

CUBOSOMES-A DRUG DELIVERY SYSTEM

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ABSTRACT

Cubosomes are the square and rounded particles with internal cubic lattices visible. The discovery of cubosomes is a unique story and spans the fields of food science, differential geometry, biological membranes, and digestive processes. Cubosomes are self-assembled nanostructured particles formed by aqueous lipid and surfactant systems. Cubosomes are thermodynamically stable; they have a structure like "honeycombed" with bicontinuous domains of water and lipid in which surfactant assembles into bilayers and twisted into a three dimension, periodic, and minimal surface, forming a tightly packed structure. They exhibit different internal cubic structure and composition with different drug-loading modalities. Overall, cubosomes have great potential in drug nano formulations for melanoma therapy owing to their potential advantages, including high drug payloads due to high internal surface area and cubic crystalline structures, relatively simple preparation method, biodegradability of lipids, the ability of encapsulating hydrophobic, hydrophilic and amphiphilic substances, targeting and controlled release of bioactive agents. Cubosome dispersions are bioadhesive and biocompatible. Because of their properties, cubosomes are versatile systems, administrable by different ways such as orally, percutaneously and parenterally. Cubosome structure by means of electron microscopy, "light scattering", x-ray and "NMR", nevertheless few researchers have been studying the potential of cubosomes as "delivery systems". Hydrating a surfactant or polar lipid that forms cubic phase and then dispersing a solid like phase into smaller particles usually forms a cubosome. Such novel particles are utilized to encapsulate guest molecules which are either hydrophilic, lipophilic or amphiphilic, due to the compartmentalization of its structure.

Keywords: Cubosomes , honeycombed , versatile , drug payloads, compartmentalization.

1. INTRODUCTION

1.1 Definitions of Cubosomes

Cubosomes are discrete, sub-micron, nanostructured particles of the bicontinuous cubic liquid crystalline phase¹. Cubosomes are nanoparticles which are self assembled liquid crystalline particles of certain surfactants with proper ratio of water with microstructure. Cubosomes are nanoparticles but instead of the solid particles usually encountered, cubosomes are self-assembled liquid crystalline particles with a solid-like rheology that provides unique properties of practical interest.

1.2 History

Despite the early recognition (in 1980) large scale manufacture of cubosomes was difficult due to their complex phase behavior and viscous properties. The cubic phases are unique as possess very high solid like viscosities because of their intriguing bicontinuous structures. Cubic phases can be fractured and dispersed to form particulate dispersions which are colloidal and/or thermodynamically stable for longer period of time. Certain surfactants spontaneously form cubic phases when mixed with water above a certain concentration. Determination of their honeycomb structure was carried out by Luzzati and Husson, Luzzati *et al.*, Larsson and Hyde *et al.* between 1960 and 1985. The term "Cubosomes" were coined by

Larsson, that reflects the cubic molecular crystallography and similarity to liposomes. Effort to develop scalable processes to produce cubosomes in large scale is under development. A few anticancer drugs have been successfully encapsulated in cubosomes and characterized¹.

1.3 STRUCTURE

The basic structure of cubosomes includes honeycombed structures separating the two internal aqueous channels along with large interfacial area. Cubosomes are nanoparticles, more accurately nanostructure particles of a liquid crystalline phase with cubic crystallographic symmetry formed by the self assembly of amphiphilic or surfactant like molecules. The cubosomes having high internal surface area (Figure-1) along with cubic crystalline structures. The cubic phases possess a very high solid like viscosity, which is a unique property because of their intriguing bicontinuous structures which enclose two distinct regions of water separated by a controlled bilayer of surfactant. Amphiphilic molecules form bicontinuous water and oil channels, where "bicontinuous" refers to two distinct (continuous, but non-intersecting) hydrophilic regions separated by the bilayer. The interconnectedness of the structure results in a clear viscous gel similar in appearance and rheology to cross-linked polymer hydrogels. However, monoglyceride-based cubic gels possess significantly more long-range order than hydrogels and, because of their composition (i.e., lipid and water), excellent biocompatibility.

2.1. ADVANTAGES OF CUBOSOMES¹

1. High drug payloads due to high internal surface area and cubic crystalline structures.
2. Relatively simple method of preparation.
3. Biodegradability of lipids.
4. Capability of encapsulating hydrophilic, hydrophobic and amphiphilic substances.
5. Targeted release and controlled release of bioactive agents.
6. While most liquid crystalline systems transform into micelles at higher levels of dilution, cubosomes remain stable almost at any dilution level because of the relative insolubility of cubic phase forming lipid in water. So, cubosomes can easily be incorporated into product formulations. Cubosomes are typically produced by high energy dispersion of bulk cubic phase, followed by colloidal stabilization using polymeric surfactants. After formation, the dispersion is

formulated into a product and is then applied to a substrate, usually skin or mucosal surface. After that materials are either absorbed or released via diffusion.

7. The cubic phases of cubosomes can be fractured and dispersed to form particulate dispersions that are colloiddally and/or thermodynamically stable for longer time.

2.2. DISADVANTAGES OF CUBOSOMES

Large scale production is sometimes difficult because of high viscosity.

3. FORMS²

Three macroscopic forms of cubic phase are typically encountered: precursor, bulk gel, and particulate dispersions (cubosomes). The precursor form exists as a solid or liquid material that forms cubic phase in response to a stimulus, such as contact with liquid. Bulk cubic phase gel is an optically isotropic, stiff, solidlike material. Cubic gel in equilibrium with water can be dispersed into particles called cubosomes, analogous to the formation of vesicles from lamellar liquid crystalline material. A recent review provides a comprehensive summary of active ingredients delivered by cubic phase. Despite intense interest in cubosome applications, we have found no work examining the practical aspects of large-scale processing and production of cubosomes.

3.1 Liquid Cubosome Precursors

Following the difficulty and expense of high-shear dispersion of viscous bulk cubic phase to form cubosomes, it is desirable to seek less aggressive processes of manufacture. High-energy processes being expensive and difficult to scale-up, also proves to be harmful to thermosensitive ingredients like proteins. In some product applications, the in situ formation of cubosomes is desired, such as during hand washing or mouth rinsing. To avoid high-energy processing and produce them in situ a strong driving force exists resulting in the development of a liquid phase precursor to cubosomes. The hydrotrope dilution process is found to consistently produce smaller, more stable cubosomes. In this process the particles are formed by nucleation and growth, as employed in crystallization and precipitation processes. This is achieved by dissolving the monoolein in a hydrotrope (ethanol) which prevents liquid crystalline formation. All this is achieved without the need of high shear, minimizing the risk of degrading the cubic liquid crystalline structure. The liquid precursor process allows for easier scale up of cubosome

preparations and avoids bulk solids handling and potentially damaging high energy processes.

3.2 Powdered Cubosome Precursors

Powders composed of dehydrated surfactant coated with polymer are termed as powdered cubosome precursors. Hydration of the precursor powders forms cubosomes with a mean particle size of 600 nm, as confirmed by light scattering and cryo-TEM. A water-soluble non-cohesive starch coating on the waxy lipid prevents agglomeration and allows control of particle size. The lipids used to make cubosomes are waxy, sticky solids, rendering them unable to form small discrete particles. Spray drying technique is an excellent process to produce these particles. Spray drying produces encapsulated particles from an emulsion of liquid droplets or a dispersion of solid particles in a concentrated aqueous polymer solution. Nozzle is used for the continuous and dispersed phases spraying throughout to create suspension droplets that are contacted with a heated, dry air stream flowing in the opposite direction. As a result of this excess water immediately evaporates, leaving dry powder particles composed of the dispersed phase encapsulated by a shell of the formerly dissolved polymer. Spray-drying processes are easily scaled up and are already widely employed for manufacturing consumer products like detergents and foods. Moreover, the process provides an easy route to preload active drug into the cubosomes prior to drying. Finally, the polymer coating on the powder imparts surface properties to the hydrated cubosomes that can be tailored by proper selection of the encapsulating polymer. Such powders offer some process and performance advantages to liquid phase hydrotropic cubosome precursors.

4. MANUFACTURE OF CUBOSOMES

Cubosomes can be manufactured by two distinct methods:

1. Top down technique
2. Bottom up technique
3. Preparation of ALA loaded cubosome dispersions
4. From Pseudo-Binary Systems
5. In the Presence of Hydrotrope

4.1 Top-Down Technique³

It is the most widely used procedure initially reported in 1996 by Ljusberg-Wahren. Bulk cubic phase is first produced and by application of high energy such as high pressure homogenization it is processed into cubosomes nanoparticles. Bulk cubic phase resembles a clear rigid gel formed by water-swollen cross-

linked polymer chains. The cubic phases differ in that they are a single thermodynamic phase and have periodic liquid crystalline structure. Cubic phases rupture in a direction parallel to the shear direction, the energy required is proportional to the number of tubular network branches that rupture. It is the most widely used in research area, where by bulk cubic phase is first produced and then dispersed by high energy processing into cubosomes nanoparticles. Bulk cubic phase is resembling a clear rigid gel formed by water swollen cross linked polymer chains; whereas cubic phases are like liquid crystalline structure. The cubic phases exhibit yield stress that increases with increasing amount of bilayer forming surfactant and oils. Warr & Chen gave the cubic phases may behave as lamellar phases during dispersion with increasing shear, dispersed liquid crystalline particles are forming at intermediate shear rates, whereas defect free bulk phase reforms at higher shear rates. Based on most existing studies comparison of dispersion produced by sonication and high pressure homogenization suggests the formation of complex dispersions containing vesicles and cubosomes with time dependent ratios of each particle type (Figure-2). Coarse cubosomes on micron scale possess the same D-surface structure as their originating bulk cubic phase, but after homogenization, the P-surface dominates because of added polymers.

4.2 Bottom-Up Technique³

In this cubosomes are allowed to form or crystallize from precursors. The formation of cubosomes by dispersing L2 or inverse micellar phase droplets in water at 80°C, and allow them to slowly cool, gradually droplets get crystallized to cubosomes. This is more robust in large scale production of cubosomes. The cubosomes at room temperature is by diluting monoolein-ethanol solution with aqueous poloxamer 407 solution. The cubosomes are spontaneously formed by emulsification. Another process is also developed to produce the cubosomes from powdered precursors by spray drying technique. Spray dried powders comprising monoolein coated with starch or dextran form cubosomes on simple hydration. Colloidal stabilization of cubosomes is immediately provided by the polymers. In this cubosomes are allowed to form or crystallize from precursors. The bottom-up approach first forms the nanostructure building blocks and then assembles them into the final material. It is more recently developed technique of cubosome formation, allowing cubosomes to form and crystallize from precursors on the molecular

length scale. The key factor of this technique is hydrotrope that can dissolve water insoluble lipids into liquid precursors. This is a dilution based approach that produces cubosomes with less energy input when compared top down approach(Figure-3).

4.3 Preparation of ALA loaded cubosome dispersions⁴

Cubosome dispersions were fabricated using two different methods. The first method was through fragmentation of GMO/P407 bulk cubic gel. GMO (5.0%) and P407 (1.0%) were firstly melted at 60°C in a hot water bath, after which ALA (25,50 or 100 mg) was added and stirred continuously to dissolve. Deionized water was gradually added and vortex mixed to achieve a homogenous state. After equilibration for 48 hrs at room temperature, an optically isotropic cubic gel phase was formed. After addition of 10 ml of deionized water, the cubic gel was first disrupted by mechanical stirring. The crude dispersion was subsequently fragmented by intermittent probe sonication at 200 W energy input under cooling in a 20 °C water bath for 20 min. The second method was achieved through the emulsification of GMO and P407 in water followed by ultrasonication. Dispersion is composed of 5% GMO (with 1% P407 and 5% ethanol) in 89% water. GMO and P407 were gently melted at 60°C and mixed; ALA ethanolic solution was then added to the melt. The resultant mixture was then added dropwise to deionized water preheated at 70°C and ultrasonicated at maximum power of 130 kW for 15 min at the same temperature. All dispersions were stored in glass vials at ambient temperature (23 °C) protected from light (Table 1).

4.4 Cubosomes from Pseudo-Binary Systems⁵

Cubosomes were first made in a pseudo-binary system of monoolein-water (including polymer at low levels) using the conventional technique of energetic dispersion of bulk cubic gel. Melted Poloxamer 407 (8% w/w) and monoolein (92% w/w) were combined to form a homogeneous solution. The monoolein-polymer solution was then added to deionized water to form a 1.8% mixture of monoolein containing 98% water and 0.2% Poloxamer 407. The mixture was sonicated for 60 min in a controlled temperature ultrasonic bath, maintained at 25°C, to disperse the cubic liquid crystalline gel. Cryo-TEM (Figure. 4) revealed mostly square cubosomes that were about 100-300 nm along an edge. The unit cell structure is evident from alternating water (light gray dots) and oil channels (dark matrix). Fourier analysis of the

periodicity results in 150 Å, which is consistent with SAXS for monoolein water cubic phases. The three-dimensional shape of the aggregates, however, is elusive. Stereographic images taken at 0 and 15° from the normal (Fig.4) show some blurring in the well-defined matrix of water channels from visualizing successive layers below the top layer. However, the length of the particle edge does not change upon tilting. This is peculiar because rotating a cube by 15° should result in an increase of 22% in size along the direction of rotation. This suggests that the aggregate might be more spherulike or relatively flat, although more distinctly cubic cubosome aggregates have been documented.

4.5 Cubosomes in the Presence of Hydrotrope⁵

Cubosomes were also formed in the presence of significant levels of hydrotrope by sonication based methods. Bulk cubic gel was fabricated by the combination of molten monoolein (93% w/w) and ethanol (7% w/w) to form a low-viscosity isotropic liquid. A 1.2% Poloxamer 407 solution was added to the liquid, forming a viscous, cubic liquid crystalline gel in the presence of excess water (final composition: 68% monoolein, 26.7% water, 5% ethanol, and 0.3% Poloxamer 407). The mixture was sonicated for 5 min.

Fig.5 shows cryo-TEM photographs of two cubosomes about 200 nm in diameter. These cubosomes are similar in size and shape to those formed without ethanol, although more circular than square. Also visible is a larger region of cubic liquid crystal attached to the support and displaying a well-defined cubic lattice. The larger pieces of cubic gel form as a result of incomplete dispersion by the short application of ultrasonic energy, this dispersion was macroscopically more opaque (a dispersion formed after 60 min of sonication). Large amounts of energy per unit volume are clearly necessary to completely disperse the cubic gel into cubosome nanoparticles when starting from bulk cubic gel. Finally, note that both cubosomes in have a hemispherical-shaped vesicle extending from an edge. The formation of a vesicular coating on cubosomes has been suggested as a thermodynamic means of avoiding exposure of lipid hydrocarbon chains as the cubic liquid crystalline gel is fragmented during dispersion. Formation of cubic liquid crystals in the presence of hydrotrope was confirmed by SAXS measurements on ethanol-containing cubic phase gels. SAXS measurements were made on cubic phase gels of 2% ethanol (i.e., 50% monoolein, 48% water, and 2%

ethanol) and compared to those without ethanol (i.e. 50% monoolein and 50% water).

5. DRUG LOADING CAPACITY OF CUBOSOMES¹

The cubosomes generally have different internal cubic structure along with variant composition related to the drug loading modalities. The cubosomes have huge potential in drug nano formulations for melanoma therapy due to their potential advantages consisting high drug payloads (fig. 6).

6. METHODS FOR CHARACTERIZATION AND EVALUATION OF CUBOSOMES

1. Gel permeation chromatography or ultra filtration techniques & UV spectrophotometer or HPLC analysis¹

Entrapment efficiency and drug loading of cubosomes can be determined using gel permeation chromatography or ultra filtration techniques. In the later technique, untrapped drug concentration is determined, which is subtracted from the total drug added. The amount of drug is analyzed by using UV spectrophotometer or HPLC analysis.

2. Photon correlation spectroscopy¹

Particle size distributions of cubosomes are mainly determined by dynamic laser light scattering using Zeta sizer (Photon correlation spectroscopy). The sample diluted with a suitable solvent is adjusted to light scattering intensity of about 300 Hz and measured at 25°C in triplicate. The data can be collected and generally shown by using average volume weight size. The zeta potential and polydispersity index can also be recorded.

3. Polarized light microscopy¹

Polarized light microscopy can be used to reveal the optically birefringent (possibly vesicular) surface coating of the cubosomes and also can distinguish between anisotropic and isotropic substances.

4. X-ray scattering¹

Small angle X-ray scattering (SAXS) can be used to identify the spatial arrangements of different groups in the sample. The diffraction patterns obtained are converted to plots of intensity versus q value, which enable

the identification of peak positions, and their conversion to Miller Indices. The Miller Indices could then be correlated with known values for different liquid crystalline structures and space groups to identify the dominant internal nanostructure of the sample.

5. Transmission electron microscopy¹

Transmission electron microscopy can be used to view the shape of the cubosomes. Kim et al. described that the suspensions of cubic phase nanoparticles were negatively stained with freshly prepared phosphotungstic acid solution (2%, pH 6.8) and were transferred onto a formvar/carbon coated grid (200 mesh), air dried at room temperature. The electron microphotographs were taken on an electron microscope. SEM analysis may not be performed on cubosomes or some vesicular systems since the integrity and robustness of the formulation may be lost during the procedure while exposing to electron array.

6. Pressure Ultrafiltration Method¹

Drug release measurement of cubosomes can be done by pressure ultrafiltration method. It is based closely on that proposed by Magenheimer et al. using an Amicon pressure ultrafiltration cell fitted with a Millipore membrane at ambient temperature (22±2) °C.

7. Stability studies¹

The physical stability can be studied by investigation of organoleptic and morphological aspects as a function of time. Particle size distribution and drug content can be assessed at different time intervals can also be used to evaluate the possible variations by time.

8. Visual inspection⁴

About 1 week after preparation, the dispersions were visually assessed for optical appearance (e.g., colour, turbidity, homogeneity, presence of macroscopic particles).

9. Light microscopy⁴

Samples of the prepared cubosomes were suitably diluted with deionized water and examined using an optical microscope (Lecia DMRXP) calibrated

with a micrometer slide at magnification of x 400 and x1000.

10. Entrapment efficiency⁴

For determination of entrapment efficiency (EE), it was mandated to separate free ALA from cubosome associated ALA. The amount of free drug in the dispersion was then analyzed spectrophotometrically at λ_{max} 250 nm, this was then subtracted from the total amount of drug initially added. A volume of 1 ml from each of the dispersions was diluted with 4 ml of deionized water. Then a volume of 1 ml from this diluted dispersion was further diluted with another 4 ml of deionized water. The resulting diluted dispersion was then passed through a syringe filter having a pore size of 0.1 μm . The filtrate was then analyzed spectrophotometrically at λ_{max} 250 nm. This concentration was then multiplied by the total volume of the dispersion produced, considering the dilution factor. This represented the concentration of free drug (C_f , namely that not encapsulated in cubosomes). This was then subtracted from the total drug concentration (C_t) in the formulation to give the amount of drug that was successfully entrapped inside the cubosomes. Each experiment was repeated three times.

$$\text{EE \% of cubosomes} = [(C_t - C_f) / C_t] \times 100$$

11. Viscosity⁴

The viscosity of the prepared formulations was determined at different angular velocities at 25°C using a rotary viscometer (Brookfield). The rotation speed was 20 rpm, with spin # 18. The average of three readings was used to calculate the viscosity.

12. In-vitro drug release⁴

Samples of ALA cubosome dispersions for release studies were prepared containing 50 mg ALA using both approaches. Each sample was placed in a disc and covered by a membrane then placed at the bottom of the dissolution vessel using dissolution tester type II (Varian, VK 7000). The membrane was used to retain the formula inside the disc. The dissolution medium used was 700 ml of hydroalcoholic solution (1:1). The apparatus was equilibrated to 32 $\pm$

0.5 °C and the stirrer paddle speed was set at 50 rpm. Aliquots were withdrawn at appropriate time intervals (0.5, 1, 2, 3, 4, 5 and 6 hrs) and filtered through a syringe filter having a pore size of 0.1 μm then analyzed spectrophotometrically at wavelength of 250 nm (according to the method of drug assay). The amount of drug released was calculated from the standard curve. This procedure was performed in triplicates for each formulation.

$$Q = [DmCd(2A - Cd)t]^{1/2}$$

where "Q" is the mass of ALA released at time "t", and is proportional to the apparent diffusion coefficient of the drug in the matrix, "Dm". "A" is the initial amount of ALA in the matrix, and "Cd" is the solubility of the drug in the matrix. Data from the release studies were plotted as amount released against square root of time to interrogate the release mechanism, to provide an indication of relative release rates.

7. CUBOSOME APPLICATIONS

7.1 Melanoma (cancer) therapy⁶

Recently few anticancer drugs have been successfully encapsulated in cubosomes and characterized physicochemically. The unique structure of this promising nanocarrier suggests its application in melanoma therapy. In order to specifically target nanomedicines to tumours, different approaches have been envisaged, with passive and active targeting of cancer cells having been shown to be valid approaches in preclinical and clinical studies. Passive targeting exploits the pathophysiological properties of the tumour vasculature which is generally highly disorganised with enlarged gap junctions between endothelial cells and compromised lymphatic drainage allowing for the extravasation of nanocarriers with size up to several hundred nanometres. Objects of this size cannot pass through the tight junctions that exist within the endothelial cell lining of the vessels of healthy tissues (Figure.7). Passive targeting is largely dependent on the ability of a drug nanocarrier to exhibit an increased circulation lifetime resulting in enhanced accumulation at the target site. Circulation time is dictated by the nanoparticle physicochemical properties (size, charge, biodegradability, solubility, shape,

rigidity), which can be easily manipulated in the majority of the delivery systems described. The most common modification used to evade macrophage capture and increase circulation time is accomplished by making the nanoparticle surface hydrophilic through the addition of a polyethylene glycol (PEG) coating on the surface. The majority of the nanoparticle-drug formulations used clinically and in development rely mainly on passive targeting. As a means of increasing recognition of target cells by nanoparticles, active targeting has been implemented. Active targeting utilizes specific ligands such as peptides or antibodies that bind to molecules specifically expressed or overexpressed on target cells. Thus, active targeting does not actually improve overall accumulation at the tumour site, but rather enhances cellular uptake of the particles following their passive extravasation due to the leaky vasculature. Transferrin and folate ligands are two examples of commonly used active targeting moieties in nanomedicine formulations targeting tumours. The only clinically approved actively targeted nanomedicines are antibody-drug conjugates used in the treatment of leukemias and lymphomas. Currently, no actively targeted nanoparticle formulations are approved for clinical use, with only a small number in clinical trials. Despite the ample evidence and extensive research efforts supporting the benefits of both passively and actively targeted nanomedicines in the treatment of cancer, clinically, both strategies have met with only moderate success. This is likely due to the fact that the complexity of the tumour microenvironment (tumour heterogeneity, vascularity, location) is commonly overlooked and will have a major effect on nanoparticle extravasation, accumulation, and penetration into the tumour. The tumour microenvironment is highly heterogeneous in composition with as much as half of its volume occupied by noncancerous cells and dense extracellular matrix. Furthermore, the hyperpermeable nature of the tumour vasculature, while being ideal for allowing nanoparticles to enter into tumour tissue, also allows fluid to leak from the vessel into the tumour microenvironment, thereby causing extraordinarily high interstitial pressure throughout the tumour interior. The interstitial pressure tends to increase with increasing tumour volume and remain lower in the outermost areas of the tumour. Finally, malignant cells within solid tumours tend to be tightly packed and are heterogeneous in nature. Thus, while the leaky nature of tumour vessels

can promote nanoparticle deposition and accumulation, the microenvironment creates a number of barriers that prevent these delivery systems from effectively accessing tumour cells and thus reaching their full potential as the 'silver bullets' of anticancer therapies. Comparison of drugs with and without cubosomes refer table no 2.

7.2 Oral drug delivery¹

Cubosomes address the varied challenges in oral delivery of numerous promising compounds including poor aqueous solubility, poor absorption, and large molecular size. In an alternative application large proteins have been encapsulated for local activity in the gastrointestinal tract. Liquid crystalline nanoparticles technology carriers can be combined with controlled release and targeting functionalities. The particles are designed to form *in situ* in a controlled rate, which enables an effective *in vivo* distribution of the drug. Cubosomes technology carriers can also be released at different absorption sites, for example in the upper or lower intestine, which is important for the drugs that have narrow regional absorption window.

7.3 Intravenous drug delivery systems¹

Lipid nanoparticles comprising interior liquid crystal structures of curved lipid membranes are used to solubilize encapsulate and deliver medications to disease areas within the body. While emulsions and liposomes have found use as intravenous carriers in drug products, liquid crystal nanoparticle structures increased payloads of peptides, proteins and many insoluble small molecules, and are ideal carriers for injection or infusion of many actives.

7.4 Topical drug delivery systems¹

Cubic phases are more bioadhesive in nature, so that they can conveniently be used in topical and mucosal depositions and delivery of different drugs. Topical delivery systems are based on the exploitation of unique properties of liquid crystal (LC) and liquid crystal nanoparticle (LCNP) technologies. Topical drug delivery systems are unique *in situ* forming bioadhesive LC systems facilitate controlled and effective drug delivery to mucosal surfaces (buccal, ophthalmic, vaginal and others). This fascinating system forms a thin surface film at mucosal surfaces consisting of a liquid crystal matrix which nanostructure can be controlled for achieving an optimal delivery profile and provides good temporary protection of sore and sensitive skin.

7.5 Drug delivery vehicle

Drug delivery vehicle is a common application for such new materials. The rapid expansion of the life-sciences industry is expected to drive previously "exotic" delivery vehicles and ingredients into broader marketplaces, such as personal care and consumer products. Consequently, self-assembled surfactant phases have been extensively examined for compatibility with numerous medical active ingredients and their applications. The number of research in association with cosmetic companies like L'Oreal and Nivea are trying for the use of cubosome particles as oil-in-water emulsion stabilizers and pollutant absorbents in cosmetics. Moreover, these researches have also discovered that a second amphiphile, phytantriol (Fig. 13), has an aqueous phase behavior sufficiently close to that of monoolein to form cubosomes under similar conditions.

7.6 As sustained release behavior

Even more recent patent activity by points to cubosome use in personal care product areas as varied as skin care, hair care, cosmetics, and antiperspirants. Despite recent activity, there remains a lack of the practical elements like manufacturing scalability and material customization that is necessary to lead formulators to consider using cubosomes in commercial products. The cubic phase has been shown to provide a vehicle for several *in vivo* delivery routes, including depot, transdermal, mucoadhesion and ophthalmic. Because of fusogenic property of monoolein it increases the penetration of macro molecules. A wide variety of drugs with different physicochemical properties have been incorporated in cubosomes, and their sustained release behavior was also studied. Sustained behavior of cubosomes was because of cubosome remnant particles. Monoglyceride based cubosome dispersion can be proposed for topical use, such as for percutaneous or mucosal applications.

7.7 In treatment of viral diseases

Because of the microbicidal properties of monoglycerides, could be used to design intravaginal treatment of sexually transmitted diseases caused by viruses (e.g. HSV, HIV) or by bacteria (e.g. *Chlamydia trachomatis* and *Neisseria gonorrhoeae*). Due to similarity between the cubic phase structure and the structure of the stratum corneum, it is reasonable to suppose the formation of mixture of cubosomal monoolein with stratum corneum lipids. This kind of interaction might lead to the formation of a cubosome depot in this layer, from which drug can be released in a controlled

fashion. The cubosome technology is used to develop a synthetic vernix– the cheesy white substance that coats infants in late gestation – to help premature infants who are born without it. The vernix is a complex mixture of lipid (fats), proteins and water. It is formed late in gestation and has an integral role in normal skin development.

7.8 In topical and mucosal depositions

Cubic phases are more bioadhesive in nature, so that they can conveniently used in topical and mucosal depositions and delivery of different drugs.

7.9 Controlled-Release Drug Delivery⁷

Controlled release of solubilized actives is the most popular application pursued by cubosome researchers, and excellent reviews exist of attempted delivery applications as well as pharmaceutical actives that have been solubilized in bulk cubic phase and cubosomes. Cubic phase is attractive for controlled release because of its small pore size (5–10 nm); its ability to solubilize hydrophobic, hydrophilic and amphiphilic molecules; and its biodegradability by simple enzyme action. Cubic phase is strongly bioadhesive and is thought to be a skin penetration enhancer, suggesting excellent compatibility with topical and mucosal deposition and delivery of active ingredients. Recent studies have emphasized similarities between the bicontinuous structures formed in human skin layers and those comprising cubic phases, offering the promise of better skin transport understanding and treatment. The tortuous structure of cubic phase lends itself well to slowing diffusive release of solubilized actives. Theory predicts the minimum reduction of a solute's free solution diffusivity by 33%. Experimental measurements of small molecule diffusivity in cubic phases give values on the order of 10^{-10} m²/sec. No commercial applications of cubic phase delivery vehicles are known other than a treatment developed for periodontal disease that is based on triglyceride–monoolein mixtures combined with the drug metronidazole. The lipid–drug mixture forms a low-viscosity liquid that, when applied to the gums and placed in contact with saliva, hydrates to form a bulk cubic phase that then delivers the drug to the gum. Despite the potential of bulk cubic phase as a delivery vehicle, some applications are not compatible with the extremely high viscosity of the bulk cubic phase and require the use of cubosomes. Although the above controlled-release limitations exist for small molecule solutes and

unmodified cubosomes, other routes may still exist for controlled-release applications of cubosomes.

For example, large poly(amidoamine) dendrimer molecules exhibit a 100% reduction in free diffusivity when entrapped in cubic phases. Modification of the cubic phase environment is also a potential method of regulating solute transport. Puvvada et al. find that aqueous alginate molecules can be gelled in cubic phase pores by ionic strength changes, increasing local viscosity and drastically slowing active release.

Other studies have found that the partitioning and release kinetics of ionic solutes in cubic phases are significantly altered by incorporating oppositely charged surfactants into the cubic bilayers. In such cases, the functionalizing surfactant must be chosen carefully to avoid disturbing the cubic phase structure, but guidelines exist for additive selection. The contorted but regular structure of the cubic phases is also of interest to material science researchers as a template for complex solid materials.

7.10 In Materials Synthesis⁷

From a materials science perspective, the creation of ordered structures with nanoscale pore geometries is of great interest to numerous fields including electronics, photonics, catalysis, and medicine. The creation of solid structures using cubic phases as a template usually entail either polymerization or reaction to form solids from precursors that are solubilized in, or comprise, the cubic phase matrix. One of the earliest and most successful materials formed in a cubic phase template is the aluminosilicate zeolite MCM-48, used for catalytic processing of petroleum. Yang et al. successfully carried out polymerization inside cubosomes, yielding a solid nanostructured particle with cubic symmetry. Such particles hold promise for use in photonic and semiconductor applications. Lu et al. have developed novel aerosol processes that create particles with nanometer-scale structure by evaporation of solvent from isotropic phase liquid droplets, simultaneously driving them into cubic phase structures and solidifying the particles. As sophistication in the cubic phase template area builds, optimization of the structures will be a leading interest area. Along these lines, Larson suggests the possibility of aligning the cubic phases by steady or large-amplitude oscillatory shearing prior to templating, forming materials with unique and highly anisotropic properties.

7.11 As biologically active substances⁸

Cubic phases were produced at 25°C in water monoolein-alcohol mixtures. Ethanol was found to be more efficient than propanol and butanol. In the composition range of 49 to 56 wt% water, 31 to 40 wt% monoolein and 10 to 13 wt% ethanol we identified a new transparent, low-viscosity (flowing) phase that we called OL. No structures were found by bright field light microscopy and polarized light microscopy, indicating that OL is an isotropic phase. Cryo-TEM showed large domains of this ordered phase, which by Fast Fourier Transformation was identified as cubic phase. The symmetry was also confirmed by SAXS. Bioactive compounds were incorporated into the OL phase, and the phase was then dispersed into cubosomes of 100 – 250 nm in diameter by homogenization, in the presence of Pluronic 127 as the stabilizing agent. Several guest molecules were solubilized, including drugs (diclofenac and carbamazepin) and nutraceuticals (phytosterols, lycopene, and coenzyme Q10). Typically, only trace amounts of a given bioactive compound could be solubilized in the cubosomes before the ordered phase was broken and vesicles formed. However, synergistic effects that significantly increased the loading and the order of the nanovehicles were found, when two guest molecules of different character were solubilized together.

7.12 Current application

1. An application area under current development by L'Oréal is the use of cubosome particles as oil-in-water emulsion stabilizers and pollutant absorbents in cosmetics.
2. In melanoma therapy.

8. PHARMACEUTICAL PREPARATIONS ENCLOSING CUBOSOMES²

Refer table no: 3

9. CONCLUSION

Cubosome nanoparticles formed from cubic liquid crystalline phases are a unique and intriguing self-assembled material with enormous potential in areas as diverse as medicine, materials science, and consumer products. The relatively recent discovery of cubosomes has spurred a broad level of investigation that, as proposed applications become financially attractive, will continue to narrow and fill in many of the current gaps in our knowledge of cubosome formation and performance. Interdisciplinary research in engineering, biology, medicine, and chemistry will be especially crucial to tie together existing

cubosome research and to provide a consistent understanding of these fascinating particles. The use of nanomedicines in localised drug delivery has received a lot of attention over the past couple of decades and resulted in several clinically approved formulations. These systems have been shown to have a number of advantages over conventional chemotherapeutics; however, they have not yet reached their full potential as anticancer agents. This is likely due to the fact that until more recently, features of the tumour microenvironment that can create barriers to

effective nanoparticle delivery have been largely overlooked. With improved understanding of how the tumour microenvironment affects nanoparticle delivery and distribution within tumours, strategies can be developed to better address and overcome the shortcomings of current delivery systems. Thus, future anticancer therapies using nanomedicine can be envisioned to specifically kill all cancer cells within the tumour while leaving normal tissue in the body virtually untouched.

Table 1: Composition of ALA cubosome dispersion

Dispersion	GMO (% w/w)	P407 (% w/w)	Ethanol (% w/w)	Water (% w/w)
D1 ^a	5.0	1.0	-	94.0
D2 ^b	5.0	1.0	5.0	89.0
D3 ^b	10.0	1.0	5.0	84.0
D4 ^b	15.0	1.0	5.0	79.0
D5 ^b	15.0	2.5	5.0	77.5
D6 ^b	15.0	5.0	5.0	75.0

a: Top down technique b: Bottom up technique

Table 2: Comparison of drugs with and without cubosomes

Drug alone	Drug enclosed in cubosomes
Fail to distinguish normal cells from cancer cells	Distinguishes normal cells from cancer cells.
Low efficacy.	More efficacy.
Less biodistribution.	More biodistribution.
Severe toxic side-effects.	Reduced side-effects.
Affect healthy tissues.	Prevent affecting healthy tissues.
Eg: Cisplatin	Eg: Doxorubicin
Tamoxifen	

Table 3: List of drugs incorporated in cubosome for sustained drug delivery

S. No.	Researcher	Drug	Category	Associated Disease
1	Engstrom et al.	2-amino-1-phenylpropanol HCl	Antidepressant	Mania, depression
		Nitroglycerin	Anti-anginal	Angina pectoris
		Oestriol	Hormonal therapy	Atrophic vaginitis, pruritus
2	Sadhale et al.	Cefazolin	Antibiotics	Genito-urinary, respiratory tract infection
		Cefuroxime	Antibiotics	Meningitis, bone and soft tissue infection
		Prilocaine	Local anesthetic	In Dentistry
3	Damani	Clindamycin phosphate	Antibiotics	Peritonitis, staphylococcal bone and joint infection
4	Engstrom et al.	Clomethiazole	Psychotropic	Insomnia
5	Engstrom et al.	Clotrimazole	Antifungal	vagina, mouth, and skin infection
6	Engstrom et al.	Gramicidin	Topical steroid	Corticosteroid sensitive dermatoses
		Insulin	Hypo/Hyper glycaemics	Diabetes mellitus
7	Nielsen et al.	Indomethacin	NSAIDs	Gout, rheumatoid arthritis
		Isosorbide mononitrate	Anti-anginal	Angina pectoris
		Lidocaine hydrochloride	Oral preparation	Fungal infection of external ear
8	Boyd	Diazepam	Sedative-hypnotic	Anxiety, insomnia, seizures
		Rifampicin	Bactericidal antibiotic	Tuberculosis
		Griseofulvin	Antifungal	Fungal infection of skin
		Propofol	Hypnotic	Procedural sedation, to induce and maintain General
				Anesthesia

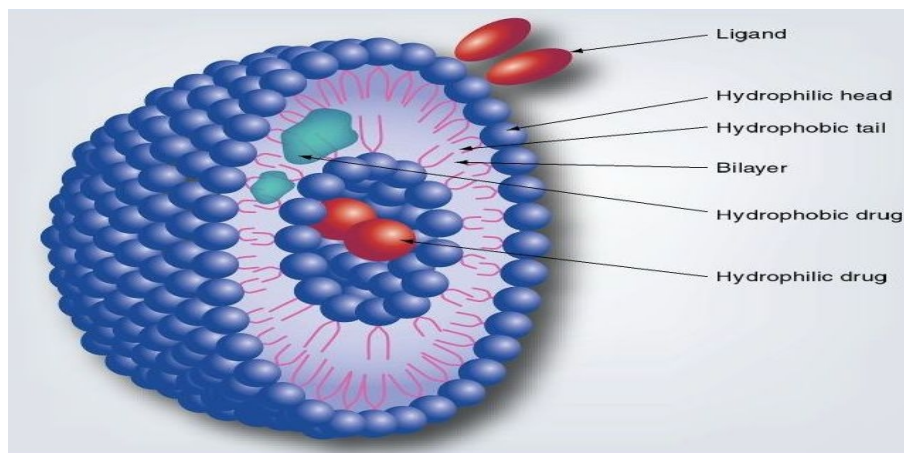


Fig. 1: Honeycombed structure separating two internal aqueous channels

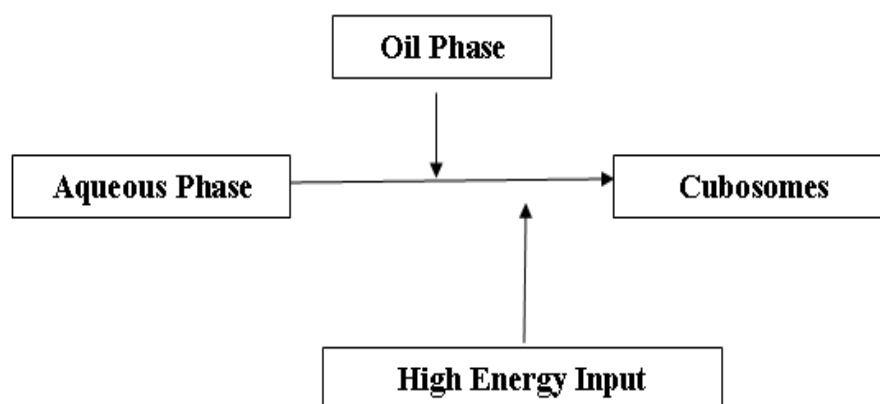


Fig. 2: Illustration of the top-down approach⁴

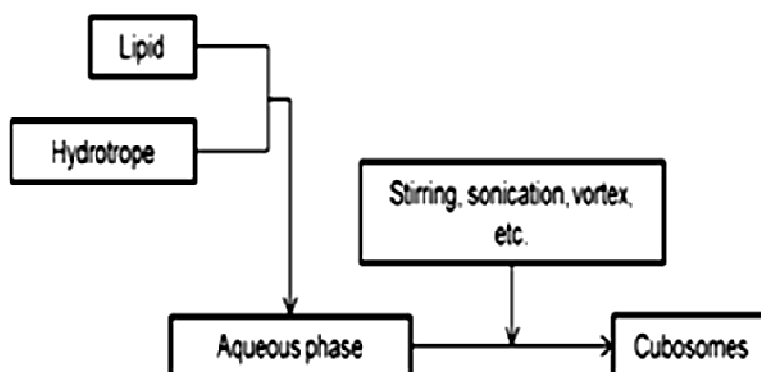


Fig. 3: Illustration of the bottom-up approach⁴

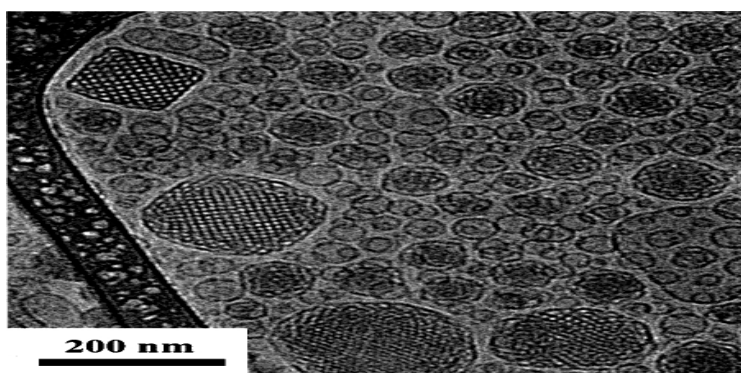


Fig. 4: Cryo-transmission electron micrograph of cubosomes⁵

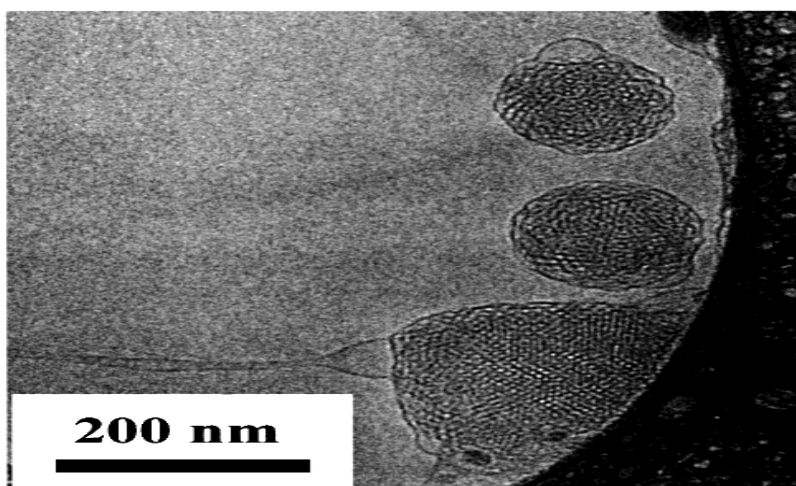


Fig. 5: Cryo-TEM of cubic gel containing ethanol hydrotrope

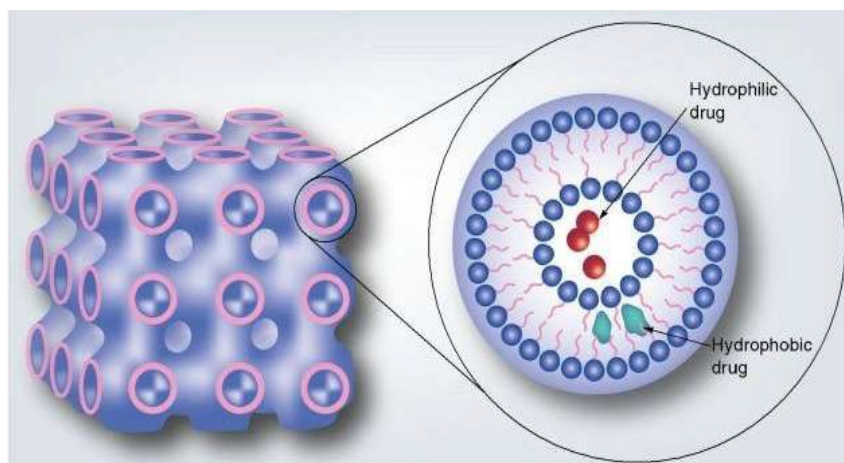


Fig. 6: Cubosomes with different drug loading modalities

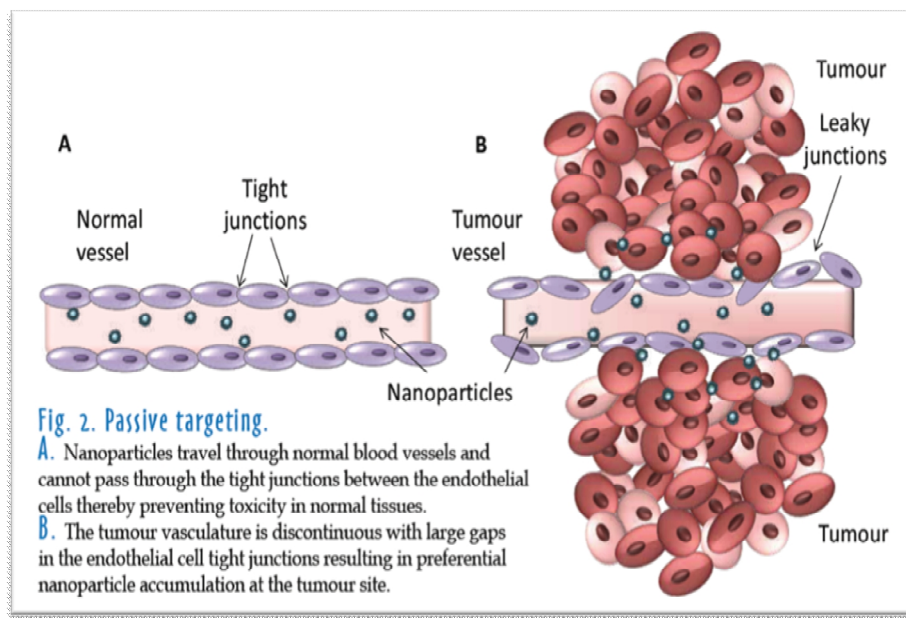


Fig. 7: Action of cubosome incorporated drugs on tumors

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