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Cubosomes: Future of Therapeutics

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ABSTRACT

Recently, increasing attention has been paid to liquid crystal formulations, such as cubosomes and hexosomes, because of their remarkable structural complexity and usefulness in various pharmaceutical applications. Cubosomes represent an important class of biocompatible amphiphiles and their application extends to several fields. such as cosmeceutical, dietary, pharmaceutical technologies. Controlled drug delivery system are advanced methods for the transport of pharmaceutical compounds within the body and can be use to overcome many of the limitations of conventional drug formulations. The advantages of cubosomes as a drug delivery system are high biocompatibility and the self-assembly ability of their structure. This ability opens new possibilities for pharmaceutical applications several studies have reported the usefulness of cubosomes to enhance the drug absorption after oral and transdermal application.

INTRODUCTION

A crystalline solid contains a structural lattice which runs in all the three dimensions and has a positional and orientational order whereas, a liquid does not contain a lattice or any structure. Liquid Crystals are defined as the state of matter existing between the liquid and the crystalline solid. They possess fluidity like liquids and have a specific arrangement of molecules like a lattice as it is observed in crystals. Liquid Crystals arecharacterized[1] by the partial or complete loss of positional order in crystalline solids while retaining the orientational order of the constituent molecules.

Liquid crystals is a unique state shown by organic compounds. Since liquid crystals are intermediate between solids and liquids, they are also called as mesophases. Liquid crystals are anisotropic; which means they have different chemical and physical properties in different axes. It is important to note that[1] not all anisotropic materials are liquid crystals but all liquid crystals are anisotropic compounds. Due to anisotropy of liquid crystals, they also exhibit birefringent i.e. having two refractive indices. Having an anisotropic molecular shape associated with polarizability is the basic requirement for liquid crystals.[2]

Liquid crystals are classified into two types according to their method preparation:

1. Thermotropic liquid crystals

2. Lyotropic liquid crystals

Thermotropic Liquid Crystals: The liquid crystallinity of these compounds are a function of temperature.[12] If the temperature is too high, it will destroy the ordering of the liquid crystals converting it into normal anisotropic form. On the other hand, at very low temperatures the materials will form conventional crystals.[6,11] Unlike lyotropic substances, they do not require solvent for formation.[4,8] There are three types of Thermotropic LCs:

a. Nematic phase: In Greek, *nema* means thread.[3] Under a microscope, when using polarized light, Nematic LCs appear thread-like structures.[7] In this phase, the rod-shaped organic molecules have no positional order but they align themselves to have long range direction order.[3] Their physical properties like refraction index, permeability dielectric constant, etc. are anisotropic[7],[9]. These crystals are widely used in electronic display.

b. Smectic phase: These are found at even lower temperatures than nematic phase. The word *smetics* means cleaning or rather having a soap like properties.[5] The long axes of all the molecules in a particular layer are parallel to one another and perpendicular to the plane of layer. True to its name, the layer slides over one another similar to that of soap's. This phase is viscous, fluid and ordered.[7]

c. Cholesteric phases: This phase is called cholesteric phase because it was first discovered in cholesterol derivatives.[6] This arrangement to extent is a combination of nematic phase and smectic phase. The layers are able to slide over one another and are parallel to each other; a property similar to nematic phase. The layers are also perpendicular to the plane which is similar to the smectic phase.[8,10]Cholesteric phase is only observed in chiral compounds hence, it is also called Chiral phase.[6]

Lyotropic Liquid Crystals: Liquid crystallinity of lyotropic compounds depends upon its concentration and temperature.[12] They consists of two or more components; which show liquid crystallinity property, in different concentrations. In this phase, the solvent system fill up the space around the components and provide fluidity to the system.[13]They consists of rod-shaped amphiphilic micelles, which means they are surfactant type molecules and can absorb water from the environment.[17] Formation of these lyotropic molecules depend upon factors like temperature, the structure of organic molecule, and the water/amphiphile ratio.[15,16]Depending of the water content of the environment, it triggers spontaneous phase transitions reactions resulting lamellar, cubic, hexagonal phases.

a. Hexagonal phase: The simplest liquid crystalline phase is the micellar cubic which consists of spherical micelles arranged on a cubic lattice. At higher concentrations, the micelles fuse with each other to form cylindrical aggregates which are arranged in hexagonal lattice.[12] The long range order is two dimensional. The hexagonal shape is much stiffer than lamellar structure. It is also called as normal topology.

b. Lamellar phase: In this phase, the concentration is higher than the hexagonal phase. The amphiphiles are separated by thin layers of water. It has relatively high fluidity in spite of high surfactant content and thus the lamellae can glide over one another. Each bilayer is prototype arrangement of lipids in cells.[14]

c. Cubic phase: Amphiphiles with only one hydrocarbon chain, one or more complex structural phases are formed at concentration intermediate between hexagonal and lamellar

phase. This intermediate phase is called as *bicontinuous cubic phase.* At concentration higher than that of the lamellar phase, formation of inverse topology lyotropic phases take place, namely inverse cubic phase.[14]The interfacial area of cubic phase is larger than other phases. It is formed spontaneously in presence of excess water. The spheres forms and align themselves into a dense cubic structure. It consists of continued curved bilayers and a pair of interpenetrating, non-intersecting aqueous channels separated by each other. In practice, inverse cubic phase are readily formed by compounds having at the least two hydrocarbon chains attached to the parent group. Phosphatidylcholine, the most abundant phospholipids in mammalian cells, is the example of amphiphiles that readily form inverse topology lyotropic phases.[14]

Cubosomes: Cubosomes are discrete, sub-micron, nanostructured particles of bicontinuous cubic liquid crystalline phase.[18]They are thermodynamically stable and have the property to self assemble.[paper] They do not display optical textures. Cubosomes have honeycombed structures whose size range from 10–500 nm in diameter. They appear like dots, which are slightly spherical in shape. Each dot corresponds to the presence of pore contains aqueous cubic phase in lipid water system. It was first identified by Luzzati and Husson using X-ray scattering technique.[19]

Method of Preparation of Cubosomes: Liquid Crystals are generally made by adding liquid phase and lipid phase and blending them together by using vortex and ultrasonication.[24] However, preparation of cubosomes is a bit complicated. There are basically two methods:

i. **Top down approach:** This approach was first reported by Ljusberg-Wahren in 1996. An extreme viscous bulk phase is prepared by mixing structure forming lipids with stabilizers, the product is then dispersed into aqueous solution with the help of high input energy like high pressure homogenization (HPH) and sonication or shearing to form Lyotropic Liquid Crystals nanoparticles.[20] Parameters like temperature and the concentration of stabilizers are very important. Shearing produces highly stable nanoparticles than ultrasonication.[21]

ii. **Bottom up approach:** The key factor in the bottom-up approach is hydrotrope, which can dissolve water-insoluble lipids to create liquid precursors and prevent the formation of liquid crystals at high concentration.[25] Compared to the Top down approach, this method requires less input of energy. This approach is more efficient in generating small particles. This approach is analogous to precipitation process in nanoparticles. In addition, cubosomes

prepared through dilution show long-term stability, which might be attributed to the homodisperse stabilizers onto the surface of cubosomes.[22]

Applications of Cubosomes in drug delivery:

There are multitude of in vitro examples of applications of cubosomes. Therapeutic delivery is to date the key biomedical application of cubosomes. Cubosomes have been investigated for treatment of fungal infections[23] and as antimicrobial peptide, carriers to treat pneumonia and infected wounds.[26]Some evidence was also seen that cubosomes had some ability to protect one of the peptides from proteolytic degradation by elastases.[30]

Cancer therapeutics

Cancer therapeutics are the most widely reported application of cubosome systems. In vitro cubosomes have been loaded with cancer drugs including Doxorubicin,[27] Sorafenib,[28] 5-Fluorouracil,[29] and Quercetin[31] and delivered to cell lines, including human hepatocellular carcinoma (HepG2) cells, glioblastoma T98G cells, and mouse 3T3 fibroblasts.[31] Tumor cells have more acidic environments making pH stimuli useful for the payload delivery of chemotherapeutics.

Significantly more in vivo work is needed to establish cubosomes as viable options in cancer therapeutics although early indications are promising.

Vaccines

Apart from cancer therapeutics, another key application of cubosomes is as agents in vaccines. Cubosomes can be loaded with antigens and/or adjuvants and subsequently delivered appropriately. Incorporating immunostimulants such as polysaccharides into the cubosome membrane. In a study, phytantriol cubosomes containing polysaccharides were co-delivered with inactivated viruses in a subcutaneous injection. It was found that cubosomes containing polysaccharides were able to potentiate the immune properties of immunostimulants by promoting antigen transport into lymph nodes and enhancing the immune response.[32]

Oral Treatment

Cubosomes, owing to some unique advantages, seem to be promising oral delivery vehicles for poorly water-soluble actives. First of all, as a result of being lyotropic, cubosomes encapsulate poorly water- soluble drugs in the lipid bilayer part of their structure in a solubilized form, avoiding the drug precipitation in the GI tract. Second, the close contact of drug-loaded cubosomes with intestinal cell membrane can be provided as a consequence of their bioadhesion property. Third, cubosomes are lipid-based nanoparticles assumed to have significant roles in the process of drug absorption. Jin et al. [33] incorporated 20(S)-protopanaxadiol (PPD), an anticancer agent, to GMO cubosomes, intended for its improved oral absorption. According to the results, the PPD- loaded cubosome association could increase the permeability values from the Caco-2 cell monolayer model of PPD at 53%. Pharmacokinetic study in rats demonstrated that the extent of bioavailability of the PPD-loaded cubosome association (AUC0–1) was 169% compared with the free PPD.

Topical Treatment

In transdermal delivery of active molecules, the skin penetration of the drug is limited as a result of the barrier function of the highly organized structure of the stratum corneum, the most external layer of the skin. Several approaches have been presented to improve the skin permeation such as chemical modification of the active molecule, applying a skin permeation enhancer and iontophoresis. The crucial issue in topical formulations is to increase the thermodynamic activity of the active molecule in the vehicle while decreasing it in skin, which results in increasing the partition of the molecule from vehicle to skin and decreasing the barrier function of the skin.[34]Cubosomes have been investigated as topical delivery agents in part because of their ability to influence permeability. Silver sulfadiazine is one of the gold standard topical treatments for burns but a delivery agent is needed. Cubosomes formed from monoolein and stabilized with F127 and polyvinyl alcohol were loaded with silver sulfadiazine and incorporated into hydrogels (cubogels) as a potential treatment for burns.[36] In vivo study showed that the cubic nanostructured vehicle was successful in treatment of deep second degree burns, which could result in better patient compliance and excellent healing results with fewer side effects in comparison with the commercially available product.[35]

Rheumatoid arthritis is often treated with the anti-inflammatory drug Etodolac. However, when administered orally it can cause gastrointestinal disturbances, ulcers, and bleeding. Therefore, transdermal application, which could provide a stable blood concentration of drug for prolonged periods at an effective low dose, is highly preferable. Monooleincubosomes stabilized with F127 and polyvinyl alcohol were loaded with Etodolac. Skin permeation was measured ex vivo in the skin of newly born albino mice after which two formulations were chosen for a pharmacokinetic study carried out in six healthy male volunteers. It was found that topical delivery of Etodolac via cubosomes provided controlled delivery at lower dosages but over a prolonged time period in comparison to orally administered Etodolac, and therefore could be a novel method for treating arthritis.[37]

Ophthalmic delivery

Although topical application of drug solutions (eye drops) is usually employed in ocular diseases, the removal mechanisms such as blinking, tears and nasolacrimal drainage are considerable challenges. The cornea is the main route of anterior drug absorption for drugs to reach the ocular tissue. However, the lipophilicity as well as the presence of tight conjunction in the corneal epithelium makes it the major limiting barrier in corneal drug absorption, thereby leading to pulsed drug entry and poor ocular bioavailability of drugs. Owing to the interesting features of the cubosomes, mostly the bioadhesive properties, they could play a key part as an ophthalmic delivery system to reduce the ocular irritancy and to improve the ophthalmic drug bioavailability. Han et al. studied the potential of cubo-somes as an ophthalmic drug delivery system for flurbiprofen (FB). The cubosomes showed low ocular irritation as evaluated by the Draize method and histological examination.[34]

Nanocarriers for bio lipids

An interesting new application for cubosome systems is not only via loading the membrane with drugs or proteins but also to deliver bioactive lipids. The higher membrane surface area in comparison to a vesicle means that each particle contains a significantly higher number of lipid molecules enabling a higher payload per particle. Examples of cubosome formulations that include bioactive lipids such as oleoyl ethanolamide (OEA), a neuromodulatory lipid, the glycolipid monosialoganglioside GM1,and unsaturated fatty acids, all of which could be used to demonstrate cubosomes as colloidal carriers for bioactive lipids.[38]

Image Contrasting Agents

Nanoparticle contrast agents enable the specific imaging of tissues and organs for the diagnosis of disease. Key considerations for the development of these is stability, toxicity, delivery method, and the ability to use them for dual purposes to first diagnose and then treat disease. Owing to their biocompatibility and the ability to load and target them, cubosomes are an attractive option for novel contrast agents. There have to date been examples of cubosomesas contrast agents for MRI,[39] fluorescence,[40] and more recently combined MRI-fluorescence imaging.[41]

CONCLUSION

Cubosomes are nanoparticles of continuous bilayer lyotropic liquid crystalline phase, having size range between 10-500 nm. They are prepared by mixing a liquid and lipid phase and stabilized by addition of stabilizers. There are two methods of preparation i.e., top down approach and bottom up approach, both differing from each other in terms of concentration of materials and input of energy. Having unique features like lipid solubility, controlled release property, and encapsulation of hydrophilic, lipophilic and amphiphilic drugs makes cubosomes as ideal drug carriers. Its use in ophthalmic delivery, topical delivery, image contrasting systems, cancer therapy, oral administration, etc. may revolutionize the field of therapeutics. Although no such drug has been approved to be used in the market as of yet because even though many researches have been focused on cubosomes, there are still many hurdles that may need to be overcome first. However, with increasing understanding of cubosomes, there is a future full of opportunities for this novel drug delivery system.

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