

Nanocochleate – A New Drug Delivery System

Thiruganesh RAMASAMY[°], Umadevi KHANDASAMY^{**}, RUTTALA HINABINDHU*, Kiran KONA*

Nanocochleate – A New Drug Delivery System

Summary

This article focuses on the therapeutic potential of a new class of drug carrier called nanocochleate. This article highlights various methods of preparation for cochleate system. All the preparation method comprises using the charge ratio between the bridging agents and lipids to control the particle size. This cochleate or nano-cochleate system may be used for microencapsulation and delivery of therapeutics, wherein the therapeutic agents are loaded in the cochleate structure as the bridging agents between lipid bilayers. Finally, this article discusses the advantages, materials used, stability issues and other uses of these new cochleate and nano-cochleate systems. The properties that nano-cochleates can facilitate cross-membrane diffusion for charged and impermeable molecules finds wide application in drug delivery. Many therapeutic agents, such as peptides, are soluble but impermeable to tissue membranes. Cross-membrane permeation is especially important for those agents for which the binding sites are found inside the cells rather than on cell surface receptors. The system may also facilitate oral absorption for peptide drugs that possess a net positive charge. In a whole nanocochleate system increases the bioavailability, and hence the therapeutic action of drugs.

Key Words: Cochleate, nanocochleates, liposomes, phospholipid, peptides.

Received: 18.01.2011

Revised: 05.03.2011

Accepted: 15.03.2011

Nanokokleat-Yeni Bir İlaç Taşıyıcı Sistem

Özet

Bu makale yeni bir ilaç taşıyıcı sistem sınıfı olan nanokokleatların terapötik potansiyeli üzerinde durmaktadır. Bu makalede kokleat sistemlerin hazırlanmasında kullanılan bazı yollardan bahsedilmiştir. Tüm hazırlama metotları partikül büyüklüğünü kontrol etmek için köprüleme ajanları ve lipitler arasındaki yük farkı oranını kullanmayı kapsamaktadır. Kokleat veya nano kokleat sistemleri, terapötik ajanların lipit çift tabakaları arasında köprüleyici ajanlar olarak yer alması sonucu terapötiklerin taşınımı veya mikroenkapsülasyonu için kullanılabilirler. Son olarak bu makale yeni kokleat ve nano kokleat sistemlerinin kullanılan malzemeler, stabilite sorunları ve diğer kullanımları açısından avantajlarını tartışmaktadır. Bu sistemler; yükü ve geçirgenliği olmayan moleküllerin membranlar arası geçişini kontrol etmeleri açısından ilaç taşınımında geniş uygulama alanı bulabilmektedir. Peptitler gibi birçok terapötik ajan çözünür olmalarına rağmen doku membranlarına karşı düşük geçirgenlik gösterirler. Bağlanma bölgeleri hücre içerisinde olan ilaçlar için hücre dışı reseptörü olanlara göre membran geçişi daha da önem kazanmaktadır. Bu sistemler net pozitif yüke sahip peptit ilaçların oral emiliminin düzenlenmesinde kullanılabilir. Sonuç olarak nanokokleat sistemler ilaçların terapötik etkilerinin ve biyoyararlanımlarını artırmaktadırlar.

Anahtar Kelimeler: Kokleat, Nanokokleat, Lipozom, Fosfolipid, Peptit.

* Department of pharmaceuticals, Pydah college of pharmacy, Kakinada, India.

** Department of pharmaceuticals, Rao's college of pharmacy, Kakinada, India.

° Corresponding author e-mail: gracewell.nic@gmail.com

INTRODUCTION

In the present scenario, more than 50% of drug which are discovered were the product of biotechnological innovations. The progress of biotechnology has brought more and more biological therapeutics to clinical applications, however, the development of appropriate dosage forms for these agents are far behind the pace of the development of the new agents. This is due to their tissue impermeability and in vivo instability, low entrapment efficiency and, so on (1).

Among various drug delivery routes, oral delivery is the easiest and by far the most convenient method for drug administration, especially when repeated doses and prolong therapeutic periods are necessary. Oral delivery is the most suitable way of administering drugs for most non-hospitalized, non-acute care patients. Drug delivery systems that allow oral delivery improve patient compliance and facilitate treatment outside the hospital, which has a significant impact on healthcare economics. Recently, many drug delivery platforms have emerged and presently are either in preclinical stage or in an advanced clinical trial with the intent of trying to demonstrate efficient oral absorption (2-5). According to Egan and Lauri, most of the drugs are absorbed across the tissue membrane by passive diffusion. While particulate therapeutics may be taken up through endocytosis of lymphatic epithelial cells such as Peyer's patches, its efficiency is low because of the limited population of these cells (6). Hydrophilic molecules can be absorbed through the water filled tight junctions but only a small portion of drug molecules can have access to the tight junctions along the entire intestinal wall. Although some of the hydrophilic drugs such as nucleosides, anti-folates, and some small peptides are absorbed cross the membrane by attaching to membrane transporters, these transporters are specific to certain therapeutics (7-8). Moreover, structural modifications of drug molecules are often required to facilitate receptor-mediated drug molecule absorption, which may alter the pharmacological activity of the drug molecule. Therefore, there is a need to develop delivery system, which could facilitate diffusion of the drugs across the cell membrane (9).

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 (10). Particulate systems may offer good protection of delicate biological agents with no need for chemical modification of the molecules themselves. However, absorption of particles by the intestines is generally less than 1% (10). Because of the structural similarity of liposomes (phospholipid bilayer vesicles) to cellular membranes, the material had once been regarded as an ideal system for delivering therapeutic agents and attracted considerable research interest (11). However, utilization of liposomes to improve oral absorption of hydrophilic and hydrophobic drugs remains unsuccessful mainly due to their poor mechanical stability, low-drug loading capacity, and probably the lack of mechanism to facilitate cross membrane diffusion in intestine. To overcome this stability problem, several alternative lipid bilayer systems have been reported, namely, stealth™ liposomes (11), polymerized liposomes (12), polyethylene glycol coated liposomes (13), lipo-beads (14), and cochleates (15).

Structure and function

Cochleate and nanocochleate are cigar like spiral rolls (Fig.1) formed of negatively charged phospholipid bilayers, which are rolled up through the interaction with multivalent counter ions (Ca^{2+} or Zn^{2+}) as bridging agents between the bilayers (16). 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 (13, 16).

Cochleate delivery vehicles are stable

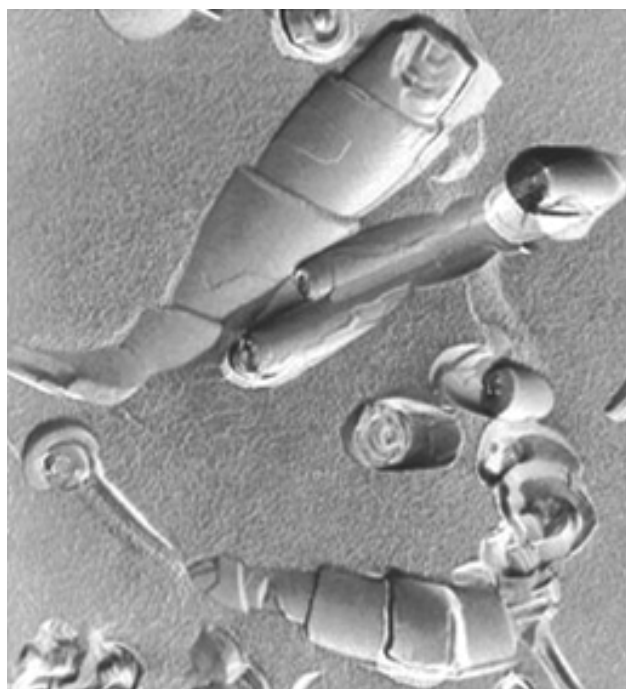


Figure 1. Cigar like structure with its double fold

phospholipid-cation precipitates composed of simple, naturally occurring materials (ie, 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 “enchochleated” molecules. Divalent cation concentrations *in vivo* in serum and mucosal secretions are such that the cochleate structure is maintained. Hence, the majority of cochleate-associated molecules are present in the inner layers of a solid, non-aqueous, stable, impermeable structure (9). A traverse section of rolled cochleate is given in Fig.2

Discovery of nano cochleates

Cochleates were discovered in 1975 by Dr. D. Papahadjoupoulos and coworkers, and have been used in the 80s and 90s to transport antigens and peptides for vaccine delivery. 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 in such a way to display a small mean particle of less than 500 nm. These nanocochleates were highly suitable for the encapsulation of hydrophobic drugs (17).

Cochleates were initially prepared in micrometer sizes (18) (Fig.3), either by direct addition of multivalent ion solution to liposome’s solution or by the dialysis method. However, the particle size could not be

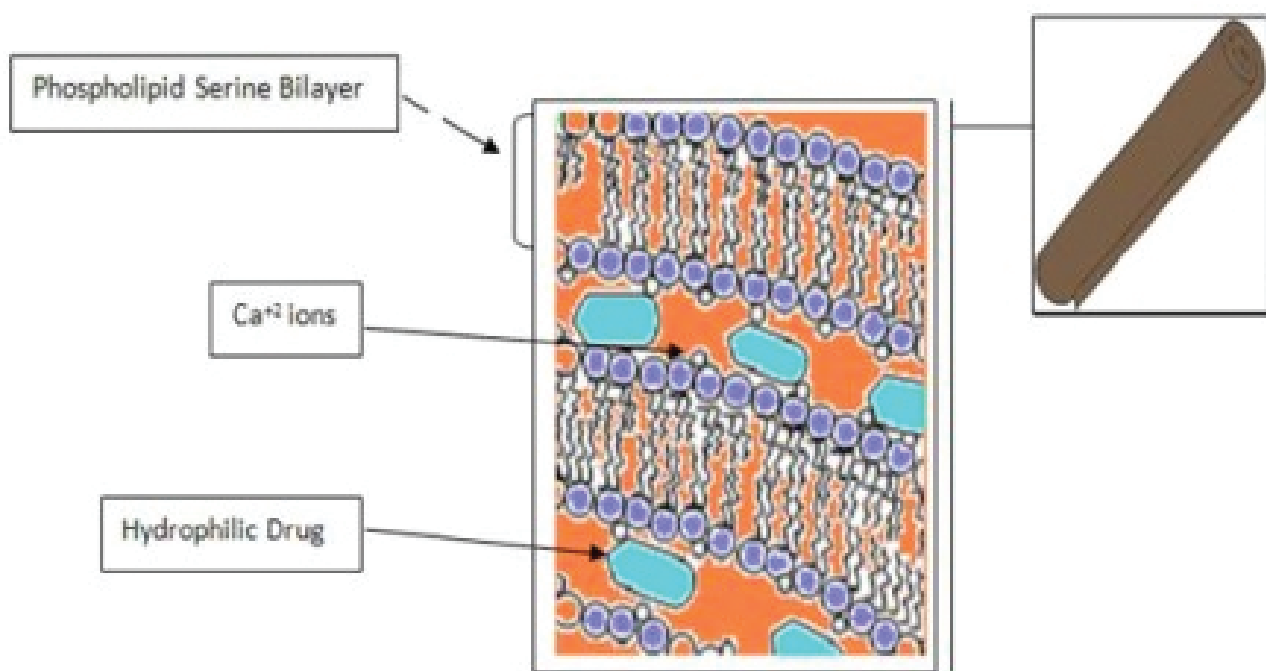


Figure 2. Traverse section of a rolled cochleate

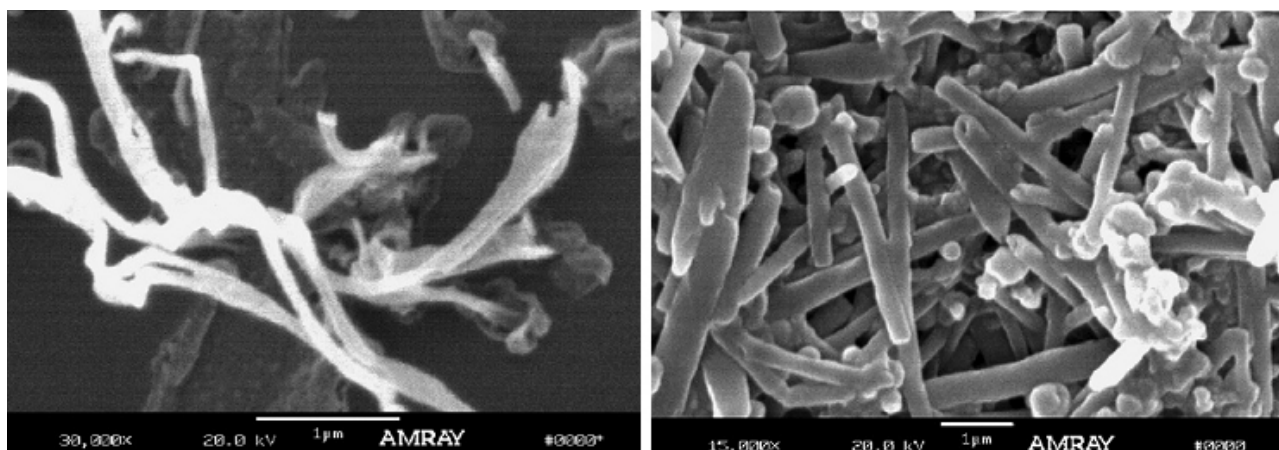


Figure 3. SEM images of cochleates formed with tobramycin, polylysine and calcium ions

reduced to nanometer range (19). Perhaps in recent past a new method named “hydrogel isolated cochleation” to prepare nanometer-sized cochleates was coined. In this method, a small amount of liposome is dissolved in dextran solution dispersed in a polyethylene glycol resulting in the formation of nanometer-sized cochleates (nanocochleates) upon interaction with multivalent ions. These nanocochleates when loaded with amphotericin B showed significantly improved oral absorption and anti-fungal activity as compared to drug solution and micrometer-sized cochleates (17, 20). This enhanced activity may be attributed to the membrane fusion capability of cochleates and an increased cell cochleate contact by increasing the number of particles due to their size reduction. The high tension at the bi-layer edges of cochleates (21) is suggested to be the driving force of cochleate’s interaction with the tissue membrane.

Advantages of nanocochleate over other drug carrier systems

The advantages of cochleates are numerous. The cochleates have a nonaqueous structure, and therefore ;

- 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 lyophilisation.

- 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.
- i) They can be produced as defined formulations composed of predetermined amounts and ratios of drugs or antigens (11, 19)

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 (22,

23). 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 .

Nanocochleate in drug delivery

Nanocochleates is an patented technology that demonstrated structure, preparation and application at nanometer level and are thus nanosized cochleates (19). These size-reduced cochleates show a capability for oral delivery of Amphotericin B (AmB), a hydrophobic drug currently administrated through IV injection in the form of liposomes or micelles. Oral availability of AmB achieved by nanocochleates encouraged the design of a new cochleate system by which highly charged and membrane-impermeable therapeutics may be encapsulated and delivered orally.

The first product employing this technique is Bioral™ Amphotericin B. This nanocochleate has a diameter of 50 nm and consists of crystalline structures with anhydrous interior that encapsulates the drug molecule and protects it from degradation in gastrointestinal tract (26). Bioral™ Amphotericin B underwent Phase I clinical trials for evaluation of its safety, tolerability and pharmacokinetics. It was found to be well tolerated in healthy human volunteers and the initial pharmacokinetic studies

revealed a measurable concentration of drug in the tissues. It is soon expected to undergo Phase II clinical trials after additional pharmacokinetic studies in healthy human volunteers (27).

In the case that cochleates are used as drug carriers, hydrophobic drug molecules are incorporated in the matrix of the phospholipid bilayer prior to cochleation (formation of cochleates by addition of metal cations) Fig. 4. It has been previously reported that cochleates were used to deliver DNA and protein vaccines (28, 29). Thus, it has been suggested that these hydrophilic macromolecules were incorporated in the cochleate structure. However, there was no solid evidence presented. The only experimental observation in this regard was that, the concentration of DNA or proteins in the supernatant was reduced after the addition of calcium ions. Since the multivalent ions (Ca^{2+} or Zn^{2+}) used in cochleate formation can form ionic complex with the proteins and DNA (since both are charged molecules.) through ionic interaction, there is a likelihood chance that these macromolecules interacted with Ca^{2+} or Zn^{2+} and were merely precipitated at the same time when cochleates were forming. Owing to the size and hydrophilic nature of these macromolecules, encapsulation of proteins and DNA into the lipid bilayer matrix or the inter-bilayer space is unlikely (29).

Lipids, cations and drugs used in Nanocochleate preparation

The various lipids which are used for the preparation

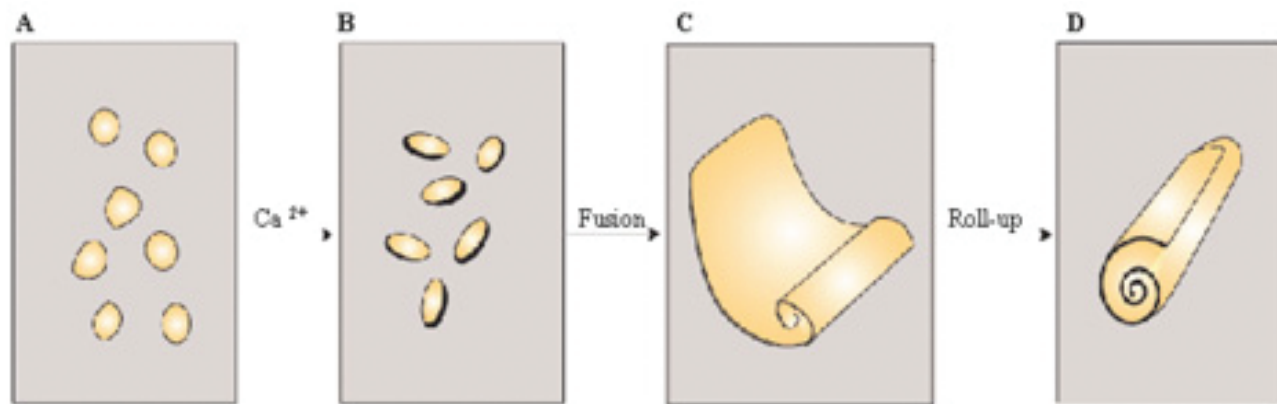


Figure 4. Cochleate formation from negatively charged vesicles, which fuse after the addition of cation to lead to fused liposomes which roll up to give cochleate cylinders

of nanocochleate are phosphatidyl serine (PS), dioleoylphosphatidylserine (DOPS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidyl glycerol (PG) and /or a mixture of one or more of these lipids with other lipids. Additionally or alternatively, the lipid can include phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), dioleoyl phosphatidic acid (DOPA), distearoyl phosphatidylserine (DSPS), and dimyristoyl phosphatidylserine (DMPS), dipalmitoyl phosphatidylglycerol (DPPG). A multivalent cation, which can be Zn^{+2} or Ca^{+2} or Mg^{+2} or Ba^{+2} and a drug, which can be protein, peptide, polynucleotide, antiviral agent, anesthetic, anticancer agent, immunosuppressant, steroidal anti inflammatory agent, non steroidal anti inflammatory agents, tranquilizer, nutritional supplement, herbal product, vitamin and/or vasodilatory agent (30).

Method of preparation

Method 1 : Hydrogel method:

In hydrogel method initially the small unilamellar drug loaded liposomes were prepared, which were added to polymer A (Which may be phosphatidyl serine, dextran, polyethylene glycol, etc.). The dispersion of two was then added to another polymer B (which may be polyvinylpyrrolidone, polyvinylalcohol, 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. 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 (31).

Method 2: Trapping method :

This method involves the formation of phosphatidylserine liposomes followed by dropwise addition of a solution of $CaCl_2$. Liposomes can be generated by either addition of water to phospholipid powder or by adding the water phase to a phospholipid film (32). A schematic presentation of the preparation methodology is given in Fig.5.

Method 3: Liposomes before cochleates (LC) dialysis method:

In this method mixture of lipid and detergent were used as the starting material and the removal of detergent was made by double dialysis. The mixture

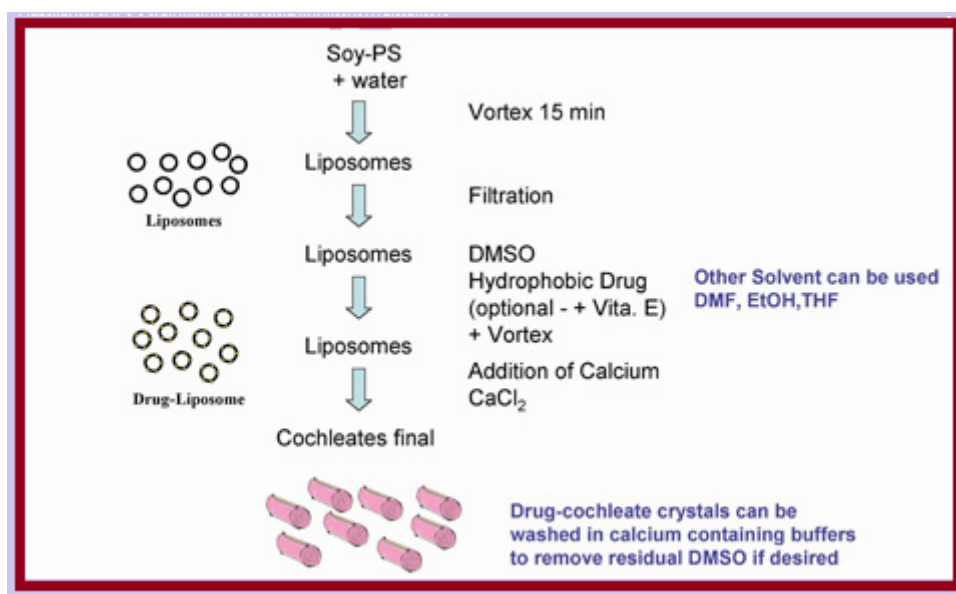


Figure 5. Schematic presentation of trapping method

was dialyzed initially with buffer and followed by calcium chloride solutions leads to formation of cochleates (32).

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. The resulting solution was vortexed for 5 minutes. The solution was dialyzed overnight using a mixture of dialysate and buffer in ratio 1:200 without divalent cations, followed by three additional changes of buffer leads to the formation of small protein lipid vesicles. The vesicles were converted to a cochleate precipitate, either by the direct addition of Ca^{2+} ions, or by dialysis against two changes of buffer containing 3 mM Ca^{2+} ions, followed by buffer containing 6 mM Ca^{2+} .

Method 4: Direct calcium (DC) dialysis method (19)

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 (2 mM TES N-Tris[hydroxymethyl]-methyl-2 aminoethane sulfonic acid, 2 mM L-histidine, 100 mM NaCl, pH 7.4) containing 3 mM CaCl_2 . The final dialysis routinely used is 6 mM Ca^{2+} , although 3 mM Ca^{2+} 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.

Method 5: Binary aqueous-aqueous emulsion system (19)

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.

Cochleates–cell interaction (33, 34)

When nanometer-sized cochleates and liposomes containing the same fluorescent labeled lipid component were incubated with human fibroblasts cells under identical conditions, cells exposed to cochleates showed bright fluorescent cell surfaces, whereas those incubated with liposomes did not. This result suggests that cochleates' edges made them fuse with the cell surfaces as compared to edge free liposomes. This mechanism of cochleates' fusion with cell membrane was supported by a bactericidal activity assay using tobramycin cochleates, which act by inhibiting intracellular ribosomes. Tobramycin bridged cochleates in nanometer size showed improved antibacterial activity than the drug's solution.

Mechanism of action

The proposed mechanism of the cross membrane delivery of lipophobic drugs loaded in the inter-bi-layer spaces of cochleates is shown in Fig. 6. We hypothesize that inter-bi-layer contents of cochleates are delivered into cells, when lipid bi-layer structure of nanocochleates fuses with the cell membrane and releases the drug.

Applications:

1. Development of a Nanocochleate based ApoA1 Formulation for the Treatment of Atherosclerosis and other Coronary Heart Diseases

Hypercholesterolemia, a condition associated with high levels of low-density lipoproteins (LDL), and

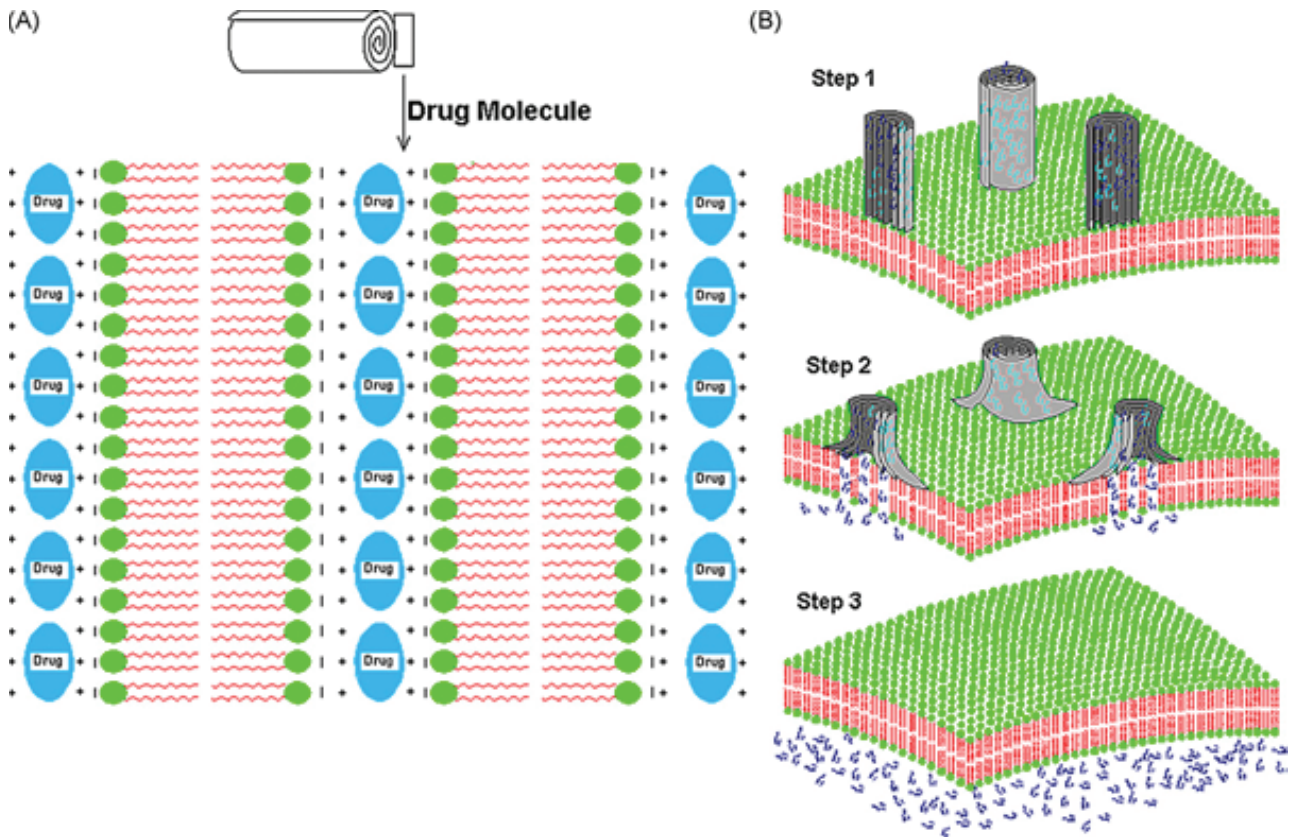


Figure 6. Illustrations of nanocochleate interaction with the cell membrane

low levels of high-density lipoproteins (HDL), is universally accepted as a major risk factor for atherosclerosis and other cardiovascular diseases. The inverse relationship between HDLs and heart diseases is well documented. HDLs facilitate the cholesterol efflux from peripheral cells and, after enzyme-mediated cholesterol esterification, transports cholesteryl esters to the body.

ApoA1 (a naturally existing lipoprotein) is an important HDL believed to be the most important in enzymatic esterification of cholesterol and then its transport to the liver, thus protecting the vessels against atherosclerosis.

Infusion or intraperitoneal administration of ApoA1 enhances the HDL ability to transport cholesterol to liver and protect against atherosclerosis but the major limitation for the use of ApoA1 as pharmacological/therapeutic agents has been the need for parenteral administration, as ApoA1 is a protein, it is rapidly degraded by GIT enzymes and

so it is not delivered to blood as intact molecule. So nanocochleates can provide a good platform for the delivery of ApoA1 by oral preparations and can bring a revolution in the treatment of atherosclerosis and other heart diseases originating from high blood cholesterol and LDL levels.

2. Cochleates for the Delivery of Anti-inflammatory Agents

Researchers are currently investigating the potential for using cochleate delivery vehicles to formulate and effectively deliver anti-inflammatory agents, including aspirin, ibuprofen, naproxen, acetaminophen, and COX-2 inhibitors. By using orally administered doses ranging from 0 to 40 mg/kg of body weight/day for 14 days in a murine model of systemic aspergillosis. The administration of oral doses of cochleate containing amphotericin B (CAMB) (20 and 40 mg/kg/day) resulted in a survival rate of 70% and a reduction in colony counts of more than 2 logs in lungs, livers, and kidneys.

Orally administered CAMB shows promise for the treatment of aspergillosis.

3. Cochleates for the Delivery of Antimicrobial Agents

Cochleates possess the advantage of reducing the toxicity and improving the bactericidal activity. For aminoglycosides and linear or cyclic peptides, cochleates should allow oral administration. The proof of principle of the efficacy of anti-TB cochleates was achieved using clofazimine as an antibacterial drug model. As using Amphotericin B (AmB) as a model, cochleates have been shown to be highly effective at mediating the oral delivery of drugs that are currently only available in injectable formulations.

4. Biogeode 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 .

7. Biodelivery Sciences International have developed nanocochleates which can be used to deliver nutrients such as vitamins, omega fatty acids more efficiently to cells, and lycopene without affecting the color and taste of food, which makes the concept 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 (16, 22, 35-38).

CONCLUSION

Nanocochleates are a lipid-based drug delivery system that shows potential in the oral delivery of many drugs. Cochleate delivery vehicles represent a new technology platform for oral and systemic delivery of drugs. They 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. Since cochleates are formed by ionic interaction between negatively charged liposomes and bivalent cations, microencapsulation of cationic drugs using themselves as the bridging agents of cochleation can be rationalized. Hydrophilic and multi-cationic drugs and peptides can be encapsulated into cochleates and nanocochleate particles as cochleates' bridging agents. This new type of cochleate is inherent in the physico-chemical properties of metal ion-bridged cochleates in terms of their conversion back to liposomes upon the extraction of cation bridging agent and their ability to fuse with the cell membrane. These unique properties enable cochleates and nanocochleates to deliver charged hydrophilic drugs across the tissue membrane. Thus nanocochleate possesses strong therapeutic potential in the area of drug delivery.

ACKNOWLEDGEMENT

We would like to thank our mentor and supervisor, Dr. Anbalagan, Principal & Professor, Pydah college of pharmacy, Kakinada, India, for his technical guidance and from offering Invaluable insights and significant feedback on the progress on the article.

REFERENCES

1. Vermeij P, Blok D. "New peptide and protein drugs". *Pharm. World Sci.* 18: 87-93, 1996.
2. Leone-Bay A, Paton DR, Weidner JJ. The development of delivery agents that facilitate the oral absorption of macromolecules drugs. *Med Res Rev* 20 (2): 169-86, 2000.
3. Florence AT, Hussain N. Transcytosis of nanoparticle and dendrimer delivery systems : evolving vistas. *Adv Drug Deliv Rev* 50 (1): S69-89, 2001.
4. Han HK, Amidon GL. Targeted prodrug design to optimize drug delivery. *AAPS Pharm Sci* 2 (1): E6, 2000.
5. Modi P, Mihi M, Lewin A. The evolving role of oral insulin in the treatment of diabetes using a novel rapidmist (trade mark) system. *Diabetes Metab Res Rev* 1 (1): S38-42, 2002.

6. Egan WJ, Lauri E. Prediction of intestinal permeability. *Adv. Drug Del. Rev* 54: 273–289, 2002.
7. Salama NN. Tight junction modulation and its relationship to drug delivery. *Adv. Drug Deliv. Rev* 58: 15–28, 2006.
8. Huang Y, Sadee W. Membrane transports and channels in chemo-resistance and sensitivity of tumor cells. *Cancer Lett* 239: 181–182, 2006.
9. Morishita M, Peppas NA. Is the oral route possible for peptide and protein drug delivery. *Drug Discov. Today* 11: 905–908, 2006.
10. Chen H, Langer R, “Oral particulate delivery: status and future trends”. *Adv. Drug Delivery Rev.* 14: 339-350, 1998.
11. Gregoriadis G, in *Liposome Technology*, CRC Press, Boca Raton, Vol. I, 1993.
12. Chen H, Torchilin V, and Carpenter R. “Lectin-bearing Polymerized Liposomes as Potential Oral Vaccine Carriers,” *Pharm. Res.* 13 (9): 1378-1383, 1996.
13. Jin T, Pennefather P, Lee PI. “Lipobeads: a Hydrogel Anchored Lipid Vesicle System”. *FEBS Letters* 397: 70-74, 1996.
14. Papahadjopoulos P. “Calcium-induced phase changes and fusion in natural and model membranes,” *Cell. Surg. Rev*, 5: 765-790, 1978.
15. Papahadjopoulos P, Vail WJ, Jacobson K, and Poste G, “Cochleate lipid cylinders: formation by fusion of unilamellar lipid vesicles,” *Biochim. Biophys. Acta*, 394: 483-491, 1975.
16. Zarif L, Graybill J, Perlin D, Mannino RJ. “Cochleates: new lipid based drug delivery system”; *Journal of Liposome Research* 10: 523-538, 2000.
17. Gould-Fogerite S, Kheiri M, Zhang F, et al. Targeting Immune response induction with cochleate and liposome-based vaccines. *Adv Drug Deliv Rev* 3: 273-287, 1998.
18. Uwais MS, Amyf W, Fotios plakogiannisa, Tuo Jina B, Hua Zhuc. Cochleates bridged by drug molecules structure and function . *International journal of pharmaceutics* 363: 118–125, 2008.
19. Jin T, Zarif L, Mannino RJ. Nano-cochleate formulations, process of preparation and method of delivery of pharmaceutical agents. *USA patent* 6153217: 2000.
20. Zarif L, Mannino RJ. Cochleates: New lipid based vehicles for gene deliveries, cancer gene therapy. *Advances in Experimental Medicine and Biology*, vol. 465. kluwer academic/plenum publishers, New York, 83–94, 2000.
21. Mannino R, Gold-Foserite S, Kheiri MT, Zhang F, Wang Z. Targeting immune response induction with cochleate and liposomes based vaccines. *Adv. Drug Deliv. rev.* 32: 237–287, 1998.
22. Delmas G, Park S, Chen ZW, et al. Efficacy of orally delivered cochleates containing amphotericin B in amurin model of aspergillosis. *Antimicrob Agents Chemother* 46: 2704, 2002.
23. Santangelo R, Paderu P, Delmas G, et al. Efficacy of oral cochleate amphotericin B in a mouse model of systemic candidiasis. *Antimicrob Agents Chemother* 44: 2356, 2000.
24. Zarif L, Graybill JR, Perlin D, et al. Antifungal activity of amphotericin B cochleates against *Candida albicans* infection in a mouse model. *Antimicrob Agents Chemother*; 44: 1463, 2000.
25. Segarra I, Movshin DA, Zarif L. Pharmacokinetics and tissue distribution after intravenous administration of a single dose of amphotericin B cochleates, a new lipid-based delivery system. *J Pharm Sci* 91: 1827, 2002.
26. <http://www.understandingnano.com/nanomedicine-biodelivery-sciences.html>
27. <http://www.understandingnano.com/clinical-study-bioral-Amphotericin-B.html>
28. Gould-Forgerite S, Mazurkiewicz JE, Raska Jr K, Voelkerding K, Lehman JM, and Mannino RJ. “Chimerasome-mediated gene transfer in vitro and in vivo,” *Gene* 84: 429-438, 1989.
29. Gould-Forgerite S, Kheri M, Zhang F, Wang Z, Scolpino AJ, Feketeova E, Canki M, Mannino RJ. “Targeting immune response induction with cochleate and liposome-based vaccines” *Adv. Drug Delivery Review*, 32: 273-287, 1998.
30. Jayaraj. Nanocochleates : A Novel Drug Delivery Technology. Pharminfo.net.
31. Zarif L, Jin T, Segarra I, Mannino RJ. 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 6,153,217.

32. Zarif L. Elongated supramolecular assemblies in drug delivery, *J. Control. Rel* 81: 7-23, 2002.
33. Sampaio JL, Moreno MJ, Vaz WL. Kinetics and thermodynamics of association of a fluorescent lysophospholipid derivative with lipid bilayers in liquid-ordered and liquid-disordered phases. *Biophys. J* 88: 4064–4071, 2005.
34. Villa AM, Caporizzo E, Papagni A. Choline and phosphatidylcholine fluorescent derivatives localization in carcinoma cells studied by laser scanning confocal fluorescence microscopy. *Eur. J. Cancer* 41: 1453–1459, 2005.
35. Etc group. Down on the farm-The Impact of Nano-Scale Technologies on Food and Agriculture. Available at: <http://www.etcgroup.org/>. 2004.
36. Joseph T, Morrison M. Nanotechnology in agriculture and food: A nanoforum report. Institute of nanotechnology. Available at: <http://www.nanoforum.org/>. 2006.
37. Apoprotein cochleate compositions. United States Patent Application 20060019870. <http://www.freepatentsonline.com>
38. Zarif L, Graybill JR, Perlin D, Najvar L, Bocanegra R, Mannino RJ. Antifungal activity of amphotericin B cochleates against *Candida albicans* infection in a mouse model. *Antimicrob Agents Chemother* 44 (6): 1463-1469, 2000

