



## **Cubosomes as Potential Nanocarrier for Drug Delivery: A Comprehensive Review**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Lyotropic liquid crystalline cores are characterized as soft nanoparticles and referred as cubosomes. They are prepared to activate the natural self-assembly capability of lipids (e.g., monoolein or phytantriol) in water. Cubosomes are crystalline isotropic lipidic nanoparticles stabilized by Poloxamers such as F127, F108. It is made up of a network of two separate aqueous channels formed by a three-dimensional, non-intersecting lipid bilayer imposed over an indefinite periodic minimum surface of cubic symmetry. Cubosomes constitute unique features such as their special cubic structure which permits to incorporate highly lipophilic, hydrophilic, and amphiphilic drugs. Also, the lipids excipients used in the preparation of cubosomes such as monoolein, phytantriol are biodegradable and biocompatible so these cubic nanoparticles are referred as safe carrier for drug delivery. Cubic lipid nanoparticles have a highly stable cubic shape that allows for a slower rate of dissociation, improved drug retention, and site-specific drug delivery. The architecture of cubic particles provides suitability in the drug delivery as compared to other lipids-based drug delivery systems such as solid lipid nanoparticles (SLN), liposomes due to their drug expulsion to the surface of nanoparticles. Cubosomes with these loaded features/architectural composition led to an array of desired performance. Solvent evaporation, ultrasonication,

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hydrotrope, spray drying, melt dispersion emulsifying methods are used to prepare these carrier systems.

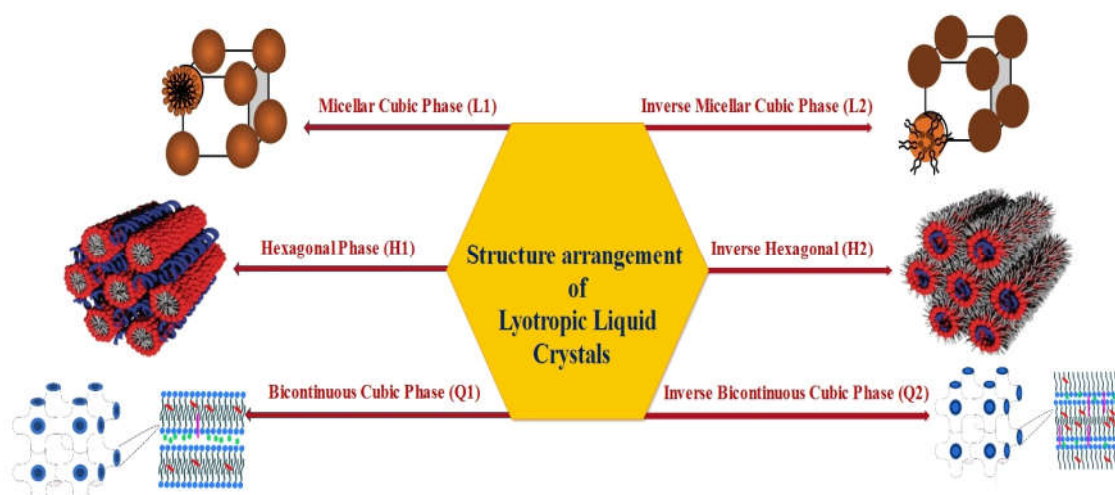
**Keywords:** Cubosomes; drug delivery; nanoparticle; pH-Sensitive.

## 1. INTRODUCTION

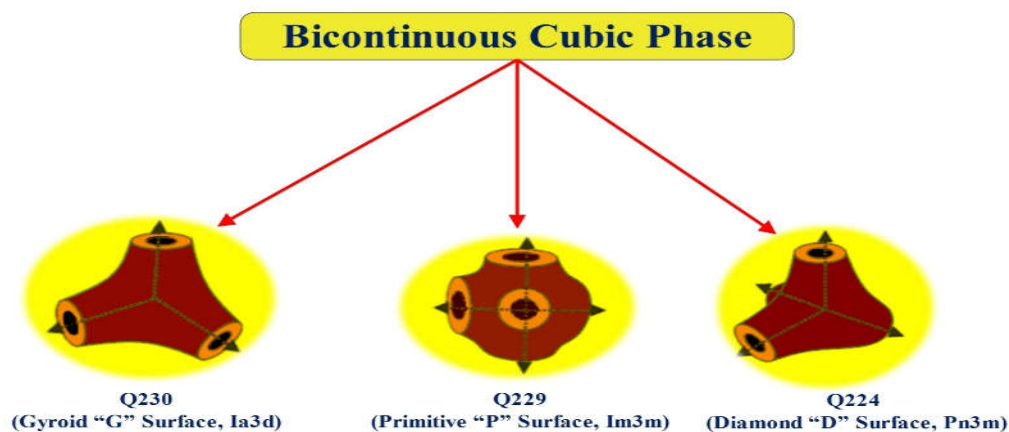
Lyotropic cubic liquid crystalline nanoparticles, also known as 'cubosomes' have gained popularity as effective carriers for solubilization of variety of drugs due to enhanced efficiency of drug and bioavailability [1-4]. Cubosomes are inverted bicontinuous curved cubic phase lyotropic liquid crystals that are formed by mixing certain amphiphiles such as glyceryl monooleate in water using appropriate stabilizers under suitable conditions of hydration and temperature [5,6]. The use of amphiphilic lipids in the formation of nanocarriers has gained traction due to their capability to self-assemble into highly organized biomembrane mimetic nanoparticles such as cubosomes under physiological conditions while protecting the active moieties against degradation and providing controlled release. Progressively increasing the amphiphile concentration and temperature allows the lyotropic liquid crystals to arrange into various structures such as micellar cubic phase ( $I_1$ ), hexagonal phase ( $H_1$ ), lamellar phase ( $L\alpha$ ), and bicontinuous cubic phase ( $Q_1$ ) as is depicted in Fig. 1 [7].

The hexagonal phase is characterized by cylindrical lyotropic liquid crystals formed by the aggregation of micelles which are arranged on a

hexagonal lattice. Cubic phases generally have higher viscosity due to their three-dimensional recurring structure as compared to lamellar and hexagonal phases [8]. In the micellar cubic phase, the micelles are crammed on a cubic lattice while the lamellar phase consists of amphiphile bilayers arranged into planar sheets divided by an aqueous compartment. In a bicontinuous structure, the hydrophilic phase and lipophilic phase are continuous, which is like the structure of a porous material having dispersed solid phase and continuous gas phase. Bicontinuous cubic phase structures such as cubosomes are generally formed by single chain amphiphiles at concentrations between those required to form lamellar phase and hexagonal phase. The three major structures of the bicontinuous cubic phase observed in a system containing lipid are  $Q^{230}$  (gyroid "G" surface, Ia3d),  $Q^{229}$  (primitive "P" surface, Im3m),  $Q^{224}$  (diamond "D" surface, Pn3m) as illustrated in Fig. 2. The structure of phase  $Q^{224}$  consists of two three-dimensional networks of rods that are joined tetrahedrally like a diamond structure. Similarly, in  $Q^{230}$  the rods are coplanar linked 3 by 3, and in  $Q^{229}$ , the rods are cubically joined 6 by 6 [9]. Increasing the ratio of water in the glyceryl monooleate-water system can result in the conversion of phase  $Q^{230}$  into phase  $Q^{224}$  due to swelling [10].



**Fig. 1. Phases of lyotropic liquid crystals**



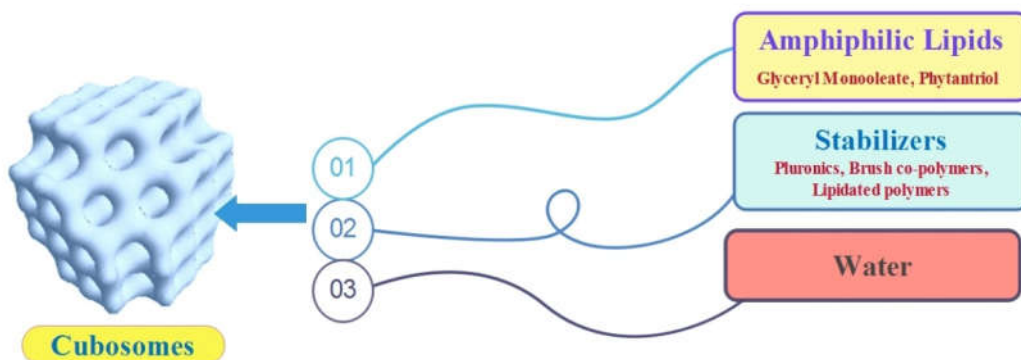
**Fig. 2. Types of bicontinuous cubic phase**

On the contrary, the formation of phase Q<sup>229</sup> necessitates a 3-component system where the third component is a surface-active polymer and cannot be attained simply by increasing the water content. The requirement of a surface-active polymer can be ascribed to the large curvature energy required for the formation of phase Q<sup>229</sup> [11]. A lipid cubic phase refers to the assembly of lipid into a structure belonging to the cubic space group and comprises a three-dimensional unceasing lipid bilayer that partition two discrete-continuous intertwining but unconnected aqueous compartments [12, 13]. The organization of interface between lipid head group and water is such that the hydrophilic head group region of the continuous lipid bilayer is curved towards the aqueous compartments [14]. Therefore, the interior of cubosomes consists of a lipid cubic phase comprising of the continuous but separate three-dimensional channels of the lipid and aqueous components. The bicontinuous lipidic cubic phase structures are optically clear and possess very high viscosity due to their three-dimensional bicontinuous nature [15-18]. The internal assembly of cubosomes carries a significantly higher surface area for the loading of active molecules as compared to liposomes [19]. Cubosomes can simultaneously solubilize hydrophobic and hydrophilic pharmaceutical actives as well as amphiphilic compounds which is convenient when compared to liposomes or emulsion-type drug delivery systems [20]. Owing to the higher lipid ratio in the bilayer membrane and stronger electrical repulsion between them, the cubosomes possess greater membrane stability than the liposomes. In addition, cubosomes exist at even extreme degrees of

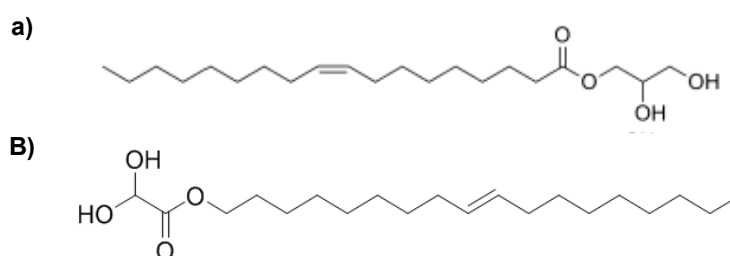
dilution in water and the leakage of a drug is generally less of a problem as compared to liposomes [21]. Several therapeutically active proteins can be easily incorporated into the cubic structure of cubosomes as compared to liposomes while retaining a better stability profile against enzymatic degradation and extremely high encapsulation efficiency [22]. As a result of their biological membrane mimetic nanostructure, cubosomes provide better transdermal drug delivery than liposomes [23]. Due to the reduced monolayer curvature energy of a cubic phase structure, as compared to other phases, cubosomes are thermodynamically stable and form spontaneously [24,25]. Cubosomes are progressively making a mark as suitable nanocarrier for drug delivery applications due to their inherent physical stability as compared to related carriers such as liposomes; simultaneous delivery of hydrophilic, lipophilic, and amphiphilic molecules; mucoadhesion properties; controlled delivery of molecules due to their porous three-dimensional topology which provides a multifaceted diffusion pathway for entrapped drug molecules [26, 27]. However, there is a drawback of cubosomes in addition to its intriguing benefits eg. - Due to the high viscosity of cubic phase, large-scale manufacturing is difficult [39].

## 2. STRUCTURAL COMPONENTS OF CUBOSOMES

The main components of cubosomes are amphiphilic lipids, stabilizers and water as shown in Fig. 3.



**Fig. 3. Composition of lyotropic cubic liquid crystalline nanoparticles also known as cubosomes**



**Fig. 4. Chemical Structure of (A) Glycerol monooleate (Monoolein), (B) Phytantriol**

## 2.1 Amphiphilic Lipids

The amphiphilic lipids mostly employed in the preparation of cubosomes are glycerol monooleate/monoolein (GMO) [28] and phytantriol (PHYT) shown in Fig. 3 [29]. Although GMO is highly biocompatible and has bio adhesive properties [30, 31], it is degraded by lipase enzymes in the gastrointestinal tract. The backbone of the phytanyl chain delivers structural stability to the PHYT, consequently, PHYT provides more stability to liquid crystalline phases than glycerol monooleate albeit at the cost of reduced biocompatibility. The structure of GMO and PHYT is illustrated in Fig. 4 [32]. Even though phytantriol and GMO have different structures, they both exhibit similar phase transitions with an increase in the water content and temperature [33, 34]. The toxicity studies of PHYT and GMO-based cubosomes have shown that PHYT based cubosomes exhibit more toxicity as compared to GMO-based cubosomes resulting in the initiation of a sustained-release inflammatory response which is not observed in the GMO cubosomes. Based on these findings, GMO-based cubosomes were found to be desirable for the efficient targeting of a lower pH

environment which corresponds to the cancer cell milieu [35, 36].

## 2.2 Surfactants/ Stabilizers

It is necessary to maintain the internal structure of cubosomes, enabling their effective use in biomedical applications. The stabilizer's major function is to establish an electrostatic barrier to avoid near particle interactions and thus to hold the dispersed particles in a stable state [37]. The most extensively used cubosomes stabilizers are Pluronic<sup>®</sup> which are self-assembling water-soluble triblock copolymers consisting of polyethylene oxide (PEO) and polypropylene oxide (PPO) arranged in PEO-PPO-PEO conformation in which hydrophobic and hydrophilic properties are imparted by the PPO and PEO portions, respectively [38,39]. The overall stability of the cubosomes depends upon the equilibrium between the size of the hydrophobic domain (PEO) and the strength of steric repulsion imparted by the hydrophilic domain (PPO) [40-44]. Chong et al. [45] in their study revealed that the Pluronic<sup>®</sup> F108 with 132 polyethylene oxide units' chain could stabilize monoolein cubosomes without affecting the

internal structure of the particles. The broadly used Pluronic® F127, with 100 PEO units, moves the symmetry of the monoolein cubic phase from its native double diamond reversed bicontinuous cubic phase ( $Q_2^D$ ) to a primitive cubic phase (cP), suggesting its role in destabilization of internal liquid crystalline structure [46,47]. Recently, brush copolymers, and lipidated polymers produced by reversible addition fragment chain transfer (RAFT) and Tween 80 [48, 49] are increasingly replacing the Pluronics®. They have several advantages over Pluronics®, such as the inclusion of biologically active diglycerides, greater drug targeting propensity, reduced toxicity, and well-defined molecular weight [50]. Lipidated polymers that are produced from the polyethylene glycol methyl ether acrylate (PEGMA) polymerized with a diglyceride initiator have also been used to stabilize PHYT based cubosomes [51]. Amphiphilic brush copolymers which are produced from RAFT have a greater chance of targeting specific cells than Pluronic® F127. Drug delivery across the blood-brain barrier has been improved by using Tween 80 as a stabilizer in PHYT based cubosomes [51]. Murgia et al. in their study observed that the GMO accentuates the toxicity of Pluronic® F127 by promoting its cellular internalization. However, brush polymers not only displayed reduced toxicity as compared to Pluronic® F127 stabilized GMO-based cubosomes, but also did not affect

the symmetry of the inverse bicontinuous cubic phase [34].

### 3. PREPARATION TECHNIQUES OF CUBOSOMES

There are range of cubosomes forming techniques are used i.e., melt dispersion emulsifying, sonication, spray drying, solvent evaporation, top-down, bottom-up, or hydrotrope method as depicted in Fig. 5. Top-down and hydrotrope method are the two most common methods employed in the formation of cubosomes.

#### 3.1 Melt Dispersion Emulsifying Method

Cubosomes were prepared by melting the amphiphilic lipids and stabilizers on a hot plate magnetic stirrer. Generally, the drug is either dispersed in the molten lipid-stabilizer mixture [52, 53] dissolved in ethanol or aqueous phase [54,55] containing a suitable surfactant or polyvinyl alcohol which acts as a dispersion stabilizer. The lipid molten mixture is then injected into the preheated aqueous phase under magnetic stirring and emulsified using a homogenizer. Aboud et al. [55] used emulsification method for the preparation of cubosomes loaded sildenafil citrate as vaginal sponges for uterine targeting.

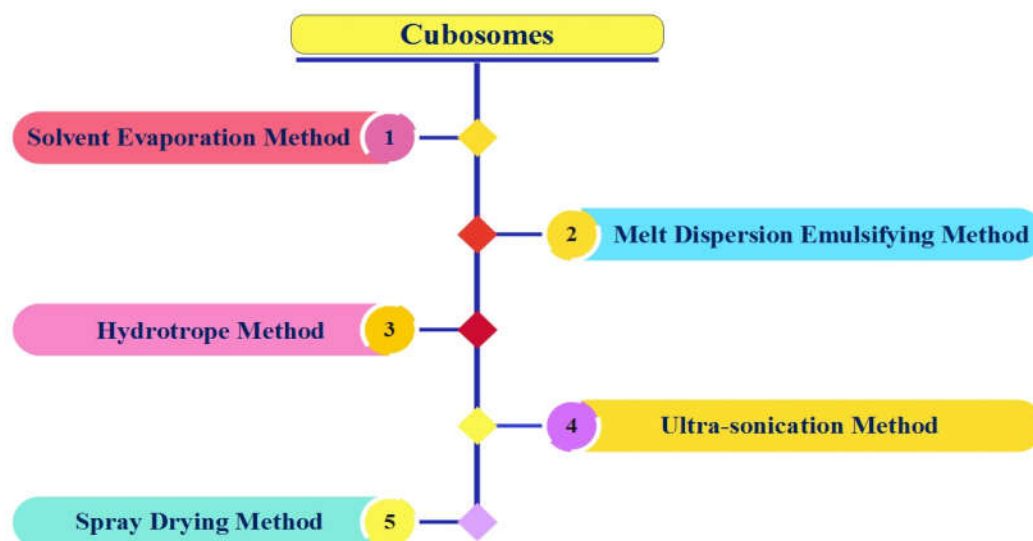


Fig. 5. Various techniques used for the formation of cubosomes

### 3.2 Solvent Evaporation Method

In this method, an organic solvent like ethanol or chloroform is used to dissolve the lipids which are then added dropwise to the aqueous solution of nonionic surfactant generally Pluronic (F108, F127, F68, P104) which act as a stabilizer. The mixture is maintained at an elevated temperature under magnetic stirring. The drug can be dispersed in the lipid or the aqueous surfactant solution [56]. Stirring under elevated temperature removes the volatile organic solvent and the mixture is homogenized by ultra-sonication or homogenizer resulting in the formation of cubosomes. The lipid-surfactant mixture can also be dissolved with an organic solvent wherein the organic solvent is removed under vacuum and the dry lipid-surfactant film is redispersed using an aqueous phase [57]. Ou et al. [56] used this technique for the preparation of cubosomes loaded *Achyranthes bidentata* polysaccharide.

### 3.3 Ultra-Sonication Method

Murgia et al. [58] prepared quercetin-loaded cubosomes stabilized by co-block polymers using this technique. Cubosomes are prepared by melting the amphiphilic lipid, generally, monoolein, and then dispersing it in an aqueous solution of Pluronic<sup>®</sup> like F-108 [58, 59] or Pluronic<sup>®</sup> F-127 [60] maintained at elevated temperature using magnetic stirring. The drug is generally dispersed in the molten lipid using a bath sonicator and the dispersion of lipid-surfactant solution is subjected to ultra-sonication to prepare cubosomes.

### 3.4 Spray Drying Method

In the spray drying process, the lipid-surfactant-solvent mixture is atomized with the wave of hot air resulting in the rapid solvent evaporation and formation of dry powder of cubosomes precursor. This technique is simple, cost effective, and normally easy to scale up. Briefly, the amphiphilic lipid with or without Pluronic is dissolved in ethanol [61, 62] or a binary solvent mixture, for example, methanol/chloroform [63]. An aqueous phase consisting of a hydrophilic solid carrier such as dextran or sorbitol is mixed with the lipid-ethanol solution under stirring which results in the development of a low viscosity emulsion. The drug can be dissolved along with the lipid in the organic solvent or mixed with an aqueous solution of the solid carrier. The lipid-ethanol-dextran-water quaternary system can then be efficiently spray-dried to evaporate the organic

solvent and water resulting in the formation of dry lipid-coated powder precursor which can be easily redispersed in water to form cubosomes.

### 3.5 Hydrotrope Method

This technique is also referred to as the solvent dilution process. Spicer et al. [64] used this method for the formation of cubic liquid crystalline nanoparticles. It involves dispersion of molten amphiphilic lipid in a hydrotrope such as ethanol or chloroform followed by dropwise addition of an aqueous phase of Pluronic (F108, F127, P105, P105) under stirring [63, 64]. Ternary phase diagrams of lipid, hydrotrope and water are plotted to determine the extent of dilution required for the formation of cubosomes. The formation of cubosomes with this method is generally achieved with low energy input methods such as vortex or magnetic stirring, however, further size reduction requires the use of a high-pressure homogenizer.

## 4. INFLUENCE OF pH-SENSITIVE POLYMERS ON STRUCTURE AND RELEASE OF DRUGS FROM CUBOSOMES

With a focus to study the effect of pH on the assembly of the cubic phases, several pH-dependent drug delivery systems are developed [65]. pH is an essential physiological condition that is required by all biological organisms. For the pathological such as inflamed or infected areas, pH-sensitive nanomedicines are used to treat these areas [66]. Improved target release of encapsulated drugs from pH-responsive nanocarriers depends on the site of application [65, 66]. Li et al. used a system of monoolein, stabilizers and, linoleic acid to develop pH-based nanocarriers. This system provides tunable release of the drug. The drug release was reversibly regulated and was found to be ideal for the targeted distribution of the drug to the various pH conditions of the gastrointestinal tract [66]. The linoleic acid undergoes both protonated and deprotonated states while shifting the pH from neutral conditions to acidic conditions. The developed nanocarriers formed experienced a phase transition from the reverse bicontinuous phase to the hexagonal phase [67].

Nakano et al. assessed the effect of linoleic acid on the blank monoolein-based cubosome structures by small-angle X-ray scattering (SAXS). The cubic phase Im3m pattern at pH 7.0 changes to a reverse hexagonal phase at pH 6.0



indicating the reactive responsiveness of linoleic acid to change with pH condition [68]. Moreover, an increased linoleic acid content was observed to change the internal liquid crystalline structure from a bicontinuous cubic phase to an inverted hexagonal phase with further inversion to an inverted cube organization phase. Therefore, their internal structures depended heavily on the existing pH condition [65]. Due to the deprotonated condition of the carboxylates, linoleic acid stabilizes the Im3m cubic symmetry through electrostatic self-repulsion in the polar portions of the lipid bilayers at pH 7.0. At pH 2.0, the electrostatic effect disappeared with the carboxylates protonation, resulting in the phase change from a cubic phase to an inverse hexagonal phase (HII) [65-68].

In order to induce pH sensitive phase transition, lipid pyridinyl methyl linoleate (PML) was integrated into monolinolein (MLO) bilayers which were recently synthesized with a precise percentage. Negrini et al. showed that the amphiphilic PML molecules have different characters and are a weak base and, neutral at pH 7.0 but showed a positive charge when exposed to the acidic pH environment which occurred due to the protonation process. [65-67] Using doxorubicin as a target, studies disclosed that at pH 5.5, the release of the drug increased ten<sup>10</sup> times in an invitro simulated tumor environment of the human colon cancer cell. Under normal physiological conditions, only a limited release of the drug occurred [69].

Nazaruk studied the electrochemical activity of doxorubicin drug integrated with monoolein cubic phases under various pH conditions [70]. The studies indicated that at pH 5.8, doxorubicin remains primarily in the cubic lattice water channels and the drug was 99.6% protonated. As a result, the release of drug at acid pH from the cubic lipid structure was faster as compared with the alkaline conditions. Thus, the uncharged molecules are predicted to exist predominantly in lipid membrane hydrophobic domains. Therefore, when DOX was kept in the alkaline condition certain changes occur such as the entrapment of the uncharged drug in the lipid cubic phase which results in a degraded release rate [65-70].

## 5. APPLICATIONS OF CUBOSOMES AS DRUG DELIVERY SYSTEM

Cubosomes which are soft nanoparticles are characterized by a lyotropic liquid crystalline core (e.g., monoolein or phytantriol) prepared in

water. Cubosomes are lipidic nanoparticles that are crystalline and isotropic stabilized by poloxamers. [71,72]. It is made up of a network of two independent aqueous channels formed by a three-dimensional, curved, and non-intersecting lipid double layer placed over an infinite periodic minimum surface with cubic symmetry. Cubic lipid nanoparticles (Cubosomes) constitute unique features such as their unique cubic structure which permits them to incorporate highly lipophilic, hydrophilic as well as amphiphilic drugs [73-75]. Furthermore, the lipid excipients employed in the creation of cubosomes, such as monoolein and Phytantriol, are biodegradable and biocompatible materials, implying that these cubic nanoparticles are safe for nanocarrier drug delivery [76]. Cubic lipid nanoparticles have a highly stable cubic shape that allows for a slower rate of dissociation, improved drug retention, and site-specific drug delivery [77]. The architecture of cubic particles provides suitability in the drug delivery system as compared to other lipids-based nanocarriers such as solid lipid nanoparticles (SLN) due to their drug expulsion to the surface of nanoparticles [73, 78]. Furthermore, the cubic structure and features are critical in achieving their critical design requirements. The optimal size of cubosomes is between 10 and 500 nm, which is desired for both oral and transdermal drug delivery [79]. These classical features of cubosomes as drug delivery systems provide effective tissue penetration, improved accumulation in the tumor site, improved pharmacokinetics and pharmacodynamic profiles [79, 80]. The polymer part used in the preparation of cubosomes is considered to provide the stability to the cubic structure. Block copolymers utilized in cubosomes exhibited di-block or tri-block hydrophilic and hydrophobic segments viz poloxamer P84, F127, F68, and P108 [81]. These block copolymers are responsible for the incorporation of the hydrophilic and hydrophobic drug in the cubic phase. The lipid part of cubosomes and the hydrophobic segment of poloxamers are usually involved in the incorporation of lipophilic drugs, while the hydrophilic portion of lipids and poloxamer provide solubilization to the hydrophilic drugs [82, 83].

The physical and biological attributes of cubic nanoparticles are mainly defined by the material being utilized in the preparation of the carrier system. Therefore, the selection of a suitable excipient is an important step required in successful delivery. Also, the clinical attributes

such as bio-distributions, pharmacokinetics, and toxicity depend on the physical or chemical properties of the delivery system. Apart from these, solubilization of hydrophobic/hydrophilic drugs and controlled release from core-shell, require the selection of a suitable polymer for the nanocarrier drug delivery. Encapsulation of hydrophilic and hydrophobic drugs inside the nanomaterial is mainly governed by the drug excipients compatibility. The hydrophilic-lipophilic balance (HLB) of the block copolymer and the polymer-to-drug ratio are other important factors in effective drug encapsulation. Monoolein has received a lot of interest in recent years because of its unique structure, which allows for the integration of both lipophilic and hydrophilic drugs with a slow-release rate [84]. Monoolein is also a biodegradable and biocompatible substance that exhibits considerable anticancer drug solubilization Exhaustive literature review reported monoolein as a comprehensive lipid excipient used in the preparation of cubosomes as a delivery system. Dawoud et al. [80]. prepared monoolein cubic nanoparticle as a novel carrier for docetaxel for improved solubilization. It was concluded that the increasing amphiphilic lipid concentration improved the solubility of a lipophilic drug. Finally, improved solubilization provides an enhanced anticancer effect as compared to an aqueous solution of free drugs. In vivo studies demonstrated that docetaxel loaded cubic nanoparticles resulted in improved anticancer effect as compare to docetaxel solution, as tumor volume and weight was significantly lower in mice injected with docetaxel loaded cubic nanoparticles compared to animals treated with docetaxel solution. Mohamed et al. [84] prepared cubic nanoparticles through the hot-melt extrusion method for effective delivery of 5-Fluorouracil for liver targeting. The prepared cubosomes were found to be effective in the liver cancer model. Histology studies showed greater 5-FU concentration in the liver tissues resulting in enhanced hepatocellular damage. In the stability testing of the 5-fluorouracil (5-FU) the gel was disrupted using water with the help of vortex mixing to obtain the cubosomal gel. The gel was kept for 3 months at a temp of 4-8°C. When formulation was tested after 3 month it was observed that mean particles size of the gel was increased when compared to the previous that is  $105.70 \pm 5.47$  nm to  $112.34 \pm 2.6$  nm. But when EE (%) was measured an observable decrease was noted that is  $31.21 \pm 2.83$  lowered to  $29.11 \pm 0.62$ . From this result it was concluded that changes were not major that would

adversely affect the formulation. Mohamed et al. [85] studied the effective treatment of gout through a transdermal route using colchicine (COL) cubosomes as drug delivery approach. In rats, the effects of transdermal administration of COL cubosomal gel on systemic drug absorption were compared to oral COL solution. The in vivo investigation showed that transdermal application of COL cubosomal gel considerably enhances drug absorption compared to oral COL solution, with 4.6 fold enhancement in relative bioavailability as compare to oral COL solution. The potential side effects of colchicine were reduced using the cubosomes as nanocarrier for drug as a targeted approach and found to improved colchicine bioavailability. Cubosomes were evaluated for their toxicity and cellular uptake potential using different state of art techniques such as dynamic light scattering (DLS), Small-angle neutron scattering (SANS), Atomic force microscopy (AFM), Fluorescence-Activated Cell Sorting (FACS), and cell line studies for effective antitumor activity. The prepared system was found to be effective for cellular uptake and improvised toxicity [86]. Phytantriol is highly recommended for the cubosomes preparation over the monoolein for the cubic lipid phase due to its more stabilized cubic structure which protects the carrier system from esterase catalytic hydrolysis. Fan et al. [87]. incorporated cefpodoxime proxetil (CP) incorporated into cubosomes using phytantriol as lipid and poloxamer 407 as a stabilizer for paediatric oral delivery. The small-sized nanoparticles were obtained from cubosomes through a top-down method with effective oral sustained delivery of CP. The electronic tongue was utilized for successful taste masking of prepared formulation resulting in the improved paediatric patient's compliance by masking the bitterness of drug. The stability of CFP-LLCNs was investigated for three months. The results indicated that sample particle size and encapsulation effectiveness was consistent throughout a three-month period. Cubosomes have also been prepared by utilizing an endogenous lipids linoleoylethanolamide (LEA) and were evaluated for their chemical stability, *in vitro* cellular toxicity studies, and CNS treating potential. LEA was protected from hydrolysis and an improved blood-brain barrier was observed through cubosomes delivery [88]. *In situ* hydrogel for ocular delivery of ciprofloxacin loaded cubic nanoparticles was prepared by Alharbi et al. [89]. to provide more retention effect of carrier system for treatment of conjunctivitis and corneal ulcers. The selection of



lipid to polymer ratios was estimated by applying the Box-Behnken experimental mixture design. The developed formulation was found to improve eye permeability, ocular retention duration and boosts antibacterial activity. The MIC values of CF hydrochloride marketed drops and Cf based cubosomes formulation in aqueous humour of rabbit eye was determined. The results showed CF cubosomes improved ocular bioavailability and reduced administration frequency from three times daily to once daily. Polygonatum sibiricum polysaccharide have also been utilized for cubosomes preparation owing to its antitumor and anti-virus potential. It was found that for rapid recovery of the spleen index

polysaccharide group could be used, also they help in increasing the proliferation of T and B lymphocytes along with the phagocytosis of peritoneal macrophages with the initiation of immune inhibitor by cyclophosphamide when equated with the cyclophosphamide group [90]. Polyphosphoester (PPEs) analog of Pluronic F127 were utilized for stabilized cubic nanoparticle preparation. With holding unique stability property and degradable nature the PPEs are polyesters which are based on phosphoric acid derivative [91]. Table 1 encompasses the list of drugs incorporated in cubosomes for various objectives.

**Table 1. Drugs incorporated in cubosomes**

Drug used	The objective of the study	The brief outcome of the study	Ref
Docetaxel	Development and evaluation of docetaxel loaded thermosensitive depot cubosomes for control release.	Thermosensitive depot cubosomes loaded with docetaxel offered gradual drug release, cubosome preparation was free flowing at room temperature and changed to the depot at body temperature.	[92]
Elesclomol	Mitochondrial targeting Elesclomol cubosomes: <i>in vitro</i> evaluation with A549 and A431 cancer cell line	A549 and A431 cancer cell line readily absorbed nanoparticles, resulting in enhanced localized distribution. Elesclomol and copper complexation provide more cytotoxic effect through the cubosomes delivery system.	[93]
Cisplatin and Paclitaxel	Dual drug-loaded system: method evaluation and anticancer activity	The top-down approach provided little aggregates with larger surface area and uniform encapsulation of the drugs without any crystallization.	[94]
Doxorubicin	Phytantriol cubosomes for reducing cardiotoxicity of doxorubicin and improved intestinal permeability using <i>in vitro</i> cell line studies	Doxorubicin fabricated cubosomes provided higher drug loading, pH sensitivity, and controlled release at the specific site. Carrier system provides enhanced cell cytotoxicity with minimum undesired side effects	[95]
Indomethacin	Evaluation of Indomethacin fabricated cubosomes for anti-inflammatory activity	Homogenized monoolein and poloxamer containing cubosomes formulation was successfully obtained with prolonged delivery of lipophilic drug through the skin	[96]
Antimicrobial peptide LL-37	The antimicrobial potential of LL-37 was evaluated through cubosomes using <i>in vitro</i> and <i>ex-vivo</i> skin irritation models	Cubic nanoparticle provides superior protection to LL-37 against enzymatic degradation and significant bactericidal effect with controlled release. LL-37 fabricated cubic nanoparticle reduces the skin irritation due to LL-37.	[97]
Ketorolac	Monoolein and poloxamer cubic nanoparticle for ocular delivery of ketorolac	Optimized formulation of Ketorolac loaded cubosomes expressed desired nano-sized particles with higher encapsulation of ketorolac. In addition, trans corneal permeation and retention	[98]

<b>Drug used</b>	<b>The objective of the study</b>	<b>The brief outcome of the study</b>	<b>Ref</b>
Tropicamide	Tropicamide loaded cubic nanoparticles were prepared for ocular application.	of ketorolac was increased with cubosomes containing ketorolac. Fast level onset and higher intensity of mydriatic actions was observed with tropicamide loaded cubic nanoparticles.	[99]
Amphotericin B	Enhancing solubilization and bioavailability of Amphotericin B using cubic nanoparticles	The formulation was suitable for oral delivery of AmB. Control release of AmB was achieved through cubosomes for oral administration	[100]
Flurbiprofen	Ophthalmic delivery of Flurbiprofen cubic nanoparticles to achieve improved bioavailability with reduced skin irritation	Improved corneal delivery and improved bioavailability of flurbiprofen was achieved through cubic nanoparticles.	[101]
Cyclosporine A	Cubic nanoparticles were prepared for ocular delivery of Cyclosporine A to achieve improved <i>In vitro</i> corneal permeation with reduced ocular irritation	A small size cubic nanoparticle was prepared with higher drug loading. Cubosomes possessed improved <i>in vitro</i> corneal permeation with enhanced retention effect and low ocular irritating properties.	[102]
Pilocarpine Nitrate (PN)	Ophthalmic delivery of pilocarpine nitrate to improve corneal permeation	PN fabricated nanoparticles exhibited improved bioavailability superior to the commercial formulation. Enhanced <i>in vitro</i> release and <i>ex vivo</i> permeation of cubic nanoparticles as compared to commercial formulation	[103]
Silver sulfadiazine	Cubosome hydrogels for topical treatment of burns and evaluated for their antibacterial potential through cubic nanoparticle	To bypass the cytotoxic effects of silver by adjusting the release of SSD the cubic liquid crystalline nanoparticles (cubosomes) Formulation was formed of SSD dispersions. It helped to reduce the dose of SSD to 0.2% and exhibited an improved and better healing with minimum side effects when compared with marketed formulation.	[104]
Dapsone	Improve skin permeation of Dapsone using cubic nanoparticles	The enhanced levels of dapsone were achieved with improved therapeutic activity at the targeted area by reducing the systemic side effects.	[105]
Fluconazole	Ocular delivery of fluconazole loaded cubosomes for the treatment of keratomycosis.	<i>In vivo</i> , ocular tolerance and histopathological studies demonstrated the effectiveness and safety of FCZ-loaded cubosome dispersion in the treatment of induced keratomycosis in rats.	[106]
Rapamycin	Transdermal and controlled delivery of rapamycin-loaded cubosomes for the treatment of psoriasis.	The rapamycin-carrying particles were eventually integrated into a polymeric matrix consisting of quickly dissolving microneedle pads. The engineered microneedles demonstrated effective piercing and deposition on a skin-imitating agarose gel of the filled cubosome-like particles. The studies illustrate the ability to transport cubosome-like particles into the skin and	[107]

Drug used	The objective of the study	The brief outcome of the study	Ref
Resveratrol	Resveratrol-loaded cubosomes were prepared for topical delivery for melanoma.	facilitate the continuous release of rapamycin The RC-Gel showed greater permeation and deposition of drugs in the skin layers of mice. The significant propensity of RC-Gel for skin localization was demonstrated by an in vivo bioavailability study. The studies showed that RC- gel cubosomes was successful topical drug delivery carrier for the treatment of melanoma.	[108]

Table 2. Various patents reported on cubosomes

S.No	Patent no/	Inventor name/ Year	Summary	Ref
1	US2007017614 3A/	Nissim et al., 2004	Ternary system having cubic-like nanosized symmetry. The ternary system may be dispersed and which can be used as a solubilizing medium for hydrophobic and hydrophilic substances.	[112]
2	US6936187B2/	Lynch et al. 2005	Cubic precursor's gels, dispersions, and cubic gel particles used to deliver active ingredients to substrates.	[113]
3	US6994862B2/	Chung et al. 2006	US patent granted for the development of novel solubilizing composition by involving monoglycerides, emulsifiers, and organic solvents. The resulted liquid formulation was claimed to disperse in water without any physical force.	[114]
4	W02007140510 A1/	Dong et al. , 2007	Agrochemical composition containing cubosome liquid crystal particles composed of phytantriol and a stabilizer.	[115]
5	WO2010060131 A1	Patrick et al. , 2009	The present patent deals with invention of a contrast agent for diagnostic imaging based on lyotropic liquid crystal phase, in which the lyotropic liquid crystal phase is dispersed as nanodroplets, and a stabiliser for providing stabilisation against re-aggregation of the nanodroplets.	[116]
6	US7807188B2/	Hoath et al. , 2010	A composition skin growth and healing when applied to the intact skin and provides a water repellent barrier and moisturizing effect.	[117]
7	KR102013804B 1/	Park et al. , 2016	A cosmetic composition for protecting the skin comprising of cubosome containing ceramide and phytosphingosine as an active ingredient. Specifically, the cubosome improve the skin moisturizing effect.	[118]

## 6. CHARACTERIZATION OF CUBOSOME

Crystalline nanoparticles prepared using different lipids and polymeric excipients are evaluated for their internalized characteristics via cryo-

transmission electron microscopy (cryo-TEM), Atomic force microscopy (AFM), and small-angle X-ray diffraction (SAXD). Compared to transmission electron microscopy, cryo-transmission electron microscopy proved to be

more significant for internalized morphological structure of cubic nanoparticles. Gel permeation chromatography or ultra-filtration procedures can be used to determine the entrapment efficiency and drug loading. The concentration of untrapped drug is measured, and deducted from the overall quantity of drug added. A UV spectrophotometer or HPLC analysis is used to determine the quantity of drug. The particle size and morphological characterization is an

important step in the design of a drug delivery system. Furthermore, the molecular behaviour of the cubosomes is widely studied using small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) depicted in fig 6. Organoleptic and morphological features are used to investigate the physical stability of cubosomes. Particle size distribution and drug content can be assessed at various time periods to assess the potential changes [109-111].

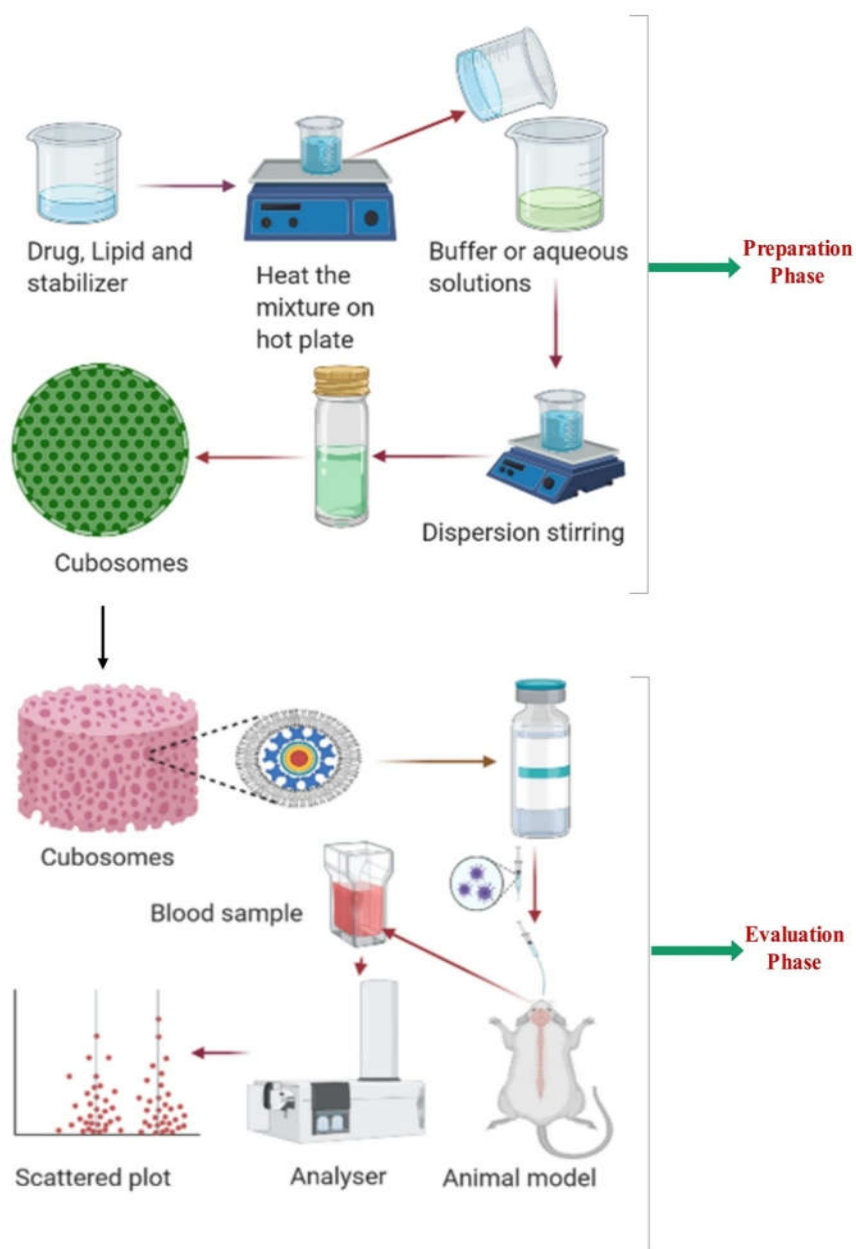


Fig. 6 Preparation and characterization of lyotropic liquid cubic nanoparticles

## 7. PATENTS ON CUBOSOMES

In the recent past, various research groups have explored the applications of cubosomes in different fields. Various patents have been filed on compositions and techniques containing cubosomes as delivery systems which are listed in Table 2.

## 8. CONCLUSIONS

It is concluded from the review of literature that cubosomes are inverse bicontinuous curved cubic phase lyotropic liquid crystals that are formed by mixing certain amphiphiles such as glyceryl monooleate in water using appropriate stabilizers under suitable conditions of hydration and temperature. The inner structure of cubosomes provides a significantly higher membrane surface area for the loading of active molecules as compared to liposomes. Cubosomes can simultaneously solubilize both hydrophobic and hydrophilic pharmaceutical actives as well as amphiphilic compounds which is convenient when compared to liposomes or emulsion-type drug delivery systems. Owing to the higher lipid ratio in the bilayer membrane and stronger electrical repulsion between them, the cubosomes possess greater membrane stability than the liposomes. Cubosomes are progressively considered as an ideal nanocarrier for drug delivery applications due to their inherent physical stability as compared to related carriers such as liposomes; simultaneous delivery of hydrophilic, lipophilic, and amphiphilic molecules; mucoadhesion properties; controlled delivery of molecules because of their porous three-dimensional architecture, which creates a tortuous diffusion pathway for entrapped drug molecules.

## CONSENT AND ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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