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Cubosomes - A Novel Drug Delivery System

Ancy Thomas*, Steffy P Raju, Aeista Thomas, Christina Das, Elessy Abraham

Nazareth College of Pharmacy, Othera, Pathanamthitta District, Thiruvalla, Kerala, India

***Corresponding author e-mail:** ancy.sarah70@gmail.com

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ABSTRACT

Cubosomes are discrete, sub-micron, nanostructured particles of the biscontinuous cubic liquid crystalline phase. It consists of honeycombed structures separating two internal aqueous channels and a large interfacial area. Cubosomes are composed of polymers, lipids and surfactants with polar and non-polar components hence said as amphiphilic. Thus cubosomes are bicontinuous cubic liquid phase enclosing two separate regions of water divided by surfactant controlled bilayers and hence the prodrugs and cyclodextrins can be incorporated into it among others. Cubic liquid crystals are physically transparent and isotropic phases that are stable in excess water and show a unique system for the production of pharmaceutical dosage forms. The structure of cubosomes generally maintains the efficacy; stability of actives such as vitamins and proteins. Cubosomes are thermodynamically stable; lasting indefinitely. Colloidal dispersions of cubosomes can be stabilized by the addition of polymers. Improved stability of drug in formulation, desired particle size range, maximum drug loading capacity and well controlled drug release made cubosomal systems superior to other novel delivery systems like solid lipid nanoparticles, microemulsions, microspheres and liposome. The choice of technique depends on the nature of polymer as well as the nature of drug and the duration of therapy. The most important physico chemical factors that may be controlled in cubosome manufacture are also being considered. The cubosomes offer well controlled delivery to variety of drug candidates like anti-inflammatory compounds, local anesthetics, antibiotics and anticancer drugs and the lipid entrapped cubosomal vaccines. The controlled release application of these nanoparticles is of a great significance in cosmeceutical and pharmaceutical fields.

Keywords: Cubosomes, Honeycombed, Versatile, Drug payloads, Compartmentalization

INTRODUCTION

Drug delivery refers to approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. It may involve scientific site-targeting within the body, or it might involve facilitating systemic

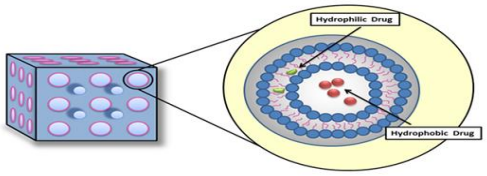

Figure 1: Diagram showing hydrophilic and hydrophobic drug site

pharmacokinetics; in any case, it is typically concerned with both quantity and duration of drug presence (Figure 1). Drug delivery is often approached via a drug's chemical formulation, but it may also involve medical devices or drug-device combination products. Drug delivery is a concept heavily integrated with dosage form and route of administration, the latter sometimes even being considered part of the definition. These include cubosomes, liposomes, proliposomes, and microspheres. Cubosomes are discrete, sub-micron, nanostructured particles of the biscontinuous cubic liquid crystalline phase. It consists of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. They contain identical microstructure as that of its parent with high surface area and their dispersions are less viscous than the parent cubic phase. Most probably cubosomes are composed of polymers, lipids and surfactants with polar and non-polar components hence said as amphiphilic. The amphiphilic molecules are driven by the hydrophobic effect into polar solvent to impulsively identify and assemble into a liquid crystal of nanometer scale. Thus cubosomes are bicontinuous cubic liquid phase enclosing two separate regions of water divided by surfactant controlled bilayers, prodrugs, and cyclodextrins, among others.

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. Cubic liquid crystals are physically transparent and isotropic phases that are stable in excess water and show a unique system for the production of pharmaceutical dosage forms. The liquid crystals of cubic phase are used in the controlled release of selected water and oil soluble molecules. They are isotropic, viscous and solid like liquid crystalline substances with cubic crystallographic symmetry. Liquid crystal is a state of matter that has properties between those of conventional liquids and solid crystals. Cubic phases have a thermodynamically stable structure consisting of two separate, continuous but non intersecting hydrophilic regions divided by a lipid bilayer. This allows the incorporation of water and oil soluble materials and also amphiphiles into the system. The structure of cubosomes generally maintains the efficacy; stability of actives such as vitamins and proteins.

Cubosomes are thermodynamically stable; lasting indefinitely. Colloidal dispersions of cubosomes can be stabilized by the addition of polymers. They also possess the potential for controlled delivery of actives, where diffusion is governed by the tortuous diffusion of the active through the “regular” channel structure of the cubic phase. Cubosomes are liquid crystalline nanostructured particles with the same unique properties of the bulk cubic phase, although cubosome dispersions have much lower viscosity. Although fundamental research has been focused sharply on bulk cubic phases, it is commercial applications that drive much of the existing and still very active research into cubosomes.

Cubosomal precursor forms

The variation in the structure of cubosomes to be the ultimate drug delivery system due to its ability to maintain the structural integrity of the ingredients that it carries. Three macroscopic forms of cubic phase are typically encountered: precursor, bulk gel, and particulate dispersions (Figure 2). The precursor form exists as a solid or liquid material that forms cubic phase in response to a stimulus, such there are three different proposed phases that these cubic structures can be in: the P-surface, G-surface and D-surface for primitive, gyroid and diamond structures respectively.

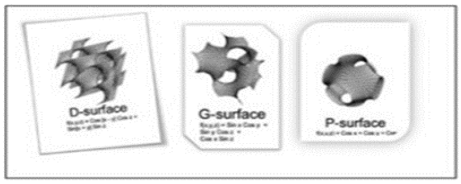

Figure 2: Diagram showing D- surface, G-surface and P-surface

1. Liquid precursors

A strong driving force is required to produce cubosomes from liquid phase precursors. Upon dilution of liquid precursors, more stable cubosomes of desired size can be produced. In hydrotrope dilution method, cubosomal particles are produced by nucleation and growth mechanism which is similar to crystallization and precipitation procedure. Liquid phase precursors are widely used in mouth washes, hand washes, where cubosomes can be formed during rinsing, washing respectively.

2. Powdered precursors

Powdered cubosomal precursors are made up of dehydrated surfactants coated with suitable polymer. Upon reconstitution of powdered precursors with water, cubosomes with an average particle size of 600 nm are formed as conformed by characterization studies such as light scattering technique and cryo- transmission electron microscopy (cryo-TEM) . Spray drying is an excellent technique to formulate cubosomal powdered precursors. It involves the production of encapsulated particles from liquid droplets in emulsion as well as from dispersed solid particles in concentrated aqueous polymer solution.

Structure of cubosomes

When cubic phase is dispersed into small particles, these particles are termed cubosomes. The internal and structural changes of cubosomes could be controlled by adjustment in lipid composition. Cubosomes are discrete, sub- micron nanostructures having the same microstructure as the parent cubic phase (Figure 3). Their size ranges from 10-500 nm in diameters. They appear like dots square shaped or slightly spherical. Each dot corresponds to the presence of pore containing aqueous phase cubic phases in lipid- water system. These were first identified using x- ray scattering technique by Luzzati and Husson. Monoglycerides are polar lipids with poor water solubility that exhibit aqueous phase behavior reflecting their structural similarity to non-ionic surfactants. Bulk cubic phase is formed by hydration of monoolein at levels between 20-40% w/w. Cubic phases are found sandwiched between lamellar and hexagonal liquid crystal several different phases is an important property of pure lipids and lipid mixtures; it depends on temperature, hydration and lipid class. In general monoglycerides exhibit different phase behaviors when they exposed to water.

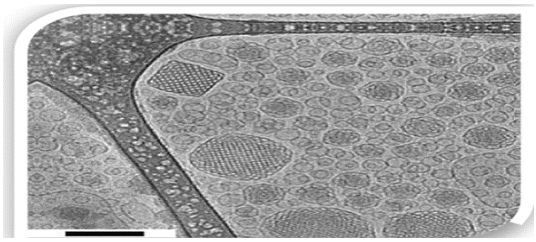

Figure 3: Square or spherical shaped cubosomes

Structural characteristics of cubosomes

1. Cubosomal personal care products are prepared by mixing biocompatible lipids and aqueous phase which promotes their use in the production of skin care, hair care and other body care products [1].
2. Cubosomal skin care products are gaining importance because of the possible interaction of stratum corneum and lipids used in cubosomal formulation promoting the permeation of drugs [2-4].
3. Cubosomes being self-assembled cubic crystals are biocompatible and bioadhesive, thereby well suitable for oral administration which was proved with the oral administration of insulin loaded cubosomes for hypoglycemic effect [3-6].
4. Phase Transition Amphiphilic lipids in aqueous environments are characteristic to establish self-assembled nanostructures and facilitate cosmetic application, drug delivery and diagnostics [7]. The phase transition and self-assembly of ionic surfactant-phytantriol cubosomal dispersion in aqueous medium depends not only on the concentration of surfactant lipid mixture but also on the ionic strength.

Advantage of cubosomes

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 [8-10].
7. 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.
8. The cubic phases of cubosomes can be fractured and dispersed to form particulate dispersions that are colloidally and/or thermodynamically stable for longer time.

9. Increased convenience and compliance (orally, topically and intravenously).
10. Improved bioavailability due to size.
11. Improved efficacy
12. Decreased side effects associated with high initial plasma levels from rapid drug release on injection (drug burst).
13. Decreased health care costs due to simplified handling and less frequent administration
14. Decreased risks of drug misuse and misdirection.

Material used in cubosomes

Bicontinuous cubic phases are found in;-

1. Natural lipids
2. Cationic and nonionic surfactants
3. Polymer systems

Natural lipids

Although the lipid most widely used to construct bicontinuous cubic phases are

1. Monoglyceride
2. Monoolein

1. Monoglycerides

Monoglycerides are spontaneously form bicontinuous cubic phases upon the addition of water, are relatively insoluble (allowing the formation of colloidal dispersions of cubosomes), and are resistant to changes in temperature.

2. Monoolein

The main precursor of cubosome formation is monoolein. Monoolein or glyceryl monooleate is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate. Commercially available monoolein may be obtained in two forms, a mixed glyceride form or as distilled monoolein; the distilled monoolein is preferred for pharmaceutical applications because of its high purity. Monoolein occurs as a waxy yellow paste with a characteristic odor. 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 in non-parenteral medicines licensed in the United Kingdom. Monoolein show the mesomorphic phase, important in making more comprehensible the potential pharmaceutical application of the lipid.

Surfactant

Surfactants, which are used in the production of cubosomes, are poloxamer 407 in a concentration range between 0% and 20% w/w with respect to the disperse phase. The concentration of the monoglyceride/surfactant mixture generally takes between 2.5% and 10% w/w with respect to the total weight of the dispersion.

Polymer system

Polyvinyl alcohol (PVA) used in addition to poloxamer as a stabilizing agent of the dispersion.

Methods for preparation of cubosomes

1. Top down technique

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 (Figure 4). The cubic phases differ in that they are a single thermodynamic phase and have periodic liquid crystalline structure. Cubic phase's ruptures in a direction parallel to the shear direction, the energy required is proportional to the number of tubular network branches that rupture.

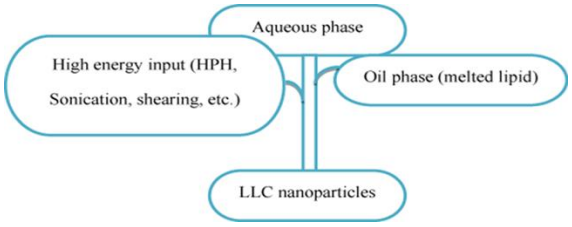

Figure 4: Top down technique

2. Bottom up technique

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 (Figure 5). 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 to op down approach.

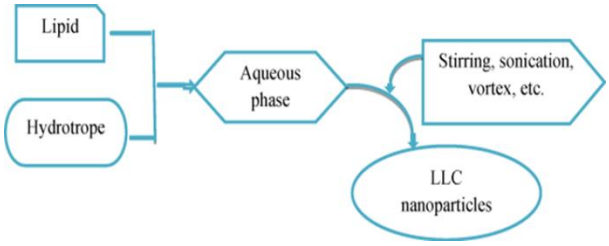

Figure 5: Bottom up technique

Drug loading capacity of cubosomes

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

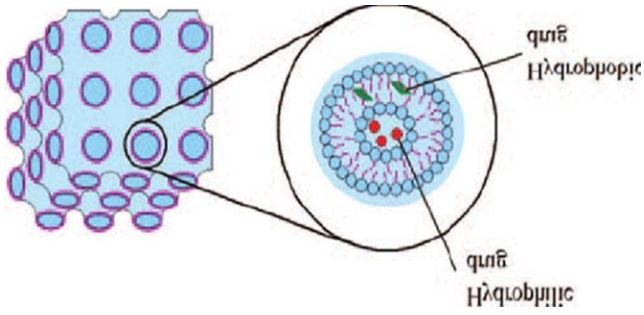

Figure 6: Cubosomes with different drug loading modalities

Drug release from cubosome

Cubosomes have been proposed as a controlled release, intravenous drug delivery system. The pressure ultra-filtration method and equilibrium dialysis were used to elucidate the *in vitro* drug release mechanisms. On dilution of cubosomes, lipophilic compounds were released rapidly when studied by the pressure ultra-filtration method. In contrast, equilibrium dialysis incorrectly indicated sustained drug release from cubosomes. Research shows that cubosomes should be burst release delivery systems where drug is released by diffusion from the cubic phase matrix, and that pressure ultrafiltration may have benefits over dialysis methods for measurement of drug release from colloidal particle-based drug delivery systems.

Mechanisms of drug transport

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 (Figure 7). 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.

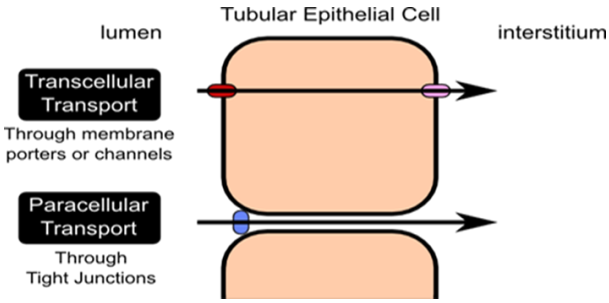

Figure 7: Transcellular and paracellular transport

Characterization and evaluation of Cubosomes

1. Ultra-filtration techniques & UV spectrophotometer

Drug loading of cubosomes and Entrapment efficiency can be determined using ultra-filtration techniques. 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.

2. Photon correlation spectroscopy

The photon correlation spectroscopy is another technique used in the evaluation of cubosomes. Particle size distributions of cubosomes are mainly determined by dynamic laser light scattering using Zeta sizer. 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 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.

4. 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.

5. 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. 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 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) °C3.

7. 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.

8. Visual inspection

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

9. Light microscopy

Samples of the prepared cubosomes were suitably diluted with deionized water and examined using an optical microscope with a micrometer slide at magnification of x 400 and x 10007.

10. Entrapment efficiency

For determination of entrapment efficiency (EE), it was mandated to separate free ALA from cubosome associated ALA. The free drug quantity in the dispersion was then analyzed spectrophotometrically at λmax 250 nm, and it should be subtracted from the total amount of drug initially added. The 1 ml volume 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. Diluted dispersion of the resulting was then passed through a syringe filter having a pore size of 0.1 µm. This concentration obtained at λmax 250 nm was then multiplied by the total volume of the dispersion produced, considering the dilution factor. Hence the concentration of free drug can be calculated. This was then subtracted from the total drug concentration (Ct) in the formulation to give the amount of drug that can entrapped inside the cubosomes. Each experiment was repeated three times.

$$EE\ \%\ of\ cubosomes = [(Ct - Cf)/Ct] \times 100$$

Cubosome applications

1. Melanoma (cancer) therapy

Recently few anticancer drugs have been successfully encapsulated in cubosomes and characterized physicochemica lly. The unique structure of this promising nanocarrier suggests its application in melanoma therapy. In order to

specifically target nanomedicines to tumors, 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 tumor vasculature which is generally highly disorganized with enlarged gap junctions between endothelial cells and compromised lymphatic drainage allowing for the extravasation of nanocarriers with sizes up to several hundred nanometers. Objects of this size cannot pass through the tight junctions that exist within the endothelial cell lining of the vessels of healthy tissues. 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.

2. 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. 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.

3. 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.

4. Topical drug delivery systems

Topical delivery systems is the another mode of cubosomal application. They are based on the exploitation of the particular properties of liquid crystal and liquid crystal nanoparticle technologies. Topical drug delivery systems are unique *in situ* forming bioadhesive LC systems used to facilitate the controlled and effective drug delivery to buccal, ophthalmic, vaginal and others. This system forms a thin surface film at mucosal surfaces which consist of a liquid crystal matrix where the nanostructure can be controlled for achieving an optimal delivery system and to provide good temporary protection of sensitive skin.

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.

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. 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. In treatment of viral diseases

Because of the microbicidal properties of monoglycerids, 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 genorrticae. 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 monolein 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.

8. In topical and mucosal depositions

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

9. Controlled-release drug delivery

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 action6. 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.

10. In materials synthesis

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 entails 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.

Future prospectives

The future prospective works of cubosomal precursors are;

- Multiple peptide agents including, Octreotide, Leuprolide, Somatostatin, Salmon Calcitonin, GLP-1 and analogues.
- Several therapeutic proteins have been used in the liquid crystalline formulations.
- Testosterone derivatives and opiates.

Two injectable products are currently in clinical development;

a) CAM2032, a long-acting LHRH agonist for the treatment of prostate cancer after a single subcutaneous administration of three different doses.
b) Another clinical-stage product is CAM2029, a long-acting formulation of Octreotide, for the treatment of acromegaly, carcinoid syndrome and vasoactive intestinal peptide (VIP) producing tumors.

CONCLUSION

As cubosomes are bicontinuous lipid cubic phases, they have capability to incorporate many hydrophilic and lipophilic drugs that get delivered to the targeted tissues such as central nervous system and brain efficiently. Stability can be imparted to potent and highly sensitive moieties by these cubic systems which contain selective and stable lipids. Two reproducible methods such as top down and bottom up approaches could be easily employed to produce cubosomes either by high pressure homogenization or ultrasonication techniques. Cubosomes are applicable to wide range of drug candidates, proteins, immunogenic substances and also to cosmetics. Due to the potential site specificity, the cubosomal preparations may be widely employed as targeted drug delivery systems for ophthalmic, diabetic and also for anticancer therapy. The cubosome technology is relatively new with high through output and would have wide scope of research in developing new formulations with commercial and industrial viability.

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 use of nanomedicine in localized 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. With the

sequence of human genome, biotechnology companies are developing a peptide and protein based drugs. It is expected that in the next 10 to 20 years, cubosomes containing protein and peptide based drugs will constitute more than half of the new drugs introduced into the market and more than 80% of these protein drugs will be antibodies due to control release activity. Further specialized studies are required to confirm this fascinating hypothesis and to better investigate the role of vesicles and cubosomes in controlling the release of the drug.

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