic drugs, which are negatively charged bilayer phospholipids rolled up to form a rigid spiral rod by interaction with multi-cationic metal ions. It differs from liposome in that it has an interior that is water-free, a rod shape and a rigid structure. These are safe delivery formulations based on lipids whose structure and properties are very distinct from liposomes. Liposomes are made up of lipid bilayer membranes bounded by the lipid bilayer with aqueous space. This bilayer of lipids is susceptible to attack by harsh environmental conditions such as pH, degradation of lipase, and temperature. The trapped molecules are shielded from harsh environmental conditions by Cochleates. Nanocochleates consist of a purified phospholipid containing lipids that can be phosphotidyl serine (PS), dioleoylphosphatidylserine (DOPS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidyl glycerol (PG) and/or a combination of one or more of these lipids[8,9].

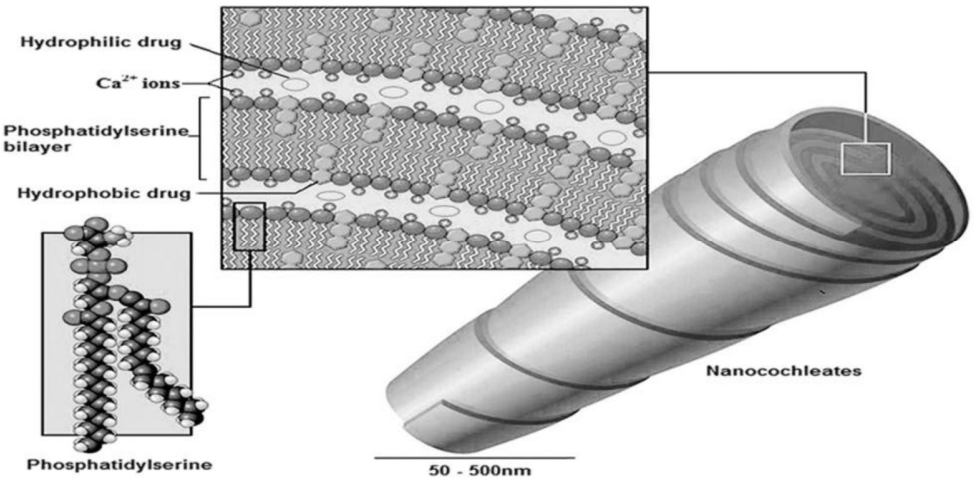

Fig. 1. Structure of Nanocochleate.

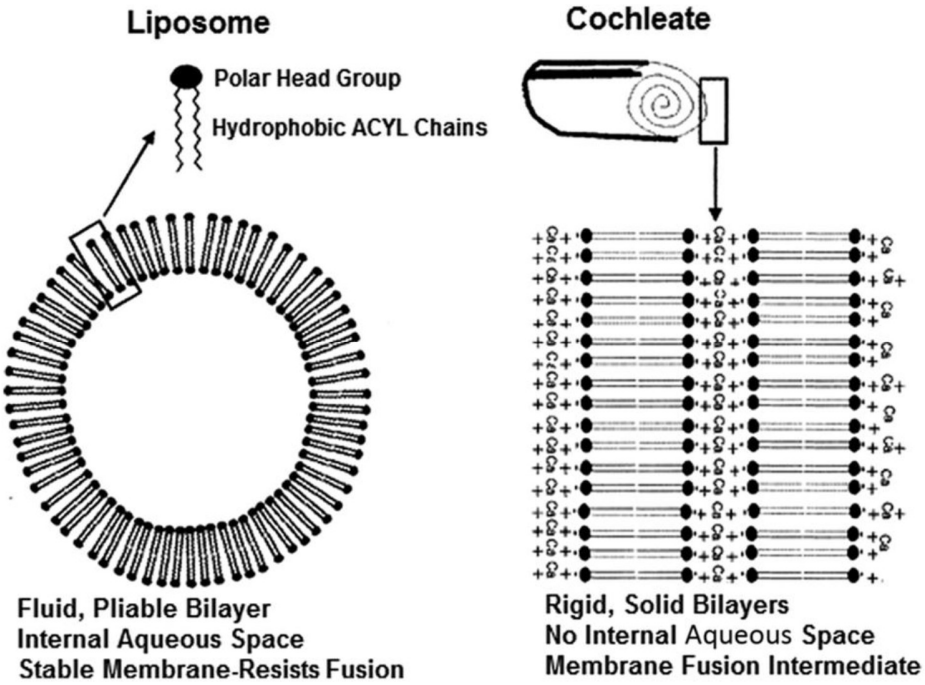

Fig. 2. Structural Difference between Liposome and Cochleate.

1. ***Routes of administration for Nanocochleate Drug Delivery*[7,10]**

The drug delivery vehicle of nanocochleates enables successful oral delivery of drugs. Parenteral, rectal, topical, sublingual, mucosal, nasal, ophthalmic, subcutaneous, intramuscular, intravenous, transdermal, spinal, intraarticular, intraarterial, bronchial, lymphatic and intrauterine administration, intravaginal or any other surface of the mucosa can be an alternative route of administration.

2. ***Dosage Forms available for Nanocochleate Drug Delivery***

Table 1. Oral Topical Parenteral Formulations.

Route of administration	Dosage Form	Reference Number
Oral administration	Capsules, cachets, pills, tablet, lozenges, powders, granules, or a solution or a suspension or an emulsion.	11,12
Topical or Transdermal administration	Powders, sprays, ointment, pastes, creams, lotions, gels, solutions, patches and inhalants.	11,12
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.	11,12

3. *Stability of Nanocochleate[*13,14]

Material encochleation gives molecules defense as well as stability. Since a collection of solid-lipid bilayers are the entire structure of these cochleates, components within the interior of this structure remain intact, even though its outer layers may be exposed to harsh external environmental conditions or enzymes. This structural interior is practically water-free and resistant to oxygen penetration, contributing to an improvement in the shelf-life of the formulation. Nanocochleates can be stored at room temperature or 4 ° C, and a type of powder can be lyophilized. Lyophilized cochleates may be reconstituted with fluid prior to in vitro use or in vivo administration. There are no harmful effects on the lyophilization of cochleate morphology, structure, or functions.

4. *Advantages of Nanocochleate Drug Delivery System*[15-18]

i. Due to the lower oxidation of lipids, they are more stable.

ii. They can be processed by freeze drying, which offers the ability to be stored at room temperature for long periods of time, which would be beneficial before administration for worldwide shipping and storage.

iii. Even after lyophilization, they can retain their structure, while liposome structures are destroyed by lyophilisation.

iv. They may demonstrate successful incorporation into the lipid bilayer of the cochleate structure of hydrophobic drugs.

v. Effective incorporation of antigens with hydrophobic moieties into the lipid bilayer of the structure of the cochleate can be demonstrated.

vi. As cochleates dissociate, they have the capacity for the gradual release of a drug, antigen or biologically important molecule in vivo.

vii. They have a lipid bilayer that functions as a carrier and consists of simple lipids contained in the membranes of animal and plant cells, so that the lipids are non-toxic.

viii. They are easily and safely made.

ix. They may be manufactured as specified formulations consisting of predetermined quantities and drug or antigen ratios.

5. *Disadvantages of Nanocochleate Drug Delivery System* [19-20]

i. They need special requirements for storage.

ii. During storage, aggregation may often occur; this may be prevented with the use of an aggregation inhibitor.

iii. Production costs are high.

6. *Mechanism of Nanocochleate Drug Delivery*[21-22]

The proposed mechanism for the delivery of hydrophobic drugs loaded into the nanocochleate interlayer spaces. The theory notes that when the lipid bi-layer structure of nanocochleates fuses

with the cell membrane, nanocochleate material is delivered into cells, so there is drug release. The schematic diagram is shown in Fig.3.

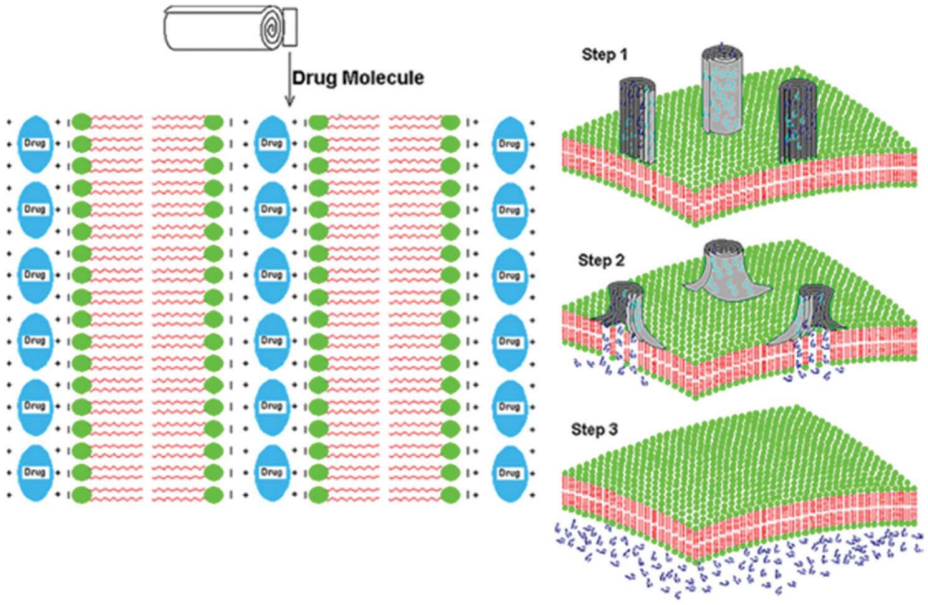

Fig. 3. Diagrammatic Presentation of Nanocochleate Interaction with the Cell Membrane.

7. *Preparation method*[23-31]

- Method of Trapping
- Method of Hydrogel
- Method of Liposomes Before Cochleate (LC) Dialysis
- Form of Direct Calcium (DC) Dialysis
- Binary aqueous-aqueous emulsion system:

8.1 Method of Trapping

This technique includes the formation of liposomes of phosphatidylserine accompanied by dropwise addition of a CaCl2 solution. Liposomes can be produced either by adding water to the phospholipid powder, or by adding a phospholipid film to the water phase.

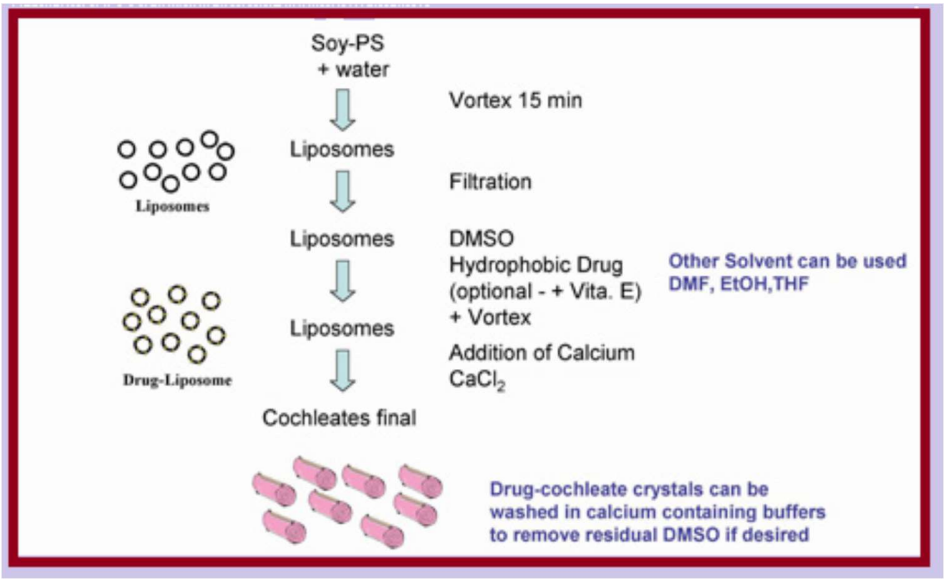

Fig. 4. Schematic presentation of trapping method.

8.2 Method of Hydrogel

Tiny unilameller drug-loaded liposomes are prepared in the hydrogel process by standard methods such as sonication & micro fludization and other similar methods. Then this liposome, including dextran, polyethylene glycol or phosphatidylserine, is blended into polymer A. The liposome / polymer suspension is ideally applied to polymer B by injection, such as polyvinyl pyrolidone, polyvinyl alcohol, ficol and polyvinyl methyl ether (pvmb) in which polymer A is immiscible and therefore polymer immiscibility contributes to the formation of a two-phase aqueous solution. This can be done mechanically with the use of a syringe pump at a controlled rate of 0.1 ml/min to 50 ml/min, for example, and ideally at 1 to 10 ml/min. In the two-phase method, a solution of cation salt is applied, so that the cation diffuses into polymer B and then into particles composed of liposome/polymer, allowing small-sized cochleates to form. In order to extract polymer which could be resuspended into physiological buffer, the formed cochleate is washed.

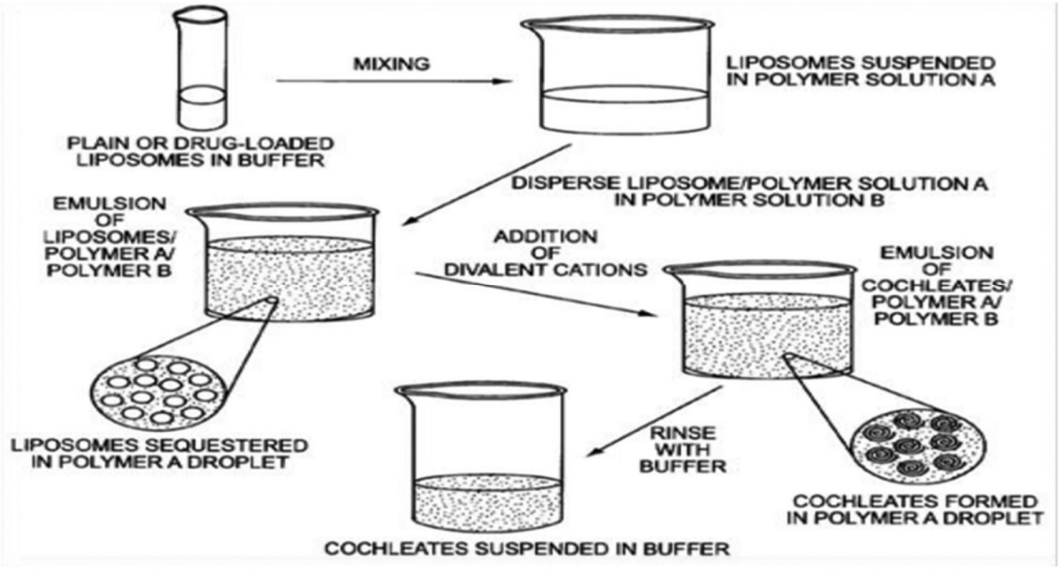

Fig. 5. Hydrogel Isolation Method.

8.3 Method of Liposomes Before Cochleate (LC) Dialysis

The mixture of lipid and detergent serving as a starting material prepares aqueous suspension in this technique. Polymer A, such as dextran, polyethylene glycol or phosphatidylserine, is combined with the detergent-lipid suspension. The suspension detergent-lipid/polymer is applied to a solution composed of polymer B, such as polyvinyl pyrolidone, polyvinyl alcohol, ficoll and polyvinyl methyl ether (pvmb), in which polymer A and polymer B are immiscible, forming a polymer system of two phases. The two-phase polymer framework is supplemented with a solution of a cationic moiety. The removal of detergent is then carried out by double dialysis. The mixture is initially dialyzed by the buffer and followed by the solution of calcium chloride that leads to cochleate formation. For encapsulation of hydrophobic material or drugs containing a hydrophobic region, such as membrane protein, this method is appropriate.

8.4 Form of Direct Calcium (DC) Dialysis

Unlike the LC process, the intermediate liposome formation does not require this method dosage, and the cochleates produced were large in size. The lipid and detergent mixture was specifically dialed against the solution of calcium chloride. A rivalry between the removal of detergent from the detergent/lipid/drug micelles and the condensation of bilayers by calcium results in broad dimensional structures formed by needles in this technique. In the extraction buffer and non-ionic detergent, the mixture of phosphotidylserine and cholesterol (9:1 wt ratio) is combined with a preselected polynucleotide concentration and vortex solution for 5min. The transparent, colorless solution resulting from this is dialled against three buffer adjustments at room temperature. 6mM Ca2+ is the final dialysis routinely used. The dial-up to buffer ratio is a minimum of 1:100 for each shift. The resulting precipitates of white calcium-phospholipids are called direct cochleates of calcium.

8.5 ***Binary aqueous-aqueous emulsion system***

Small liposomes were formed using either a high pH or a film process in this method, and then the liposomes were combined with a polymer, such as dextran. In a second, non-miscible, polymer (i.e. PEG), the dextran/liposome process is then injected. The calcium was then applied and slowly diffused, creating nanocochleates from one phase to another, after which the gel was washed out. Nanocochleates have been shown to facilitate the oral delivery of injectable medicines. The cochleates produced by this method are less than 1000 nm in particle size

9. ***Evaluation of Nanocochleates*****[32-36]**

9.1Particle Size and Size Distribution

One of the most significant parameters is the size of the atom. The particle size distribution, which involves photon correlation spectroscopy (PCS) and electron microscopy (EM), is calculated by two techniques. The above includes electron scanning microscopy (SEM), transmission electron microscopy (TEM) and techniques for freeze-fracture. With freeze-fracturing microscopy, the size assessment of nanocochleate dispersion shows better results and helps to establish the inner structure morphologically. An advanced nanoscopic technique applied for the characterization of nanocochleates is atomic force microscopy (AFM). Analysis should be carried out at a temperature of 30 ± 2 ° C, maintaining a detection angle of 90 ° C.

9.2 Specific Surface Area

In general, a sorptometer is used to calculate the specific surface area of freeze-dried nanocochleate. To measure the real surface area, the following equation can be used:

$A = 6 / \rho d$ ---Equation 1

Where A is the specific surface area, ρ is the density and d is the diameter of the cochleate.

9.3 Entrapment efficiency (EE)

In centrifugation tubes, one hundred micro litres of cochleates are aliquoted. 60 µl of pH 9.5 EDTA and 1ml of ethanol are applied to each tube when vortexing. The absorption of the resulting solution is measured using a spectroscopic technique and the efficiency of entrapment is calculated using the equation below.

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

---Equation 2

9.4 Density

With helium or air, the density of nanocochleates is measured using a gas pycnometer. Due to the particular surface area and porosity of the system, the value derived from air and helium is even more pronounced.

9.5 Molecular Weight Measurements

Using a refractive index detector, gel permeation chromatography (GPC) will determine the molecular weight of the polymer and its distribution in the matrix.

9.6 Drug content

To separate the free drug into the supernatant, the redispersed nanocochleate suspension is centrifuged at 15,000 rpm for 40 min at 25 °. The concentration of the drug in the supernatant can then be spectrophotometrically determined by UV-Vis after sufficient dilution.

9.7 Surface Charge and Electrophoretic Mobility

As it specifies their interactions with the biological environment as well as their electrostatic interaction with bioactive compounds, the existence and strength of the surface charge of Nanocochleate is very important. By calculating the particle velocity in an electric field, the surface charge of colloidal particles in general and of nanocochleates in particular can be calculated. For the determination of nanocochleate velocities, laser light scattering techniques such as Laser Doppler Anemometry or Velocimetry (LDA/LDV) are used as fast and high-resolution techniques. It is also possible to calculate the surface charge of colloidal particles as electrophoretic mobility. The composition of the charge critically specifies the bio-distribution of nanocochleate-carrying medication. By applying the Helmholtz-Smoluchowski equation, the zeta potential can be obtained by calculating electrophoretic mobility.

9.8 In-vitro Release

Using standard dialysis, diffusion cells or adapted ultra-filtration techniques that have recently been introduced and which use phosphate buffer using double chamber diffusion cells on a shake stand, the in vitro release profile of nanocochleates can be calculated. A Millipore, a low protein-binding, hydrophilic membrane, is positioned between the two chambers. Nanocochleates fill the donor chamber and the receptor compartment is assayed using normal procedures at various time intervals for the released drug. To evaluate the in-vitro release behaviour of Nanocochleates, the modified ultra-filtration method is also used. The Nanocochleate is added directly into a buffer-containing stirred ultra-filtration cell here. At different time intervals, aliquots of the dissolution medium are filtered using < 2 positive nitrogen pressure through the ultra-filtration membrane and assayed using normal procedures for the released compound.

***10. Applications of Nanocochleate*[37-42]**

- **i.** Creation of a formulation of apoa1 related nanocochleates for the treatment of atherosclerosis and other coronary heart diseases.
- **ii.** Biogeode nanocochleates are capable of stabilising and safeguarding a wide variety of micronutrients and have the ability to improve the nutritional value of processed foods.
- **iii.** For vaccine and gene therapy uses, nanocochleates have been used to deliver proteins, peptides and DNA.
- **iv.** Cochleates for anti-inflammatory agent delivery. And Agents of Antimicrobials.
- **v.** Without altering the product's taste or odour, nanocochleates can provide Omega-3 fatty acids to cakes, muffins, noodles, soups and cookies.
- **vi.** Nanocochleates show the potential for oral and parental delivery of Amphotericin B, a potential antifungal agent, with a good safety profile and reduced treatment costs.

Amphotericin B cochleate preparation shows increased stability and efficacy at low doses. They demonstrate enhanced compliance with patients.

vii. Cochleates will have the benefit of reducing toxicity and improving the efficacy of bactericides.

11. Marketed preparation [1]

Name	Trade name	Company	Indication
Liposomal Amphotericin B	Abelcet	Enzon	Fungal Infection
Liposomal Amphotericin C	Ambisome	Gilead Science	Fungal and protozol infection
Liposomal Cytarabin	Depocyt	Pacira (formerly skyepharma)	Malignant lymphomatous meningitis
Liposomal daunorubicin	Daunoxome	Gilead science	HIV –related Kaposi's sarcoma
Liposomal Doxorubicin	Myocet	Zeneus	Combination therpy with cyclophosphamide in metastatic breast cancer
Liposomal iriv vaccine	Epaxal	Berna biotech	Hepatitis a
Liposomal iriv vaccine	Inflexal v	Berna biotech	Influenz
Liposomal morphine	Depodur	Skyepharma , endo	Postsurgical analgesia

Liposomal verteporfine	Visudyne	Qit, Novartis	Age-related macular degeneration , pathologic myopia , ocular histoplasmosis
			Menopausal therapy
Micellular estradiol	Estrasorb	Novavax	

12. CONCLUSION

Nanococoleates have been widely used to deliver many active therapeutic agents, as this system can deliver hydrophilic and hydrophobic drugs due to the bilayer lipid structure. Encocleation can be helpful by improving shelf life, stability, bioavailability, reducing toxicity to improve the quality of formulation. This drug delivery system can therefore be used in the future as an alternative to delivering biological or therapeutic agents. This review article focuses on the therapeutic potential of the new class of drug carriers, i.e. nanococoleates, which can definitely lead the pharmaceutical world to the new era of highly challenging drug delivery.

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