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CUBOSOMES: A BICONTINUOUS CUBIC CRYSTALLINE PHASE

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ABSTRACT: Several liquid crystalline phases are bicontinuous cubic, reversed hexagonally, or reversed cubic phase, and several nanostructure liquid phases. Of the many liquid crystalline phases, bicontinuous cubic phases possess a special status. Dispersed particles of bicontinuous cubic liquid crystalline phase (cubosomes) are self-assembled nanostructured particles that can be formed in aqueous lipid and surfactant systems. Cubosome dispersions are thermodynamically stable, bioadhesive, and biocompatible, because of their properties, they are versatile systems, administrable by different ways such as orally, percutaneously, and parenterally. The discovery of cubosomes is a unique story. Despite the early realization of their potential, the manufacture of cubosomes on a large scale faced difficulty because of their complex phase behavior and viscous properties. The cubosome advantage is related to the simple production procedure and chemico-physical stability. Concerning the liposome, cubosome possesses a larger ratio between the bilayer area and the particle volume and larger breaking resistance. Cubosome structure studied using electron microscopy, "light scattering," x-ray and "NMR." This review focus on liquid crystalline phase and cubosomes (bicontinuous cubic shape nanoparticles) in detail.

Keywords: Liquid crystalline phase, Bicontinuous cubic phases, Cubosomes

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INTRODUCTION: Surfactants, lipids, and polymer molecules that have both polar and nonpolar components are termed amphiphilic [1], liquid crystalline phases share features from both liquids and crystalline substances. The liquid state is associated with the ability to flow, whereas the solid state is characterized by an ordered, crystalline structure. Crystalline solids exhibit short as well as long-range order about both position and orientation of the molecules.

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Liquid crystal materials generally have several common characteristics, among these are rod-like molecular structures, rigidness of the long axis, and strong dipoles and/or easily polarizable substituent, the distinguishing characteristic of the liquid crystalline state is the tendency of the molecules (mesogens) to point along a common axis, called the director [5]. The characteristic orientational order of the liquid crystal state is between the traditional solid and liquid phases, and this is the origin of the term mesogenic, so, also called as "mesophases" [2].

The tendency of the liquid crystal molecules to point along the director leads to a condition known as *anisotropy*; the term means that the properties of a material depend on the direction in which they are measured. Liquid crystalline nanoparticles possess nanocavities (an aqueous medium which is

separated by lipid bilayers) [3, 4]. Liquid crystals are typically organic molecules, ranging from small molecules, *i.e.*, detergents, to polyelectrolytes, *i.e.*, DNA; vegetable gums [7]. Liquid crystals can be classified as thermotropic and lyotropic crystals. *Thermotropic liquid crystals* exhibit phase transitions between the liquid crystalline phases with the changes of the temperature whereas *lyotropic liquid crystals* have phase transitions between the liquid crystalline phase as the temperature and the concentration of the amphiphilic molecules from which they have been formed are changed [6]. Lyotropic liquid crystals can be mainly classified into lamellar phase, cubic phase and hexagonal phase according to their different internal structures, among them cubic phase is the most important and recently have received much attention due to their highly ordered internal structures, which offers the potential as a slow release matrix for active pharmaceutical ingredients with various sizes and polarities [8, 9].

Cubic liquid crystalline phase forms via the self-assembly of certain surfactants that are combined with water and polar lipid in the proper ratio. Their unique structure makes them biologically compatible and capable of controlled release of solubilized active ingredients like drugs and proteins. Cubic liquid crystals are transparent and isotropic phases that are physically stable in excess water representing a unique system for the production of pharmaceutical dosage forms. The cubic phase structure provides some interesting and noteworthy properties that are (i) it forms spontaneously, (ii) has a gel-like texture, and (iii) has a high lipid bilayer/water interfacial area of ~400-600 m2/g lipid [10 -12].

At higher concentrations, liquid crystals are formed by polar lipids, such as GMO (glyceryl monooleate) or GME (glyceryl monooleyl ether), and water, where both components diffuse freely [35]. What structure is formed depends on the polar/non-polar shape of the lipid, and the amount of water present [36]. One application of cubic phase liquid crystals is the controlled release of selected water- and oil-soluble molecules [13]. Amphiphilic molecules form bicontinuous water and oil channels, where "bicontinuous" refers to two distinct (continuous, but non- intersecting) hydrophilic regions separated by the lipid bilayer. This allows for simultaneous incorporation of water and oil soluble materials as well as amphiphiles. The phase structure provides a tortuous diffusion pathway for controlled release and also lipid-based cubic phase liquid crystals are found to be biocompatible, digestible, and bioadhesive [14].

Molecules have higher polar groups, together with high water binding capacities forms cubic phases ("balls"). Bicontinuous cubic phases are optically isotropic, highly viscous, and solid like liquid crystalline substance with cubic crystallographic symmetry. The bicontinuous nature of such cubic phase differentiates them from the so-called micellar or discontinuous cubic containing micelles packed in cubic symmetry [15]. The structure of cubic mesophases is unique and comprises a curved bicontinuous lipid bilayer (with an estimated thickness of 3.5 nm) [8].

Information from the Past Related to the Cubic Phase: Luzzati and Husson [16] and Luzzati *et al.*, [17] reported the existence of cubic phases in lipid-water systems using x-ray scattering measurements. Fontell *et al.*, [18] drew similar conclusions regarding cubic phases in ternary systems of amphiphiles, oils, and water. In parallel, although without apparent awareness of the lipid work, biologists began documenting structures with cubic symmetry in plant leaf plastid membranes,[19] identical to what is now accepted as cubic liquid crystalline phase structures.

Around the same time, Lutton [20] published a comprehensive study of the aqueous phase behavior of monoglycerides. Kare Larsson is unanimously credited with discovering that, these phases can exist as dispersed particles as well as in bulk [21], an observation made from studies of human fat digestion [22].

Types: There are 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 a cubic phase in response to a stimulus, such as contact with liquid [23].
- The bulk phase is commonly a clear, viscous, semi-solid gel that is similar in

appearance and rheology to cross-linked polymer hydrogels [24]. Its high viscosity makes it difficult to handle and limits its application and, furthermore, the bulk phase can cause the irritation reaction when in contact with the biological epithelia [25].

- To overcome these issues, an innovative strategy has been formulated: to disperse the bulk phase into the water in the form of small particles; the *dispersed cubic particles* are denoted as 'cubosomes,' which can stably exist in equilibrium with an aqueous solution with the internal bicontinuous structure unchanged [26, 27].

Cubosomes: Cubosomes are nanoparticles whose size ranges from 10-1000 nm in diameter; 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 [15]. These are self-assembled from aqueous surfactant systems where the mesogens form bicontinuous cubic phases and typically discrete, submicron, nanoparticles. These nanostructured particles are liquids instead of solid [28]. The controlled release application of these nanoparticles is of great significance in cosmeceutical and pharmaceutical fields.

History: Despite the early recognization (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 colloidally and thermodynamically stable for longer period. 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" was coined by Larsson that reflects the cubic molecular crystallography and similarity to liposomes. The effort to develop scalable processes to produce cubosomes on a large scale is under development. A few anticancer drugs have been successfully encapsulated in cubosomes and characterized [29].

Properties:

1. Cubosome dispersions have much lower viscosity.
2. Cubosomes are discrete, sub-micron, nanostructured particles of bicontinuous cubic liquid crystalline phase [30]. Cubic liquid crystals are transparent and isotropic phases that are physically stable in excess water. Due to the small pore size of cubosomes are attractive for controlled release. It's having an ability of solubilizing hydrophobic, hydrophilic and amphiphilic molecules and its biodegradability by simple enzyme [31].

Advantages:

They can encapsulate both hydrophilic and hydrophobic also amphiphilic drugs.

Relatively simple method of preparation [29].

1. They have a sustained- release drug delivery characteristics.
2. Cubosomes have biocompatibility and bioadhesive properties.
3. Bicontinuous cubic liquid crystalline phase is of cubosomes even stable in excess water [32].
4. Cubosomes are excellent solubilizers, compared with conventional lipid or non-lipid carriers.
5. They show high drug carrier capacity for a range of sparingly water-soluble drugs.
6. These are an excellent vehicle to protect the sensitive drug from enzymatic degradation and in-vivo degradation, such as peptides and proteins.
7. The cuboidal system enhances the bioavailability range twenty to more than one hundred times of water-soluble peptides [33].
8. 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 [29].

Disadvantages:

1. Cubosomes may lead to low drug loading efficiency and drug leakage in preparation, preservation, and transport in vivo, thus the major problem of their stability acts as a barrier and thus limiting their use [34].
2. Large scale production is sometimes difficult because of high viscosity [29].

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 the high internal surface area along with cubic crystalline structures [37].

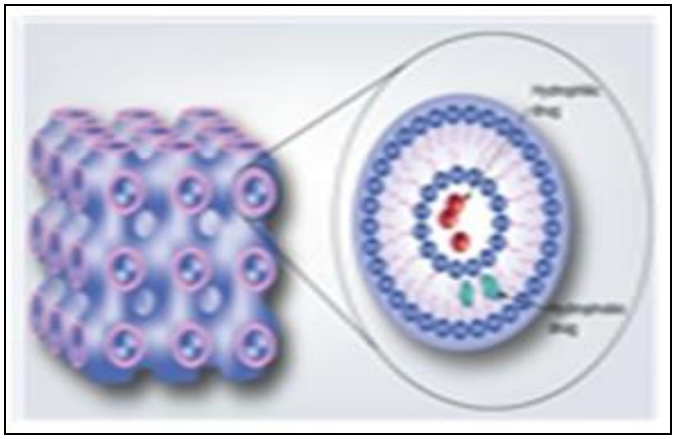

FIG. 1: STRUCTURE OF CUBOSOMES

Monoolein: According to Fontell & Drew ternary systems of amphiphiles, oil & water, some monoglycerides will exhibit cubic phases. Monoglycerides are polar lipids, having poor water solubility that exhibits aqueous phase behavior, which is structurally mimicking to non-ionic surfactants. Lutton results in the monoglycerides whose hydrocarbon chain lengths between C_{12} and C_{22} of all the monoglycerides, particularly monoolein exhibits larger region of the cubic phase. Monoolein is unsaturated, C_{18} monoglyceride.

Monoolein, or glyceryl monooleate, is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate. The acyl chain (oleic acid) is attached to the glycerol backbone by an ester bond. The two remaining carbons of the glycerol have active hydroxyl groups, giving polar characteristics to this portion of the molecule. The glycerol moiety may form hydrogen bonds with water in an aqueous environment and is commonly referred to as the head group. The hydrocarbon chain gives hydrophobic characteristics to monoolein and is often termed the tail [38-42, 10].

Monoolein occurs as a waxy yellow paste with a characteristic odor. It swells in water, giving rise to several lyotropic liquid crystalline structures. From a pharmaceutical standpoint, the phase behavior of the system shows several interesting properties. Some phases may be in equilibrium with excess water solutions, and temperature-induced transitions occur between phases of different rheology. Monoolein is a nontoxic, biodegradable, and biocompatible material classified as GRAS (generally recognized as safe), and it is included in the FDA (Inactive Ingredients Guide) and nonparenteral medicines licensed in the United Kingdom. Its biodegradability comes from the fact that monoolein is subject to lipolysis due to diverse kinds of esterase activity in different tissues [10, 41-45].

Chemical Name: 9-Octadecenoic acid (Z)-monoester with 1,2,3-propanetriol

Synonyms: Glycerol-1-oleate, glycerol oleate, glyceryl monooleate, monolein, αmonoolein glycerol

Empirical Formula: $C_{21}H_{40}O_4$

Boiling Point: 238°C–240°C

Density: 0.94 g/cm3

Melting Point: 35°C–37°C (36°C for the pure form)

Refractive Index: 1.4626

Saponification Value: 160–170

Soap Content: ≅ 0.5%

Solubility: Practically insoluble in water (≅1026 M), soluble in chloroform, ethanol, ether, mineral oil, and vegetable oils.

Water Content: ≤1%. [44, 46-47]

The monoolein-water system uniquely possesses a cubic phase region contains broad compositional and temperature range. But surfactant packing concepts are more approaching. Normally monoolein has continuous hydrophilic headed, hydrophobic tail end, producing reversed or inversed cubic phases, indicating the phases towards the 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.

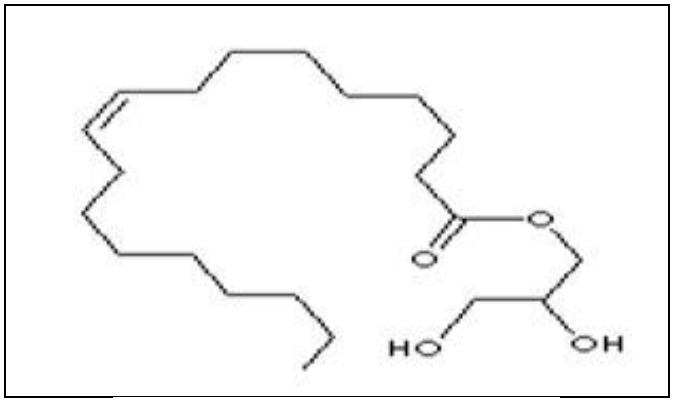

FIG. 2: GLYCEROL MONOOLEATE

Based on their curvatures, 3 types of minimal surfaces are studied in cubic phases was discovered mathematically by Schwarz. 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 a third component such as caseins or amphiphilic block copolymer is added [48].

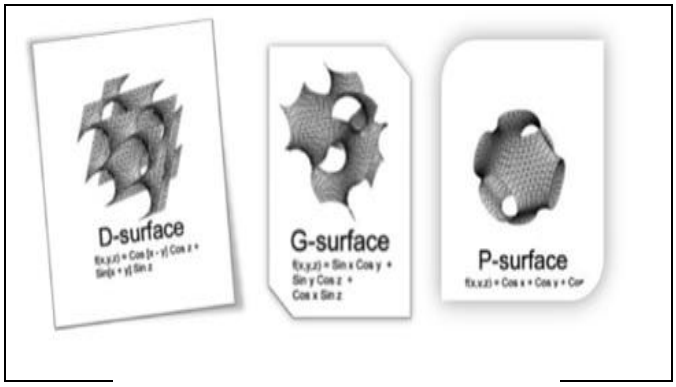

FIG. 3: GLYCEROL MONOOLEATE

Precursor forms of Cubosomes: Since high-energy processes being expensive and difficult to scale-up. To avoid such high-energy processing and to protect the thermosensitive moieties it is planned to produce them in situ resulting in developments of precursors.

Liquid Precursors: The hydrotrope dilution process is found to produce smaller, more stable cubosomes. 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 [24, 48]. 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 [15].

Powdered Precursors: Powders composed of dehydrated surfactant coated with a polymer. Hydration of the precursor powders forms cubosomes with a mean particle size of 600 nm. 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 before drying. 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. 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. 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 [15]. Such powders offer some process and performance advantages to liquid phase hydrotropic cubosome precursors [37].

Materials used for Preparing Cubosomes: The drug, glyceryl monooleate (monoolein), Poloxamer 407, Glycerol, Carbopol 934P, Alcohol (ethanol), Stabilizing agent (Polyvinyl alcohol), Bidistilled water, *etc*. [49]

Techniques used for Manufacture of Cubosomes:

General Method: Cubosomes are usually produced by combining monoolein and water at 40°C. The resultant cubic liquid crystalline gel is dispersed into particles via the application of mechanical or ultrasonic energy. High-pressure homogenizers are often employed to produce cubosomes. Finally, the cubosomes are stabilized against flocculation by polymer addition [13].

Top-down Technique: It is the most widely used in the research area and procedure initially reported in 1996 by Ljusberg- Wahren [50]. The viscous bulk cubic phase is prepared by mixing lipids with stabilizers; then the resultant mixture is dispersed into aqueous solution by the input of high energy (such as High-Pressure Homogenization [HPH], sonication or shearing) to form Lyotropic Liquid Crystal (LLC) nanoparticles. HPH is the most used technique in the preparation of LLC nanoparticles. The cubic phases differ that they are a single thermodynamic phase and have periodic liquid crystalline structure [51]. Wörl et al. investigated the parameters influencing the properties of glyceryl monooleate (GMO)-based cubosomes. Based on the results observed, the concentration of F127 and temperature during HPH were regarded as crucially important parameters [52]. The cubosomes prepared through a top-down approach are always observed to coexist with vesicles (dispersed nanoparticles of lamellar liquid crystalline phase) or vesicle-like structures [33].

Bottom-up Technique: 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 [53]. Compared with the top-down approach, this dilution-based approach can produce cubosomes without laborious fragmentation. In other words, it needs less energy input. Moreover, this approach is far more efficient at generating small particles. In this cubosomes are allowed to form or crystallize from precursors.

The formation of cubosomes by dispersing inverse micellar phase droplets in the water at 80°C, and allow them to slowly cool, gradually droplets get crystallizes to cubosomes. Spicer et al., developed cubosomes at room temperature are by diluting the monoolein-ethanol solution with aqueous poloxamer 407 solutions. 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 [15]. This approach first forms the nanostructure building blocks and then assembles them into the final material. It is a more recently developed the technique of cubosome formation, allowing cubosomes to form and crystallize from precursors on the molecular length scale [51].

Preparation of ALA Loaded Cubosome Dispersions: 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 600°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 200W 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 the 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 [54].

Nucleation: The dilution (nucleation) process provides the ability to produce cubosomes without laborious fragmentation. The best way to anticipate appropriate dilution pathways is by charting trajectories on the ternary diagram. Dilution with water is essentially equivalent to drawing a line from some composition to the water apex [24].

Heat Treatment Approach: This technique is not an integrated process for the manufacture of cubosomes because it only promotes the transformation from non-cubic vesicles to well-ordered cubic particles comprising a homogenization and heat-treatment step as a result decrease in the small particle size fraction that corresponded to vesicles and forms more cubic phases with narrow particle distribution and good colloidal stability [55].

Spray Drying Approach: Because of the less flexibility of liquid precursor for the formation of cubosomes (Spicer *et al*.) developed a dry powder precursor for cubosomes preparation. They utilized the spray drying technique for preparation of starch encapsulated monoolein precursor and dextran encapsulated monoolein precursor.

A high proportion of polymer (75% w/w for starch and 60%w/w for dextran) for encapsulation decreased the amount of loading of active materials, so the system was limited for potent medicament, Vitamins, flavors, or scents [56].

Cubosomes in the Presence of Hydrotrope: Cubosomes were also formed in the presence of significant levels of hydrotrope by sonication based methods. The 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 [24].

Advantages of cubosomes (phospholipids based carrier system) in comparison to other delivery systems:

1. These systems show enhanced permeation of drug through the skin for Percutaneous and dermal delivery.
2. These are a platform for the delivery of a large and diverse group of drugs (peptides, protein molecules). Their composition is safe, and the components are approved for pharmaceutical and cosmetic use.
3. Low-risk profile- the toxicological profiles of the phospholipids are well documented in the scientific literature.
4. High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for the production of Ethosomes.
5. The vesicular system is passive and non-invasive; it is available for immediate commercialization [57].

Evaluation: The cuboidal dispersions can be evaluated by the following parameters:

Thermal Analysis: In this study, DSC is used to evaluate the physical status of the drug within the cubosomes. Both ingredients of the cubosomes seem to melt together in temperature of around 37°C to 56°C, which may results of plasticizing of Glyceryl Monooleate (GMO) [58].

COMPARISON BETWEEN CUBOSOMES AND LIPOSOMES [49]

Cubosomes	Liposomes
Cubosomes are the formation of bicontinuous cubic liquid crystalline phase by a hydrating mixture of monoolein and poloxamer 407	Liposomes are formations of vesicles by a hydrating mixture of cholesterol and phospholipids.
Are artificial, colloidal, and spherical vesicles of 0.05-5.0 μm diameter	Are appear like dots square shaped, slightly spherical of 10-500nm in diameter.
In cubosomes active chemical constituent molecules are anchored through chemical bonds to the polar head of the phospholipids	In liposomes, the active principle is dissolved in the medium of the cavity or the layers of the membrane. No chemical bonds are formed
In cubosomes, polymer and the individual drug compound form a1:1 or 2:1 complex depending on the substance	In liposomes, hundreds and thousands of phosphatidylcholine molecules surround the water-soluble molecule

Cryo-Transmission Electron Microscopy: A small amount of prepared sample at ambient conditions is placed on a pure thin bar 600-mesh TEM grid. The solution blotted with filter paper to form a thin film. Then the sample is vitrified by immersing into liquid ethane near its freezing point. This is transferred to a transmission electron microscope for imaging using a cryo-holder with temperature maintains 175°C. Images are recorded digitally by a charge coupled device camera using an image processing system [59].

X-Ray Diffraction Measurements (XRD): The XRD is carried out using a Philips PW 1830 X-ray generator. Diffraction patterns were recorded by using an INEL Curved Position Sensitive 120 detector. Diffraction data are collected at 25°C controlling the temperature. Little structural information was derived from X-ray diffraction patterns: once the symmetry of the lipid phases was found, the unit cell dimension was calculated by using Bragg's law as follows:

$$a = (h^2 + k^2 + l^2)^{½}$$

Where S_{hkl}= 2sin ϑ/λ with 2 ϑ the scattering angle and ϑ (0.154 nm) the wavelength [60].

HPLC Procedure: Samples were assayed by using a validated HPTLC densitometric method. Developed plates were stained using a solution containing mobile phase cupric sulfate (pentahydrate): phosphoric acid: water and quantified densitometrically using a UV light source set respected wavelength [61].

Particle Size Distribution Measurement: Determination of the particle size distribution of the cubosome dispersions is carried out using laser diffraction (Horiba LA-910) to characterize both the spray dried powders and the aqueous dispersions formed upon hydration of the powders. Diffraction analysis is performed on cubosome dispersions in a re-circulation loop with the lowest ultrasonic setting applied. Dry powders are analyzed using dispersion in a non-solvent (i.e., starch powders in isopropyl alcohol) or the dry powder feeder attachment of the Horiba that allows powder feeding by vibration and air suction to allow the powders to fall through the laser light beam (in the case of the dextran–monoolein powders). The relative refractive index used to size the hydrated powders (*i.e.,* cubosomes) is 1.02 based on literature values for water (n ~ 1.33) and monoglyceride (n ~ 1.36) as well as the agreement with microscopy data [48].

Applications:

1. Control release of a solubilized substance is the most popular application of cubosomes.

2. The cubic phase is more applicable for control release because of its small pore size (5-10nm), ability to solubilize 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 be used in topical and mucosal depositions and delivery of different drugs [15].

5. Due to microbiocidal properties of monoglycerides, can 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 genorrticae*).

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

7. 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 [57-62].

8. More recent use is as skin care, hair care, cosmetics and antiperspirants [56].

CONCLUSION: Bicontinuous liquid crystalline phase in cubosome form provide unique properties of particular interest for the various applications. Cubic phase materials can be formed by a simple combination of biologically compatible lipids and water and are thus well suited for pharmaceutical and body tissue. The ability to form cubosomes either in use, during formulation or manufacture offers greatly enhanced flexibility for product development efforts. The precursor forms enhance its further scope in the technological field.

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