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CUBOSOMES: A BRIEF REVIEW

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ABSTRACT

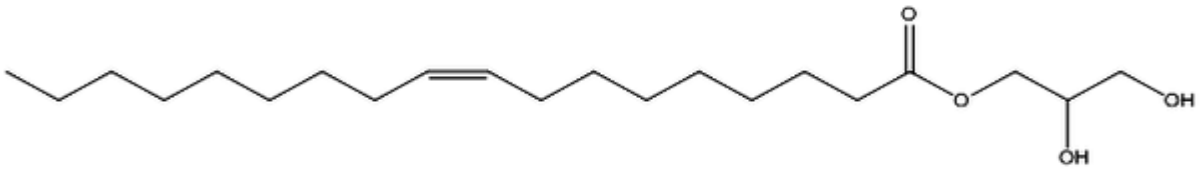
Cubosomes are small biological 'capsules' that can deliver molecules of nutrients or drugs with high efficiency. They have a highly symmetrical interior made of tiny cubes of assembled fat molecules similar to the ones in cell membranes. This also means that cubosomes are safe to use in living organisms. Such features have triggered great interest in the pharmaceutical and food industry, who seek to exploit the structure of cubosomes for the controlled release of molecules, improving the delivery of nutrients and drugs. The main ingredients of cubosomes are cube forming lipids such as glyceryl mono oleate or other phospholipids and a stabilizing polymer polaxomer. The cubic structure and nano size provide many advantages to cubosomes including high drug payloads, controlled and targeted drug delivery, ability to contain hydrophilic, hydrophobic and and amphiphilic drug molecules. Also easy availability of raw materials, and simple method of preparation make them more interesting. The suitability for administration through oral, parenteral, topical routes etc. make the area of applications of cubosomes more wide.

Introduction

"Cubosomes" are discrete, submicron nanostructured particles of bicontinuous cubic liquid crystalline phases, whose size ranges from 10-500 nm in diameter. "Bicontinuous" refers to two distinct (continuous, but non-intersecting) hydrophilic regions separated by the bilayer that is contorted into a periodic minimal surface with zero average curvature. The phase's regular structural connectivity results in a very high viscosity, whereas its tortuosity is useful for slowing diffusion in controlled transport applications. The bicontinuous nature of such cubic phases distinguishes them from the so-called micellar or discontinuous cubic phases containing micelles packed in cubic symmetry.

Structure of Cubosomes

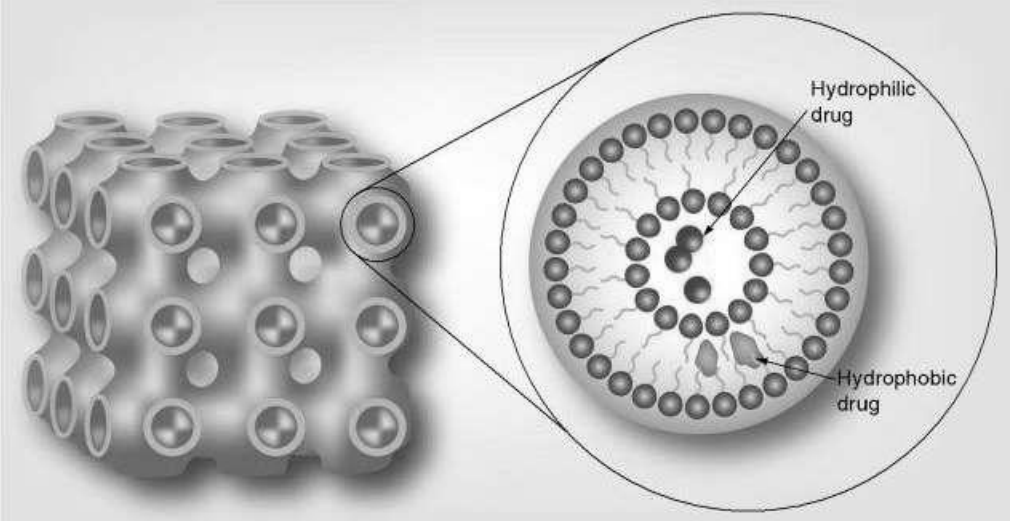
Cubosomes have honeycombed (cavernous) structures whose size range from 10–500 nm in diameter. They appear like dots, square shaped, which are slightly spherical in structure. Each dot corresponds to the presence of pore containing aqueous cubic phase in lipid water system of pore size 5-10 nm.



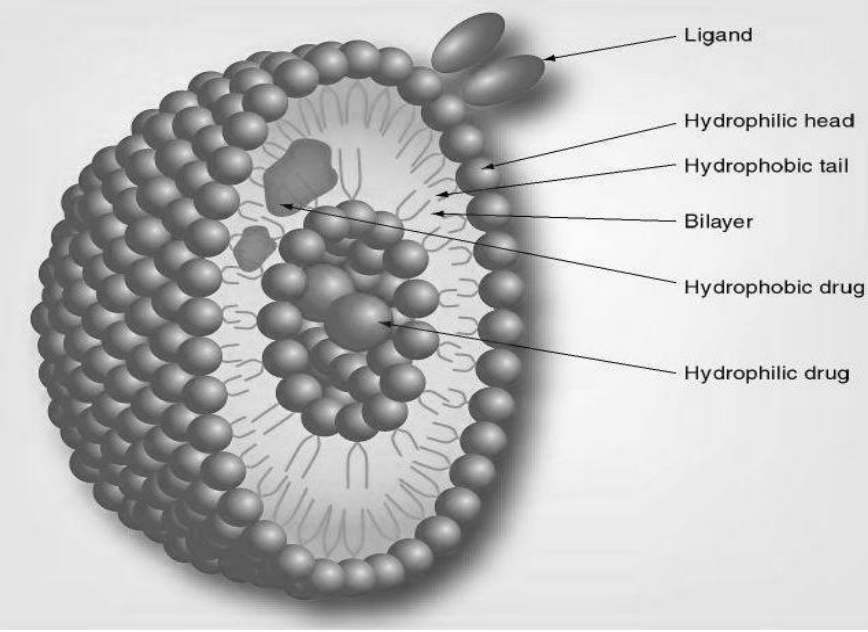
The chemical structure of 2-monoolein, the primary studied lipid building block of a cubosome. It is used to form the bilayer of the membrane. Cubosomes are formed from the cubic phase of lipids, such as monooleate, or any other amphiphilic macromolecules with the unique property to be dispersed into particles. In short, the emulsification of the cubic lipid phases in water results in production of cubosomes that can be defined as nanoparticulate dispersal systems characterized by high biocompatibility and bioadhesivity[(1)].

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 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 andrheology 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].



Cubosomes exhibiting its cavernous internal and cubic structure and its membrane composition with different drug loading modalities[3]



Honeycombed structure separating two internal aqueous channels

PRECURSOR FORMS OF CUBOSOME

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, solid like 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.

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.

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.

ADVANTAGES OF CUBOSOMES[(2)]

1. High drug payloads is possible 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 colloidally and/or thermodynamically stable for longer time.
8. Low cost of the raw materials.

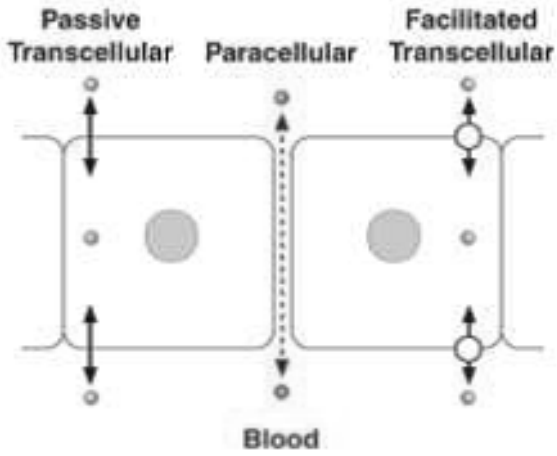
DISADVANTAGES OF CUBOSOMES

1. Large scale production is sometimes difficult because of high viscosity.
2. 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[(5).]
3. Drugs can ruin the lattice structure of the bicontinuous liquid crystalline phase and also ‘big’ drug cannot penetrate inside the channels.

MECHANISMS OF DRUG TRANSPORT[(6)]

Drug transportation across the biological membrane is dependent on the nature of the activity and composition of the carrier, the anatomy and physiology of the skin. Small ions are

transported through the hair follicles, pores of skin membranes, the tight junctions without much complex mechanism. Mechanisms involved in skin membrane transport generally involve in intra (*trans*) and inter (*para*) cellular transports. By manipulating carriers, drugs can be incorporated either in the core or as an integral part of the vesicles [7]. Paracellular diffusion is the movement of drug across a membrane by going between, rather than through, two cells. By definition, this process is solely passive and is dependent upon pore size, as well as the size and shape of the xenobiotic. Transcellular diffusion is the movement of a drug across the cell. When intestinal absorption occurs by transcellular diffusion, the drug is exposed to the enzymes within the cell, as well as any efflux pumps that are present on the apical region of the membrane. These may result in a reduction in the amount of drug that reaches the systemic circulation. Transcellular diffusion may be passive, facilitated, or active [7]. Transcellular movement, which involves the passage of drug through cells, is the most common route of drug transport. Some drugs, however, are too polar to pass across the lipoidal cell membrane and for them only the paracellular pathway, between the cells, is generally available [9].



Paracellular and Transcellular Transports

Advantages of cubosomes (phospholipids based carrier system) in comparison to other delivery systems[4, 10]

1. These systems show enhanced permeation of drug through skin for Percutaneous and dermal delivery.
2. These are platform for the delivery of large and diverse group of drugs (peptides, protein molecules).
3. Their composition is safe and the components are approved for pharmaceutical and cosmetic use.

4. Low risk profile- the toxicological profiles of the phospholipids are well documented in the scientific literature.
5. High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes.
6. The vesicular system is passive and non-invasive, it is available for immediate commercialization

Difference between Cubosomes and Liposomes[(4)].

Sl.No.	Cubosomes	Liposomes
1	Cubosomes are formation of bicontinuous cubic liquid crystalline phase by hydrating mixture of monoolein and poloxamer 407.	Liposomes are formations of vesicles by hydrating mixture of cholesterol and phospholipids.
2	Are appear like dots square shaped, slightly spherical of 10-500nm in diameter	Are artificial, colloidal and spherical vesicles of 0.05-5.0 μm diameter,
3	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 in the layers of the membrane. No chemical bonds are formed.
4	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.

Techniques used for production of Cubosomes[(4)]

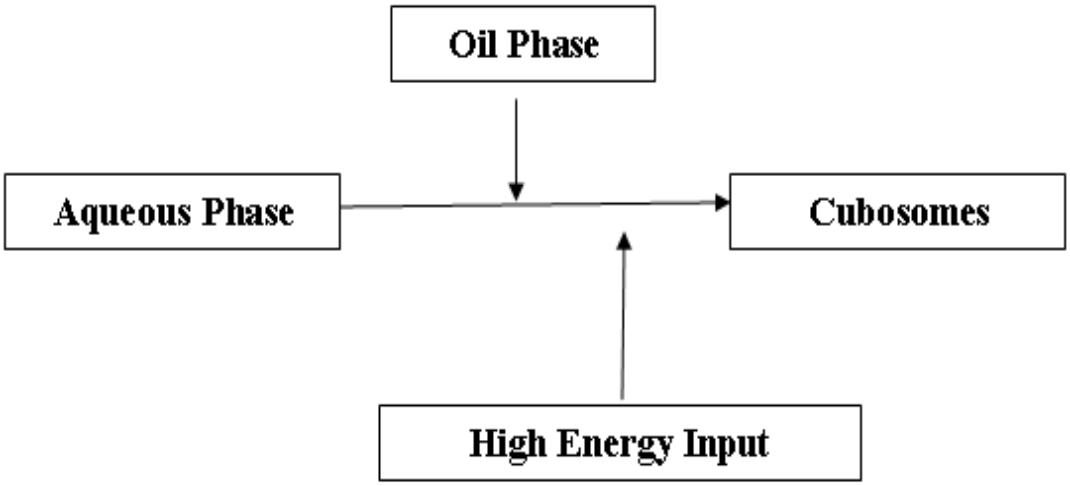
The following techniques are used to produce cuboidal and nanocuboidal drug delivery systems,

1. Top down technique
2. Bottom up technique

3. Heat treatment
4. Spray drying

Top down technique approach

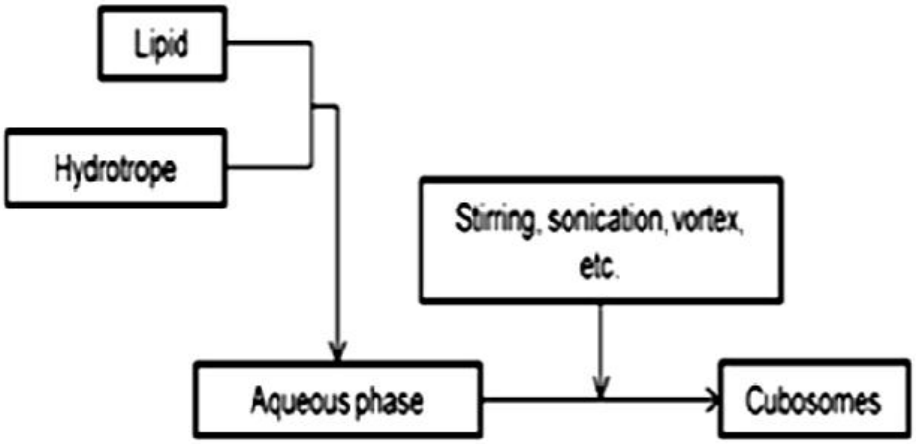
The viscous bulk cubic phase is prepared by mixing lipids with stabilizers, then the resultant mixture is dispersed into aqueous solution by the input of high energy (such as High-Pressure Homogenization [HPH], sonication or shearing) to form Lyotropic Liquid Crystal (LLC) nanoparticles (figure 4). HPH is the mostly used technique in the preparation of LLC nanoparticles. The cubosomes prepared through top-down approach are always observed to coexist with vesicles (dispersed nanoparticles of lamellar liquid crystalline phase) or vesicle-like structures [4,11].



Top down Approach

Bottom up technique approach

In the bottom-up approach the hydrotrope is dissolve in water-insoluble lipids to create liquid precursors and prevent the formation of liquid crystals at high concentration and needs less energy input (Almgren et al). Discuss the formation of cubosomes by dispersing inverse micellar phase droplets in water at 80 0C and allow them to slowly cool, gradually droplets get crystallizes to cubosomes. The cubosomes are spontaneously formed by emulsification. This bottom-up approach cannot effectively avoid forming vesicles through cryo-TEM, many vesicles and vesicle-like structures were also observed to coexist with cubosomes [4,12].



Bottom up Approach

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 form more cubic phases with narrow particle distribution and good colloidal stability [4,13].

Spray drying approach

Because of the less flexibility of liquid precursor for formation of cubosomes (Spicer et al) developed dry powder precursor for cubosomes preparation. They utilized spray drying technique for preparation of starch encapsulated monoolein precursor and dextran encapsulated monoolein precursor. 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 system was limited for potent medicament, vitamins, flavours, or scents[(4,14)]

General method of cuboidal preparation

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 [4,15].

METHODS FOR CHARACTERIZATION AND EVALUATION OF CUBOSOMES[(5)]

Photon correlation spectroscopy

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 [16, 17].

Polarized light microscopy

Polarized light microscopy can be used reveal the optically birefringent (possibly vesicular) surface coating of the cubosomes and also can distinguish between anisotropic and isotropic substances [18].

Gel permeation chromatography or ultra filtration techniques & UV spectrophotometer or HPLC analysis

Entrapment efficiency and drug loading of cubosomes can be determined using gel permeation chromatography or ultra filtration techniques. In the later technique, unentrapped 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 [16].

X-ray scattering

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 [19, 20].

Transmission electron microscopy

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 [20]. 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.

Pressure Ultrafiltration Method

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

Stability studies

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 [23].

Applications of Cuboidal drug delivery system(4)

1. Melanoma (cancer) therapy

Cubosomes are widely used in melanoma (cancer) therapy. 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 [14,24, 25].

2. **Topical drug delivery systems**

Cubic phases are more bioadhesive in nature, so that they can conveniently use 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 [24].The monoglyceride based cubosome dispersion can be used for topically, such as for percutaneous or mucosal applications.

3. Intravaginal treatment

Due to microbiocidal properties of monoglycerieds, can be used to design intravaginal treatment of sexually transmitted diseases caused by viruses (e.g. HSV, HIV) or by bacteria (eg. Chlamydia trachomatis and neisseria genorrticae).

4. Skin development

The cubosomal technology is used to develop a synthetic vernix (complex mixture of lipid (fats), proteins and water) the chessy 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.

5. Cubosome particles are used as oil water emulsion stabilizers and pollutant absorbents in cosmetics.
6. More recent use is as skin care, hair care, cosmetics and antiperspirants [14].

7. **Controlled release of drugs**

Control release of solubilised substance is the most popular application of cubosomes. 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[24]. Liquid crystalline phases can trap different substances, in particular drugs and enzymes. It has been also shown that bicontinuous structures formed in a human body are similar to the artificial ones [30, 31].

8. **Oral drug delivery**

Cubosomes address the varied challenges in oral delivery of numerous promising compounds including poor aqueous solubility, poor absorption, and large molecular size. These are both liquid and powder in capsule products comprising our self emulsifying liquid crystalline nanoparticles technology (LCNP). 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. Liquid crystalline nanoparticles 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[27].

9. **Intravenous drug delivery systems**

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 [28]. Cubosomes may be important for pharmaceutical industry in the nearest future.

10. Material synthesis

Since cubosomes have regular structure their application in the field of material synthesis can also be interesting. The creation of particles with nanostructure opens the road to the application of the cubosomes in electronics, photonics, catalysis, and medicine. Cubic phases can be used as matrices for protein encapsulation or the reaction of synthesis or polymerization. For example, aluminosilicate zeolite MCM-48 is formed in cubic phases [32].

PHARMACEUTICAL PREPARATIONS ENCLOSING CUBOSOMES[2]

Sl.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
		Cefuroxime	Antibiotics	Meningitis, bone and soft tissue infection
		Prilocaine	Local anesthetic	In Dentistry
3.	Damani	Clindamycin phosphate	Antibiotics	Peritonitis, staphylococcal bone andjoint 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

		Isosorbidemononitrate	Anti-anginal	Angina pectoris
		Lidocaine hydrochloride	Oral prepration	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 Gernal Anesthesia

CONCLUSION

As the potential advantages exhibited by cubosomes are inimitable, it may become important for pharmaceutical as well as food industry in the nearest future. The simple dispersion of glyceryl monooleate in water forms cubosomes which is then stabilized by the addition of polaxomer 407. The cubosomes are suitable carrier for hydrophilic, hydrophobic as well as amphiphilic drug moleculesfor delivery through oral, topical, parenteral routes etc..Properties like biodegradability by simple enzyme action, bioadhesive nature, high drug pay loads,low cost of raw materials make these nanostructures attractive vehicles for pharmaceutical applications.

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