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Review Article

OVERVIEW OF CUBOSOMES: A NANO PARTICLE

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ABSTRACT

Cubosomes consist of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. Self-assembled cubosomes as active drug delivery systems are receiving more and more attention and interest after the first discovery and nomination. They exhibit different internal cubic structure and composition with different drug-loading modalities. Overall, cubosome 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. The preparation mostly involves simple emulsification of monoglyceride and a polymer, accompanied by sonication and homogenization. The preparation methods fall into two categories, including top-down and bottom-up techniques. The aim of review article includes manufacturing techniques, system forming cubic phase, mechanism, applications of cubosomes.

Keywords: cubosomes, top-down and bottom-up techniques, bioactive agents.

INTRODUCTION

Lipids, surfactants and polymer molecules have both polar and non polar components are termed as amphiphilic.The hydrophobic effect drives amphiphilic molecules in polar solvents to spontaneously self assembling in to a array of thermodynamically stable liquid crystalline phases with lengths on nanometer scale. These liquid crystalline phases possess a sufficient average degree of molecular orientation and structural symmetry. An interesting example is the bicontinous cubic liquid crystalline phase.

Bicontinous cubic phases are optically isotropic, very viscous, and solid like liquid crystalline substance with cubic crystallographic symmetry. Bicontinous cubic phases consist of two separate, continuous but nonintersecting hydrophilic regions divided by a lipid bilayer in to a periodic minimal surface with zero curvature. The bi continuous nature of such cubic phases differentiate them from the so-called micellar or discontinuous cubic containing micelles packed in cubic symmetry. A special property of the cubic phases formed by certain classes of amphiphiles is their ability to be dispersed in to particles, termed as cubosomes. This chapter reviews about the historical research of cubosomes, describing the initial discovery and its differential geometry. Cubosome properties are reviewed in the context of structure of cubosome and applications.

CUBIC PHASE STRUCTURE AND ITS MECHANISM

Cubosomes are nanoparticles whose size ranges from 10-500nm in diameter (fig.1) they appear like dots square shaped, slightly spherical. Each dot corresponds to the

presence of pore containing aqueous phase cubic phases in lipid water system in X-ray scattering technique was first identified by Luzzati & Husson.

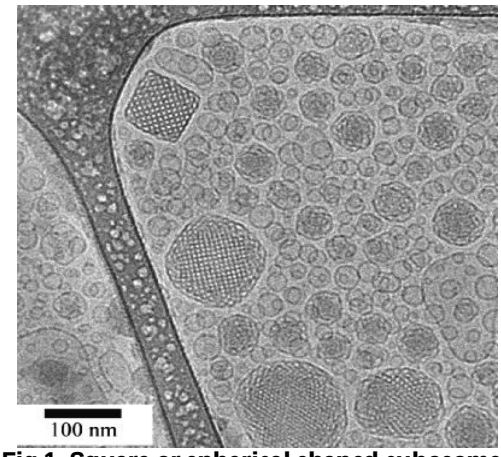

Fig.1: Square or spherical shaped cubosomes

According to Fontell & Drew ternary systems of amphiphiles, oil & water, some mono glycerides will exhibits cubic phases. Monoglycerides are polar lipids, having poor water solubility that exhibits aqueous phase behaviour, which are structurally mimicking to non-ionic surfactants. Lutton results the monoglycerides whose hydrocarbon chain lengths between c12 and c22 (Fig.2) of all the monoglycerides, particularly monoolein exhibits larger region of cubic phase. Monoolein is unsaturated, c18 monoglyceride.

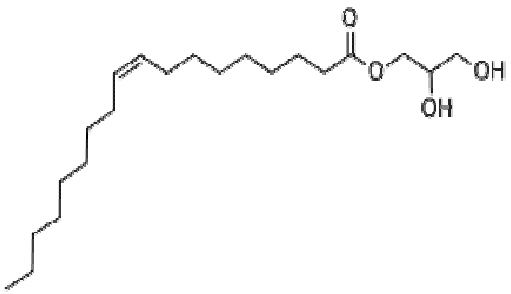

Fig. 2: Monoolein structure

MONOOLEIN PROPERTIES

Melting point: 35-37 °c
FEMA: 2526
Flash point: 180 °c
Storage temp: −20°c
Solubility: chloroform: 50 mg / ml clear, colorless

Cubic phases are often founded sandwiched between lamellar and hexagonal liquid crystalline phases, especially in non ionic surfactant systems. The monoolein-water system uniquely possess a cubic phase region contains broad compositional and temperature range. But surfactant packing concepts are more approaching. Normally monoolein has continous hydrophilic headed, hydrophobic tail end, producing reversed or inversed cubic phases, indicating the phases towards polar medium .so that the cubic phase structures can be described using the concept of differential geometry and periodic minimal surfaces. Minimal surfaces are best described by analogy with soap films. Based on their curvatures, 3 types of minimal surfaces are studied in cubic phases was discovered mathematically by Schwarz.

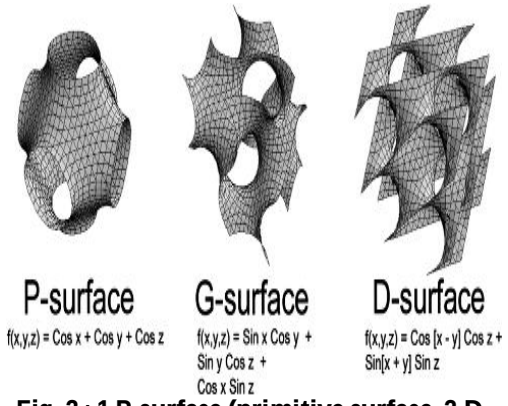

Fig. 3 : 1.P-surface (primitive surface, 2.D-surface (diamond surface), 3.G-surface (gyroid surface)

The monoolein water system forms the D-surfaces at high water levels, and the G-surface at lower levels. The p-surface is formed in the monoolein water system, but only when third component such caseins or amphiphilic block copolymer are added. In some cases the cubic phase can exist in a particulate form possessing the same bicontinous structure as the bulk cubic phase. Normally, using copolymers like poloxamer407-monoolein-water system because of the polymers utility it provides colloidal stability to cubosomes by re coalescence to bulk cubic phases.

Poloxamer (peo_{99}-ppo_{67}-peo_{99}), the ppo copolymer exists either at surface of cubic phase particle or with in bilateral structure, where as the peo chain remains in the bulk

water phase resulting in the formation of 3 phased regions, disordered bicontinous phase, cubic phase, water phase.

Jacob & Anderson gave cubic phases existed can be measured by X-ray scattering technique. Transmission electron microscopy (TEM), freeze fracture electron microscopy is used to visualize the cubosomes.

According to Gustafsson et al., cubosomes are single crystal structures, with visible uni lamellar vesicles, dispersed lamellar liquid crystalline phase particles. Increasing polymer-to-monoolein ratios leads to formation of larger vesicles. ulttrasonication of bulk cubic phases produces mostly vesicles on due course of time they get trace formed into cubosomes via membrane fusion such meta stability is characteristic of cubosome systems because of slow transport processes involved in forming high viscous crystalline structure and high energy is required to fragment them(bulk cubic phase).vesicles also give colloidal stabilization of cubosomes.

LIQUID CUBOSOME PRECURSORS

In view of 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 can be expensive, difficult to scale-up, and harmful to fragile temperature-sensitive active ingredients like proteins. In some product applications, it is also advantageous for cubosomes to form only upon use, such as during hand washing or mouth rinsing. A strong driving force exists for development of a liquid phase precursor to cubosomes to avoid high-energy processing and produce them in situ. The hydrotrope dilution process is found to consistently produce smaller, more stable cubosomes. In concept, particles are formed by nucleation and growth, as employed in crystallization and precipitation processes. This is achieved by dissolving the monoolein in a hydrotrope, such as ethanol, that prevents liquid crystalline formation. Subsequent dilution of this mixture spontaneously “crystallizes” or precipitates the cubosomes. This is all done without the need for 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.

POWDERED CUBOSOME PRECURSORS

Powdered cubosome precursors are powders composed of dehydrated surfactant coated with polymer. Such powders offer some process and performance advantages to liquid phase hydrotropic 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. The lipids used to make cubosomes are waxy, sticky Solids, rendering them unable to form small discrete particles. It is found that a water-soluble non-cohesive starch coating on the waxy lipid prevents agglomeration and allows control of particle size. Spray drying 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. The continuous and dispersed phases are sprayed through a nozzle to create suspension droplets that are contacted with a heated, dry air stream flowing in the opposite direction. 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. Further, the process provides an easy route to preload active 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.

The liquid feed to the spray-dryer can be tailored to adjust the resultant powder properties. The production of starch-coated cubosome powder precursors requires high shear treatment of monoolein in aqueous starch solution to form a coarse cubosome dispersion that is then pumped through a nozzle and dried. Full operating conditions are given in Spicer et al. (2002a). The initial composition pumped into the spray-drier is 60% w/w water, 30% starch, and 10% monoolein. Drying removes almost all water present and gravimetric tests of the powder generally indicate a final composition of about 4% w/w water, 72% starch, and 24% monoolein in the product powders. Although the relative fraction of starch is high (3:1 starch : monoolein), the level is necessary to preserve powder quality. Figure 5 compares SEM

photographs of starch-encapsulated monoolein with a 3:1 (left hand side) and 1:1 (right hand side) starch : monoolein ratio. The powder with the 3:1 ratio exhibits good encapsulation of the monoolein and small particle size. However, the 1:1 ratio exhibits poor morphology and larger particle size. In the latter case, the bulk powder is more cohesive as a result of poor encapsulation of the sticky monoolein by the starch.

The production of starch-coated powder precursors from a hydrotropic solution of monoolein emulsified in water makes the spray-drying step easier. Ethanol is known to act as a hydrotrope and dissolve viscous cubic liquid crystalline phase to form a low-viscosity liquid and ease processing. Re-application of the hydrotrope effect in the spray-drying process avoids formation of a dispersion of cubosomes and eases spray drying. However, other changes in the formula are also required to accommodate the ethanol. A new polymer is needed for encapsulation of the monoolein, as the insolubility of starch in ethanol prevents its use. A useful alternative to the starch is dextran. The material to be spray-dried contains 37.5% water, 25% dextran, 22.5% ethanol, and 15% (w/w) monoolein. The quaternary system is prepared by first dissolving the dextran in the water and the monoolein in the ethanol. Thereafter the two solutions are combined and mixed. Once mixed, the quaternary system forms an emulsion of two distinct phases. One phase is optically isotropic and the other is optically birefringent. The emulsion of both phases has low viscosity and is easily spray dried.

The type of encapsulating starch also affects powder quality. Drying occurs as the dispersion is sprayed into droplets and moisture rapidly evaporates by convective heating. The cubosomes in the dispersion form the nucleus of many of the sprayed droplets, surrounded by aqueous starch solution. As drying proceeds, the starch remains and forms a coating on the cubic gel particle, thereby encapsulating it. Because the cubic phase itself contains 40% (w/w) water, some drying must also occur at the core of the particles. Low molecular weight starches (84,000 MW) produce superior powders when compared to those made using high (335,000 MW) molecular weight starches. Spicer et al. (2002b) provide a more comprehensive listing of feasible polymers and other materials for use as polymeric coatings to encapsulate cubosomes.

The application of the hydrotrope method to spray-drying for production of cubosome precursors significantly eases processing. Thermal gravimetric analysis indicates the presence of 16% (w/w) volatile materials remaining in the powders following drying. Of this fraction, 3% is water and 13% is ethanol. The volatile content remains constant for several months, indicating good encapsulation of both the ethanol and the monoolein by the dextran. Depending on the application, the powders can be produced with varying amounts of ethanol by tailoring the film properties of the polymer in order to take advantage (during hydration) of the nucleation of small cubosomes from monoolein-ethanol solution.

The large proportion of polymer required for encapsulation (~75% w/w for starch and ~60% for dextran), limits the amount of active material incorporated for subsequent delivery. Assuming (as an upper feasible limit) a 10% w/w dispersion of starch-stabilized cubosomes is desired and a 1:1 ratio of monoolein-to-active is used, the maximum weight percent of active in the dispersion is 1.25%. Such a low level is useful only for high value-added materials like pharmaceuticals, vitamins, flavors, or scents. The process described demonstrates the feasibility of forming dry powders with the ability to form cubosomes upon hydration.

MANUFACTURE OF CUBOSOMES

Cubosomes can be manufactured by two distinct methods

1. Top down technique
2. Bottom up technique

TOP DOWN TECHNIQUE

It is the most widely used in research area, where by bulk cubic phase is first produced and then dispersed by high energy processing in to cubosomes nanoparticles. Bulk cubic phase is resembling a clear rigid gel farmed by water swollen crossed linked polymer chains, where as cubic phases are like liquid crystalline structure. The cubic phases exhibits a 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, where as 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. 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.

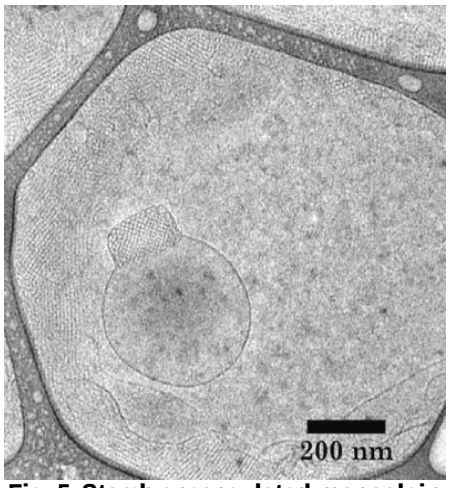

Fig. 5: Starch encapsulated monoolein particles by cryo-(TEM)

BOTTOM UP TECHNIQUE

In this cubosomes are allowed to form or crystallize from precursors. Almgren et., al. discuss the formation of cubosomes by dispersing L_2 or inverse micellar phase droplets in water at 80ºc,and allow them to slowly cool, gradually droplets get crystallizes to cubosomes. This is more robust in large scale production of cubosomes.

Spicer et., al developed 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.

FORMULATION 1
Cubosome dispersion formed by dilution of an isotropic solution

S. No.	INGREDIENT	WT. PERCENT
1.	Monoolein	10
2.	Ethanol	5
3.	Water	1.8
4.	Poloxamer 407	1
5.	Water	82.2
	Total	100

MIXING INSTRUCTIONS

Weigh the Part A ingredients into a suitable vessel equipped with a mixer. The materials form a clear, low viscosity isotropic liquid. Combine the Part B ingredients into a separate vessel and stir until all polymers are dissolved. Inject Part B solution into Part A and mix only as much as needed to produce cubosomes of the desired size. A colloidally stable dispersion of cubosomes forms.

FORMULATION 2
Powder cubosome precursor

S. No.	INGREDIENT	WT. PERCENT
1.	Monoolein	10
2.	HICAP 100 Starch	30
3.	Water	60
	Total	100

MIXING INSTRUCTIONS

Weigh the Part A ingredients into a suitable vessel equipped with a high-shear mixer. Upon shearing the materials will form a relatively low viscosity coarse dispersion of cubosomes. Spray-dry to produce a powder with a final composition of about 4% w/w water, 72% starch, and 24% monoolein.

FORMULATION 3
Bulk cubic phase gel for skin treatment

S. No.	INGREDIENT	WT. PERCENT
1.	Monoolein	57
2.	Glycerin	5
3.	Water	38
	Total	100

MIXING INSTRUCTIONS

Melt the Part an ingredient into a suitable vessel by heating above 40° C and stirring. In a separate vessel, mix the Part B ingredients until completely homogeneous. Add the Part B ingredients to the Part A ingredients and mix well. The mixture forms a very viscous, clear gel that needs to be mixed well to ensure uniform incorporation of all Part B ingredients.

SYSTEM FORMING CUBOSOMES

The system forming cubosomes is possible in binary and ternary systems with a sufficiently large miscibility gap between the cubic phase and the solvent. Colloidal stabilization of cubosomes is good when poloxamer 407 is used to provide stabilization against aggregation and coalescence. Cubosomes can be coated with lamellar bilayered 'caps' covers the cubic bilayer opening which is formed by fragmentation which prevents the exposure of hydrocarbon chains to water and provides colloidal stability. Coating of cubosomes with solid crystalline bilayers provides more colloidal stability, where as lamellar liquid crystalline will give rigid coatings. In addition coating of the sponge phase have been proposed as a stabilizing coating for cubosomes. The following figures will shows two general forms of the ternary phase diagrams exhibited by system forming cubosomes. Another molecule with great potential for cubosome formation is phytantriol.

FORMATION OF CUBOSOMES AS VEHICLES OF BIOLOGICALLY ACTIVE SUBSTANCES

Cubic phases were produced at 25 ºC 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% monooleine and 10 to 13 wt% ethanol we identified a new transparent, low-viscosity (flowing) phase that we called O_L. No structures were found by bright field light microscopy and polarized light microscopy, indicating that O_L is an isotropic phase. Cryo-TEM showed large domains of this ordered phase, which by Fast Fourier Transformation was identified as a cubic phase (Figures A and B). The symmetry was also confirmed by SAXS.

Bioactive compounds were incorporated into the O_L phase, and the phase was then dispersed into cubosomes of 100 – 250 nm in diameter by homogenization, in the presence of Pluronics 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 where found, when two guest molecules of different character were solubilized together.

APPLICATIONS OF CUBOSOMES

1. Control release of solubilised substance is the most popular application of cubosomes.
2. Cubic phase is more applicable for control release because of its small pore size (5-10nm), ability to solubilise hydrophilic, hydrophobic, amphiphilic molecules and its biodegradability by simple enzymes.
3. Cubosomes are most widely used in melanoma (cancer) therapy.
4. Cubic phases are more bioadhesive in nature, so that they can conveniently used in topical and mucosal depositions and delivery of different drugs.

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