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Cubosomes as Bicontinuous Cubic Liquid Crystalline Nanocarriers: A Comprehensive Review of Composition, Preparation, Characterization, Therapeutic Applications and Translational Prospects

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Abstract: Lipid-based nanocarriers have reshaped pharmaceutical development, yet most clinically established systems, such as liposomes and solid lipid nanoparticles, are built on lamellar or crystalline architectures that offer a limited internal surface for drug loading. Cubosomes are colloidal dispersions of bicontinuous inverse cubic liquid crystalline phases in which a single, continuously curved lipid bilayer is folded in three dimensions to separate two interpenetrating but non-intersecting networks of water channels. This honeycomb-like architecture provides a very large interfacial area, compartments for hydrophilic, lipophilic and amphiphilic molecules within one particle, and a tortuous diffusion path that slows drug release. This review consolidates current knowledge on cubosomes for pharmaceutical scientists. It first describes the structure-forming lipids, mainly glyceryl monooleate and phytantriol, the role of steric stabilizers such as Poloxamer 407 and Poloxamer 188, and the crystallographic features of the Im3m, Pn3m and Ia3d phases. It then compares top-down, bottom-up and emerging microfluidic and continuous manufacturing routes, and outlines a characterization workflow that combines dynamic light scattering, cryogenic electron microscopy, small-angle X-ray scattering, zeta potential, entrapment, release, rheology and storage stability. Applications in oncology, dermal and transdermal delivery, ocular, nasal and mucosal delivery, delivery of peptides, proteins and nucleic acids, cosmeceuticals and nutraceuticals, and theranostics are critically discussed. Finally, the review examines persistent barriers to translation, including phase transformation in biological media, scale-up and sterilization, stabilizer-associated toxicity and regulatory expectations for nanomaterials, and outlines future directions in stimuli-responsive, ligand-targeted, data-driven and personalized cubosome design.

Keywords: *Cubosomes; Lyotropic Liquid Crystals; Bicontinuous Cubic Phase; Glyceryl Monooleate; Phytantriol; Poloxamer 407; Small-Angle X-Ray Scattering; Nanomedicine; Drug Delivery.*

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I. INTRODUCTION

➢ *Overview of Nanotechnology-Driven Drug Delivery*

The therapeutic value of a drug depends not only on its intrinsic pharmacological activity but also on whether an adequate concentration reaches the site of action for an adequate time without causing unacceptable toxicity elsewhere. A large fraction of new chemical entities are poorly water soluble, chemically labile or rapidly cleared, and many biological agents such as peptides, proteins and nucleic acids cannot cross epithelial or cellular barriers unaided. Nanotechnology addresses these limitations by packaging the active ingredient into carriers with dimensions typically between ten and a few hundred nanometers, whose surface chemistry, size and internal structure can be tuned to control solubilization, protection, biodistribution and release [1, 2].

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Over the past three decades, polymeric nanoparticles, micelles, dendrimers, inorganic particles and lipid assemblies have been explored, and the field has progressively moved from simple encapsulation towards "precision" carriers engineered to overcome specific biological barriers and to respond to patient-level heterogeneity [3].

Despite this progress, the efficiency with which systemically administered nanoparticles reach their intended target remains modest, and a meta-analysis of tumor-targeting studies highlighted that only a small proportion of the injected dose typically accumulates in solid tumors [4]. This observation has shifted attention towards carriers that combine high payload capacity, biocompatible and biodegradable constituents, controllable release kinetics and amenability to surface functionalization. These requirements explain the sustained interest in lipid-based systems, whose components are frequently endogenous or generally regarded as safe, and whose self-assembly can be exploited to create well-defined internal nanostructures.

➢ *Importance of Lipid-Based Nanocarriers*

Lipid-based nanocarriers represent the most clinically mature class of nanomedicines. Liposomes, the first nanocarriers approved for parenteral use, demonstrated that encapsulation could alter the pharmacokinetics and toxicity profile of cytotoxic drugs, as illustrated by PEGylated liposomal doxorubicin [5, 6]. Solid lipid nanoparticles and nanostructured lipid carriers were subsequently developed to provide a solid lipid matrix with improved physical stability and scalability by high-pressure homogenization [7]. More recently, ionisable lipid nanoparticles enabled the clinical delivery of small interfering RNA and messenger RNA, culminating in the rapid deployment of mRNA vaccines and demonstrating the capacity of lipid assemblies to deliver fragile macromolecules at a global scale [8, 9]. Collectively, these platforms have shown that lipid carriers can be manufactured reproducibly, sterilized, characterized and approved by regulatory agencies [10].

Nevertheless, the dominant lipid carriers have structural limitations. Liposomes possess a single aqueous core enclosed by one or a few lamellar bilayers, so the space available for membrane-associated or amphiphilic drugs is restricted to a relatively small bilayer volume, and leakage of small hydrophilic molecules can be rapid. Solid lipid nanoparticles may expel drug on polymorphic transition of the lipid matrix during storage [7]. These constraints have motivated interest in non-lamellar lyotropic liquid crystalline phases, in which amphiphilic lipids self-assemble in water into ordered, highly curved, nanostructured mesophases that are thermodynamically stable in excess water [11, 12]. Among these, the bicontinuous cubic and inverse hexagonal phases can be dispersed into nanoparticles termed cubosomes and hexosomes, respectively [13, 14].

➢ *Emergence and Relevance of Cubosomes in Pharmaceutics*

The structural basis of cubosomes lies in the bicontinuous cubic phases first described for lipid–water systems by Luzzati and later analyzed in detail by Larsson, who related their geometry to triply periodic minimal surfaces and proposed their biological relevance [15, 16]. The term "cubosome" was coined to describe discrete, submicron particles that retain the internal cubic lattice of the parent bulk phase. Gustafsson and co-workers demonstrated that a bulk monoolein–water cubic phase could be fragmented into stable submicron particles in the presence of a polymeric stabilizer, providing the first practical route to colloidal cubic dispersions [17, 18]. Shortly thereafter, Spicer and colleagues introduced a low-energy hydrotrope dilution process and powder precursor approaches that made cubosome production more amenable to industrial processing [19–21].

Since then, cubosomes have attracted wide attention because of a combination of properties that is rare among nanocarriers: a large internal surface area, the ability to co-encapsulate hydrophilic, hydrophobic and amphiphilic molecules, a viscous, bioadhesive and biodegradable lipid matrix, protection of labile actives from enzymatic and chemical degradation, and sustained release governed by the geometry of the water channels [22–24]. Their nanostructure can be modulated by temperature, pH, ions, enzymes and light, which opens possibilities for responsive and on-demand delivery [25, 26]. As shown in Fig. 1, the number of publications on cubosomes indexed in OpenAlex has risen steadily, from only a few papers per year in the late 1990s to more than a hundred per year in the 2020s, reflecting a transition from fundamental colloid science to applied pharmaceutics.

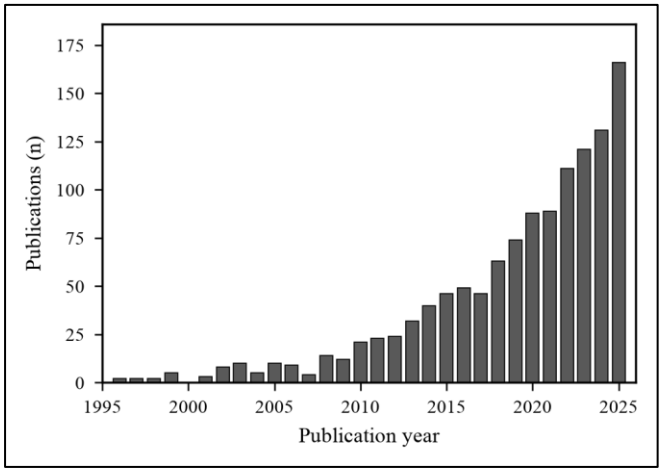

Fig 1 Annual Number of Publications on Cubosomes, 1996–2025 (OpenAlex Records with 'Cubosome' or 'Cubosomes' in the Title or Abstract; n = 1,210; Retrieved 18 November 2026).

Several reviews have addressed individual aspects of cubosome science, including colloidal design principles [21, 27], liquid crystalline particles for drug delivery [28, 29], drug delivery and imaging [14, 30, 31], comparisons with other lipid nanoparticles [32], structural components and preparation techniques [33], cancer therapy [34, 35] and food or nutraceutical applications [36]. The present review aims to provide an integrated and practically oriented account that links molecular composition and phase behavior to preparation methods, critical characterization techniques and therapeutic performance, and that places specific emphasis on the barriers that must be overcome for clinical translation. The review is

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structured as follows: Section II describes composition and structure; Section III compares methods of preparation; Section IV discusses characterization and evaluation; Section V reviews therapeutic applications; and Section VI considers challenges, limitations and future perspectives.

II. COMPOSITION AND STRUCTURAL CHARACTERISTICS

Cubosomes are usually composed of three classes of components: a structure-forming amphiphilic lipid that generates the bicontinuous cubic phase, a stabilizer that prevents aggregation and coalescence of the dispersed particles, and the aqueous medium, to which drugs and functional additives are added. The principal components and their functions are summarized in Table 1.

Table 1 Principal Components of Cubosome Formulations and their Functions

Component	Examples	Function	Remarks
Structure-forming lipid	Glyceryl monooleate (monoolein, GMO)	Forms Ia3d and Pn3m cubic phases; Pn3m coexists with excess water	Biodegradable, food grade; susceptible to hydrolysis, lipolysis and batch variability [37, 38]
Structure-forming lipid	Phytantriol	Forms Pn3m phase in excess water at body temperature	Non-ester, chemically stable; higher cytotoxicity than GMO [39, 40]
Other cubic-phase lipids	Glycerate surfactants, selachyl alcohol, phospholipid–glycerol dioleate mixtures	Tune mesophase, lattice parameter and degradation	Broaden the choice of lipids for depots and brain delivery [41–43]
Steric stabilizer	Poloxamer 407 (Pluronic F127)	PPO block anchors in bilayer; PEO chains give steric barrier	Partitions into bilayer; may swell Pn3m to Im3m and form vesicles [44, 45]
Steric stabilizer	Poloxamer 188 (Pluronic F68)	Weaker anchoring; used alone or as co-stabilizer	Generally less efficient than Poloxamer 407 [46]
Alternative stabilizers	Polysorbate 80, PEG-lipids, brush copolymers, polyphosphoesters, citrem	Reduce toxicity, avoid complement activation, enable targeting	Lower cytotoxicity and new functionality [47–51]
Functional additives	Fatty acids, vitamin E acetate, cationic lipids, ligand-lipids	Confer pH or temperature response, charge, nucleic acid binding, targeting	Basis of responsive and targeted cubosomes [52–55]

➢ *Lipids Used in Cubosome Formation (Monoolein, Phytantriol)*

The phase adopted by an amphiphile in water can be rationalized by the critical packing parameter, CPP = v/(a_0·l), where v is the volume of the hydrophobic chain, a_0 the effective head-group area at the interface and l the chain length [56]. Lipids with a CPP close to unity form flat bilayers (lamellar phase), whereas lipids with a bulky hydrophobic region relative to their head group (CPP slightly greater than one) prefer interfaces curved towards the water, giving rise to inverse bicontinuous cubic phases; further increase of the CPP favors the inverse hexagonal phase [57, 58]. Because CPP depends on hydration, temperature and the presence of additives, the same lipid can adopt different mesophases under different conditions, which is the physical basis of both the versatility and the instability of cubosomes [59].

Glyceryl monooleate (GMO, monoolein), a monoglyceride of oleic acid, is the most widely used cubosome-forming lipid. It is biodegradable, is hydrolysed in vivo by lipases to glycerol and oleic acid, and is included in pharmaceutical and food products [38]. The GMO–water phase diagram is rich: as water content increases at physiological temperature, GMO transitions from a lamellar crystalline and fluid lamellar phase through the gyroid Ia3d cubic phase to the diamond Pn3m cubic phase, which coexists with excess water and is therefore the phase retained in dilute dispersions [37]. Commercial GMO grades are mixtures of mono-, di- and triglycerides and free fatty acids, and batch-to-batch variation in their composition can alter phase behavior, a practical issue that formulators must control [38]. GMO is also susceptible to ester hydrolysis during storage and to lipolysis in biological fluids.

Phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) is a branched, non-ester amphiphile used for decades in cosmetic products. Its phytanyl chain and triol head group produce a Pn3m cubic phase in excess water at physiological temperature, which transforms into the inverse hexagonal phase at moderately higher temperatures [39]. Because it lacks an ester bond, phytantriol is chemically more stable than GMO and is not susceptible to enzymatic hydrolysis, which enables longer-lasting depots but raises questions about elimination and cytotoxicity [40, 45]. Other cubic-phase formers include monolinolein, phosphatidylethanolamines, glycerate surfactants, mixtures of phospholipids with glycerol dioleate, and blends of GMO with unsaturated fatty acids, triglycerides or citrem, which are used to tailor mesophase, lattice dimensions and biological interactions [41, 51, 60].

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➢ *Role of Stabilizers (Poloxamer 407, Poloxamer 188)*

Bulk cubic phases are highly viscous gels, and their fragments tend to fuse back into the bulk phase. Colloidal stability is therefore achieved with steric stabilizers that adsorb to or insert into the particle surface. Poloxamer 407 (Pluronic F127), a triblock copolymer of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) with long hydrophilic blocks, has been the stabilizer of choice since the earliest studies [18]. Its hydrophobic propylene oxide block anchors in the lipid bilayer, while the ethylene oxide chains extend into the surrounding water to provide a steric barrier [44]. However, a fraction of the polymer also partitions into the internal bilayer, where it can swell the lattice and drive the transition from Pn3m to the more hydrated Im3m phase, and excess polymer may produce coexisting vesicles [44, 45].

Poloxamer 188 (Pluronic F68), which has a shorter hydrophobic block, anchors less strongly and generally produces less efficient stabilization, although it is sometimes combined with Poloxamer 407 to modulate particle size and phase [46]. High-throughput screening of block copolymers has shown that the balance between the hydrophobic anchor and hydrophilic shell governs both stabilization and preservation of internal order [46, 61]. Alternative stabilizers including polysorbate 80, PEGylated lipids, RAFT-synthesized brush copolymers, polyphosphoester analogues of Poloxamer 407, citrem and even protein or polysaccharide coatings have been introduced to reduce the stabilizer content, improve biocompatibility, avoid complement activation or enable targeting [47–51, 62].

➢ *Bicontinuous Cubic Phases (Im3m, Pn3m, Ia3d)*

The bilayer in a bicontinuous cubic phase is draped over a triply periodic minimal surface, a surface with zero mean curvature at every point and negative Gaussian curvature, which divides space into two congruent, interwoven water networks [15, 16]. Three such phases are commonly encountered in lipid–water systems (Fig. 2):

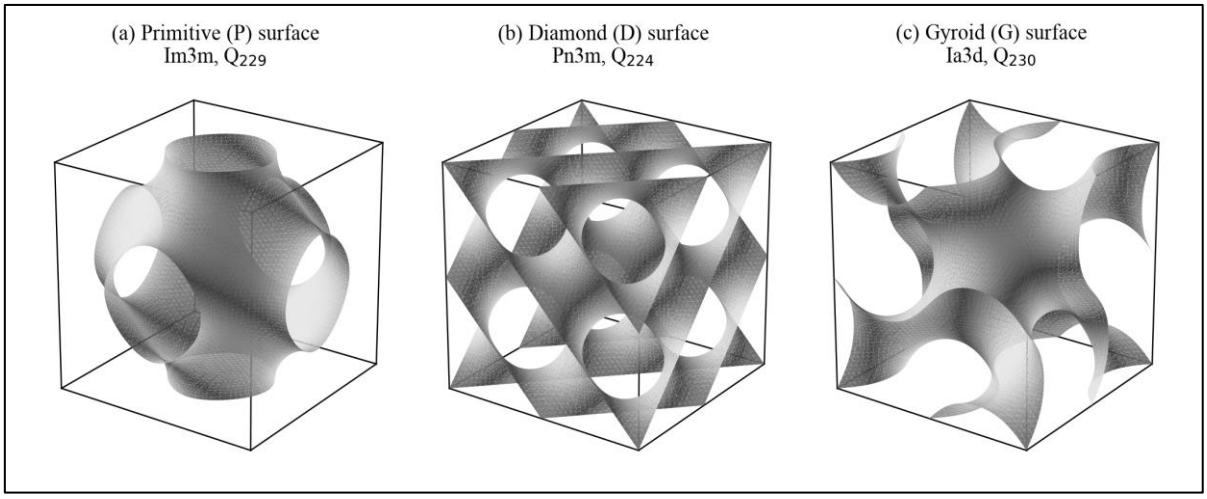

Fig 2 Unit Cells of the Three Bicontinuous Cubic Phases Found in Cubosomes, shown as Nodal Approximations of the Triply Periodic Minimal Surfaces: (a) Primitive Surface, Im3m; (b) Diamond Surface, Pn3m; (c) Gyroid Surface, Ia3d. The Lipid Bilayer Follows Each Surface and Separates Two Interpenetrating Water Networks.

- The primitive or P-surface phase, crystallographic space group Im3m (Q229), in which each water channel node is connected to six neighbours; it is typically the most hydrated cubic phase and is often stabilized in dispersions containing high Poloxamer 407 contents [45].
- The diamond or D-surface phase, space group Pn3m (Q224), in which each node connects four channels in a tetrahedral arrangement; it is the equilibrium phase of GMO and phytantriol in excess water at body temperature [37, 39].
- The gyroid or G-surface phase, space group Ia3d (Q230), in which three channels meet at each node; it occurs at lower hydration and is generally not stable in excess water [37].

The three surfaces are related by the Bonnet transformation, so that for a lipid of fixed geometry the lattice parameters of the Im3m, Pn3m and Ia3d phases are approximately in the ratio 1.279:1:1.576 relative to Pn3m [57, 58]. This relationship is often used to confirm that an Im3m phase observed in a dispersion has arisen from swelling of the Pn3m phase by the stabilizer. Typical lattice parameters for GMO and phytantriol cubic phases in excess water are in the range of about 6–15 nm, corresponding to water channel diameters of a few nanometers [37, 39].

➢ *Internal 3D Architecture and Its Importance*

The internal architecture of cubosomes underpins their pharmaceutical attributes. First, the bilayer is continuous throughout the particle and folded on a nanometer scale, giving an interfacial area that is much greater than that of a liposome of the same size and allowing amphiphilic and membrane-associated molecules to be loaded at high levels [27, 63]. Second, the two water networks are continuous but closed from each other, so hydrophilic molecules can reside in the channels while lipophilic molecules partition into the hydrocarbon region, enabling co-delivery within a single carrier [13]. Third, the channel diameter is comparable to the size of small proteins, and diffusion through the tortuous network results in release kinetics that depend on the size of the solute relative to the channel and on the lattice parameter [64, 65]. Finally, the bilayer geometry resembles that of fusogenic intermediates, which may favor membrane fusion and intracellular delivery

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[66, 67]. The capacity of the lipidic cubic phase to accommodate and stabilize membrane proteins has been exploited in membrane protein crystallography, underlining the gentle, membrane-mimetic environment that cubic phases provide to macromolecules [68, 69]. Extremely swollen cubic phases with channels large enough to host large proteins have also been reported, pointing towards future carriers for biologics [70].

III. METHODS OF PREPARATION

Cubosome preparation methods are conventionally classified as top-down or bottom-up, depending on whether particles are produced by fragmentation of a pre-formed bulk cubic gel or by self-assembly from molecular solution [21, 23]. Emerging approaches based on microfluidics and continuous processing aim to combine the advantages of both. The general scheme is shown in Fig. 3 and the methods are compared in Table 2.

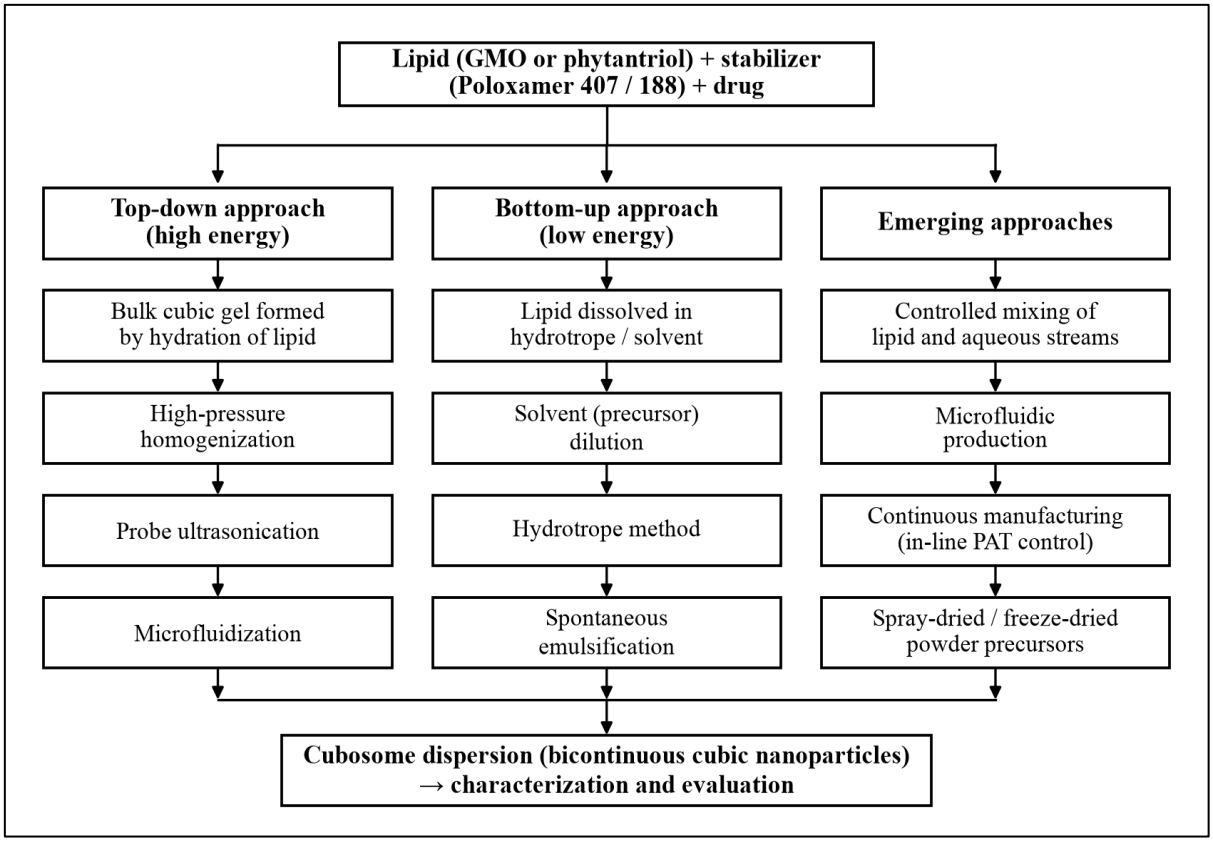

Fig 3 Classification of Cubosome Preparation Methods into Top-Down (High-Energy), Bottom-up (Low-Energy) and Emerging Approaches. PAT: Process Analytical Technology.

➢ *Top-Down Techniques*

In top-down processes, the molten lipid is first hydrated with an aqueous stabilizer solution to form a viscous bulk cubic phase, which is then dispersed by application of high mechanical energy [17, 71]. These methods are robust and solvent-free, and they are well suited to GMO- and phytantriol-based systems, but the high viscosity of the gel and the energy input may degrade labile actives and generate heat.

- *High-Pressure Homogenization*

High-pressure homogenization forces the coarse dispersion through a narrow gap at high pressure, where cavitation, shear and turbulence fragment the cubic gel. Repeated cycles reduce particle size and narrow the distribution, and the process is directly scalable because homogenizers are already used industrially for emulsions and solid lipid nanoparticles [7, 71]. Systematic studies have shown that the number of cycles, pressure, temperature and Poloxamer 407 concentration influence size, the proportion of vesicular by-products and whether the Pn3m or Im3m phase is retained [71, 72]. Moderate heating during homogenization can facilitate dispersion, but excessive temperature may transiently shift the system towards other mesophases.

- *Probe Ultrasonication*

Probe sonication delivers acoustic energy that disrupts the cubic gel through cavitation, and it is the most common laboratory method because it is simple and requires small volumes [73, 74]. Its drawbacks include local heating, possible metal shedding from the probe, limited reproducibility between operators and difficulty in scale-up. Comparative studies with phytantriol–Poloxamer 407 systems have demonstrated that the method and duration of energy input determine internal structure, morphology and the presence of liposome-like vesicles [74].

- *Microfluidization*

Microfluidizers pump the coarse dispersion through fixed-geometry interaction chambers in which two high-velocity streams collide. The constant geometry provides more

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uniform shear than sonication and produces narrow size distributions in a continuous, scalable fashion. Microfluidization and related high-shear processes are therefore attractive for industrial cubosome manufacture, although process heat and the need for pre-dispersion must be controlled [23, 73].

➢ *Bottom-Up Techniques*

Bottom-up methods form cubosomes by nucleation and growth from a solution of the lipid in a water-miscible solvent or hydrotrope. They require less energy, can be performed at room temperature and frequently yield smaller particles, which makes them suitable for heat-sensitive drugs and biologics [19, 75].

- *Solvent Dilution*

In solvent (precursor) dilution, the lipid and drug are dissolved in a small amount of a water-miscible solvent such as ethanol, and the solution is injected into an aqueous stabilizer solution under stirring. On dilution, the solvent diffuses away and the lipid self-assembles into cubic nanoparticles. Rizwan and co-workers used liquid precursors containing phytantriol or GMO, Poloxamer 407 and a hydrotrope such as ethanol, polyethylene glycol or propylene glycol to form submicron particles spontaneously on dilution, and achieved the greatest entrapment of the model protein ovalbumin in phytantriol cubosomes, demonstrating the suitability of the approach for fragile macromolecules [75]. Residual solvent must be removed or controlled, and the process parameters, including the lipid-to-solvent ratio and mixing rate, influence the final size and phase [76].

- *Hydrotrope Method*

The hydrotrope method introduced by Spicer and colleagues dilutes a liquid precursor containing GMO, Poloxamer 407 and a hydrotrope such as ethanol into water [19]. The hydrotrope dissolves the lipid at high concentration and prevents formation of the viscous gel; upon dilution, the lipid becomes insoluble and spontaneously forms cubosomes without high-shear processing. The method produces small particles with low energy input and is conceptually simple to scale, although it may yield a higher proportion of vesicles and requires control of residual hydrotrope [19, 21].

- *Spontaneous Emulsification*

Spontaneous emulsification exploits the interfacial turbulence generated when a lipid-rich organic phase containing a water-miscible solvent or surfactant comes into contact with an aqueous phase. Diffusion of the solvent produces local supersaturation and spontaneous fragmentation into droplets that subsequently restructure into cubic particles [76]. Related approaches include the use of dry powder precursors, in which lipid and stabilizer are spray dried or freeze dried with hydrophilic carriers and hydrated before use to form cubosomes, which also offers a route to solid dosage forms and improved storage stability [20].

➢ *Emerging Advanced Methods*

- *Microfluidic Production*

Microfluidic mixing, already established for the manufacture of ionisable lipid nanoparticles, provides precise control over mixing time, flow-rate ratio and total flow rate. Hydrodynamic flow focusing and staggered herringbone mixers dilute an ethanolic lipid stream into an aqueous stream within milliseconds, enabling reproducible production of nanostructured lipid particles with narrow size distributions [8, 77]. Microfluidic devices coupled with synchrotron small-angle X-ray scattering have also been used to observe phase transitions of cubosomes in real time on addition of ions, demonstrating the value of microfluidics as both a manufacturing and analytical tool [78]. Time-resolved studies with rapid mixing have shown that protein-loaded cubic nanoparticles can form within milliseconds [79].

- *Continuous Manufacturing Systems*

Continuous manufacturing integrates mixing, dispersion, purification and, potentially, drying in a single uninterrupted process with in-line monitoring of critical quality attributes. For cubosomes, high-throughput formulation and characterization platforms with automated dispensing and synchrotron SAXS have accelerated mapping of composition–structure relationships [80], and parallel microfluidic channels or scaled-up homogenization trains provide routes to larger volumes. Machine learning trained on such datasets has begun to predict the mesophase formed by lipid mixtures as a function of composition and temperature, which could support quality-by-design and real-time release testing in future continuous processes [81].

Table 2 Comparison of Cubosome Preparation Methods

Method	Principle	Advantages	Limitations
High-pressure homogenization	Fragmentation of bulk cubic gel by cavitation and shear at high pressure	Solvent-free; narrow size distribution; industrially scalable	Heat generation; high energy; possible vesicle formation [71, 72]
Probe ultrasonication	Acoustic cavitation disperses the gel	Simple; small volumes; common in laboratories	Local heating; probe contamination; poor scalability [74]
Microfluidization	Collision of high-velocity streams in fixed chambers	Uniform shear; continuous; reproducible	Requires pre-dispersion; heat control needed [73]
Solvent (precursor) dilution	Injection of lipid solution in water-miscible solvent into aqueous stabilizer	Low energy; small particles; suitable for biologics	Residual solvent; sensitive to mixing conditions [75, 76]

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26nov975

Hydrotrope method	Dilution of lipid–hydrotrope–stabilizer liquid precursor	No high shear; simple; scalable concept	Vesicle by-products; hydrotrope removal [19]
Spontaneous emulsification / powder precursors	Interfacial turbulence on solvent diffusion; hydration of dried precursors	Low energy; route to solid dosage forms	Control of size and phase more difficult [20, 76]
Microfluidic production	Millisecond mixing of lipid and aqueous streams in microchannels	Precise control; reproducible; compatible with in situ SAXS	Throughput per chip limited; device cost [77, 78]
Continuous manufacturing	Integrated mixing, dispersion and monitoring with in-line analytics	Consistent quality; real-time control; quality by design	Still emerging for cubosomes; validation required [80, 81]

IV. CHARACTERIZATION AND EVALUATION

Because the therapeutic performance of cubosomes depends on both their colloidal properties and their internal nanostructure, characterization requires a combination of techniques. A practical workflow is shown in Fig. 4, and the main techniques are summarized in Table 3.

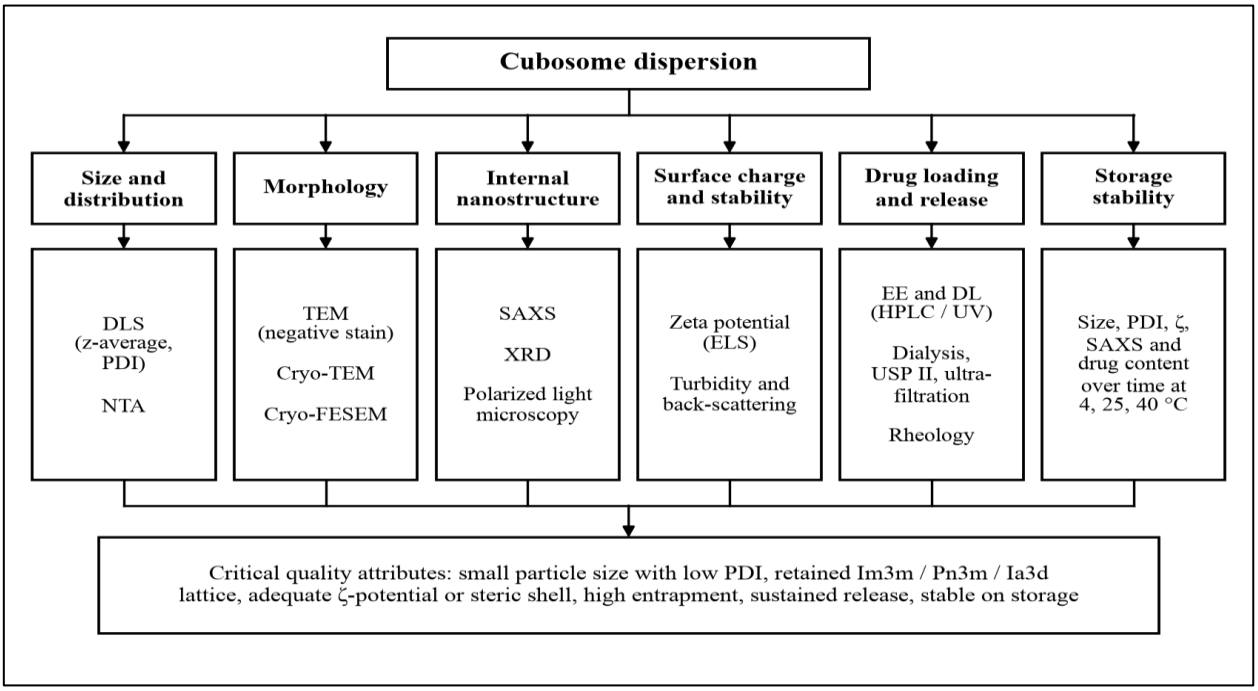

Fig 4 Workflow for the Characterization and Evaluation of Cubosome Dispersions. DLS: Dynamic Light Scattering; NTA: Nanoparticle Tracking Analysis; ELS: Electrophoretic Light Scattering; EE: Entrapment Efficiency; DL: Drug Loading; PDI: Polydispersity Index.

➢ *Particle Size and Distribution Analysis (DLS)*

Dynamic light scattering (DLS), also termed photon correlation spectroscopy, measures the fluctuations in scattered light intensity caused by Brownian motion and calculates the hydrodynamic diameter (z-average) and polydispersity index (PDI) [23]. Most pharmaceutical cubosome dispersions have mean diameters between about 100 and 300 nm. DLS is rapid and non-invasive, but it is intensity weighted and therefore biased towards larger particles and aggregates; it also assumes spherical particles, whereas cubosomes are often faceted. Nanoparticle tracking analysis and laser diffraction are useful complements for detecting sub-populations and large particles respectively [82].

➢ *Morphological Assessment (TEM, Cryo-TEM, SEM)*

Conventional transmission electron microscopy (TEM) with negative staining is widely used, but drying and staining can collapse the hydrated structure and create artifacts. Cryogenic TEM (cryo-TEM), in which thin films of the dispersion are vitrified, preserves the native hydrated state and reveals the typical square or hexagonal particle profiles, internal periodicity and the presence of coexisting vesicles [83]. Fast Fourier transforms of cryo-TEM images can be indexed to identify the cubic space group, and cryo-electron tomography has provided direct three-dimensional visualization of the bicontinuous network inside dispersed particles [84]. Cryo-field emission scanning electron microscopy (cryo-FESEM) has also been used to visualize the surface topology of phytantriol cubic phases [85].

➢ *Internal Cubic Phase Confirmation (SAXS, XRD)*

Small-angle X-ray scattering (SAXS) is the definitive method for identifying the internal mesophase of cubosomes [86]. Each space group gives Bragg reflections at characteristic

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ratios of the scattering vector q, determined by the allowed Miller indices (h, k, l). For the Pn3m phase, peaks appear at relative positions √2:√3:√4:√6:√8:√9; for Im3m at √2:√4:√6:√8:√10:√12; and for Ia3d at √6:√8:√14:√16:√20:√22 (Fig. 3). The lattice parameter a is calculated from the position of each indexed peak as:

$$a = 2\pi \cdot \sqrt{(h^2 + k^2 + l^2)} / q_hkl \quad (1)$$

Assuming a constant lipid bilayer thickness, the radius of the water channel, r_w, can be estimated from the lattice parameter as:

$$r_w = a \cdot \sqrt{(-A_0 / 2\pi\chi)} - L \quad (2)$$

where A_0 and χ are the dimensionless surface area and Euler characteristic of the underlying minimal surface (1.919 and −2 for Pn3m; 2.345 and −4 for Im3m; 3.091 and −8 for Ia3d), and L is the length of the lipid chain [58, 59]. Laboratory wide- and small-angle X-ray diffraction can also detect crystalline drug or lipid, and polarized light microscopy is a simple screening test, since cubic phases are optically isotropic whereas lamellar and hexagonal phases are birefringent. Synchrotron SAXS, especially when combined with temperature, pH or microfluidic control, allows phase transitions to be followed in real time [78, 87].

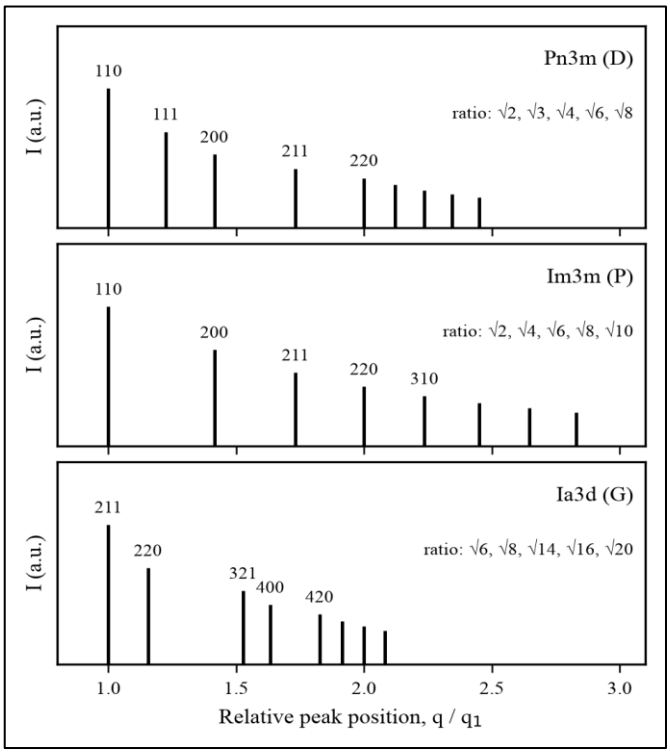

Fig 5 Characteristic SAXS Bragg Reflections of the Pn3m, Im3m and Ia3d Cubic Phases, Plotted Relative to the First Allowed Reflection (q_1). Miller Indices (HKL) are Given for the First Five Peaks of Each Phase; Stick Heights are Schematic.

➢ *Surface Charge and Colloidal Stability*

Zeta potential, measured by electrophoretic light scattering, reflects the charge at the shear plane and indicates the likelihood of electrostatic stabilization. Poloxamer-stabilized GMO and phytantriol cubosomes typically show slightly negative zeta potential, as their stability is primarily steric rather than electrostatic [23]. Charge can be deliberately modified by incorporating fatty acids, cationic lipids or coatings such as poly-ε-lysine to improve mucoadhesion, cellular uptake or nucleic acid complexation [88, 89]. Because the cubic lattice is sensitive to ions, the stability of charged cubosomes in physiological salt and buffer solutions must be checked directly [87].

➢ *Drug Loading, Release Studies, and Rheology*

Entrapment efficiency and drug loading are usually determined by separating free drug through ultrafiltration, dialysis, size-exclusion chromatography or centrifugation, followed by quantification with high-performance liquid chromatography or spectrophotometry. Release studies are complicated by the small particle size and the need to separate particles from released drug; dialysis-bag methods may underestimate release rates because the membrane becomes rate limiting. Boyd applied a pressure ultrafiltration method that rapidly separates particles from the release medium and showed that, on dilution, lipophilic compounds were released rapidly from cubosomes, consistent with diffusion over the short path length of a nanoparticle, whereas equilibrium dialysis incorrectly suggested sustained release [90]. Cubosomes intended for intravenous use should therefore be regarded primarily as burst-release carriers unless the drug is strongly retained in the bilayer. Release of drugs from bulk cubic phases is typically diffusion controlled and follows square-root-of-time kinetics, and the release of macromolecules depends strongly on the ratio of molecular size to water channel diameter [64, 65]. Rheological characterization is essential for topical, ocular and depot formulations, because the bulk cubic phase is a stiff viscoelastic gel, whereas cubosome dispersions are fluid; in situ gelling systems that transform into cubic or hexagonal phases on contact with water can combine ease of administration with sustained release [63, 91].

➢ *Storage Stability Evaluation*

Storage stability is evaluated by monitoring particle size, PDI, zeta potential, internal structure by SAXS, drug content and chemical degradation over time at refrigerated, room and accelerated conditions according to ICH principles. GMO is prone to hydrolysis and oxidation of the unsaturated chain, which can change the phase behavior; phytantriol is more chemically robust [38, 39]. Phase transitions, Ostwald ripening and aggregation may occur during storage, and lyophilization with cryoprotectants or the use of powder precursors are strategies to prolong shelf life [20, 82].

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Table 3 Characterization Techniques for Cubosomes

Attribute	Technique	Information obtained	Practical notes
Size and distribution	DLS, NTA, laser diffraction	Hydrodynamic diameter, PDI, sub-populations	DLS intensity weighted; assumes spheres [23, 82]
Morphology	TEM, cryo-TEM, cryo-electron tomography, cryo-FESEM	Particle shape, internal periodicity, coexisting vesicles	Cryo methods avoid drying artifacts [83–85]
Internal structure	SAXS, XRD, polarized light microscopy	Space group, lattice parameter, water channel size	SAXS is definitive; cubic phases are optically isotropic [58, 86]
Surface charge	Electrophoretic light scattering	Zeta potential; effect of coatings	Stability of poloxamer systems is mainly steric [87, 88]
Loading and release	Ultrafiltration, dialysis, HPLC/UV, pressure ultrafiltration	Entrapment efficiency, drug loading, release kinetics	Dialysis may misrepresent release from nanoparticles [64, 90]
Rheology	Oscillatory and flow rheometry	Viscoelasticity of gels and in situ depots	Critical for topical, ocular and depot systems [63, 91]
Storage stability	Size, PDI, zeta potential, SAXS and assay over time	Physical and chemical stability under ICH conditions	GMO hydrolysis and oxidation; drying prolongs shelf life [20, 38]

V. THERAPEUTIC APPLICATIONS

The structural features described above translate into a broad spectrum of therapeutic applications. Representative studies are summarized in Table 4 and discussed below according to the therapeutic area and route of administration.

Table 4 Representative Therapeutic Applications of Cubosomes

Area	Payload	Formulation	Model / route	Main finding
Oncology	5-Fluorouracil	GMO / Poloxamer 407	Rats, in vivo	Higher hepatic drug levels; hepatotoxicity noted [92]
Oncology	Doxorubicin	Phytantriol, hydrotrope method, lyophilized	Oral; breast cancer model	Higher oral bioavailability and efficacy; lower cardiotoxicity [93]
Oncology	Paclitaxel	Cubic/sponge-phase NPs, EGFR antibody fragments	Ovarian cancer xenograft	Reduced tumor burden; longer survival [94]
Oncology	Copper acetylacetonate	Im3m cubosomes, Affimer-tagged	Colorectal cancer xenograft	Preferential tumor accumulation [55]
Oncology	AT101 (gossypol)	GMO / Pluronic F127	Glioblastoma cells	High entrapment; sustained release; efficient uptake [95]
Dermal	Capsaicin	Phytantriol or GMO / poloxamer, Im3m	Skin in vitro; mouse skin	Higher stratum corneum retention than cream [96]
Dermal (PDT)	Aminolevulinic acid and methyl ester	GMO or phytantriol cubic phase	Topical; nude mice	Faster photosensitizer formation than ointment [97]
Ocular	Dexamethasone	GMO / Poloxamer 407, Pn3m	Ocular, in vivo	Longer preocular retention; higher ocular bioavailability [98]
Ocular	Flurbiprofen	Cubosomes, P-type cubic phase	Ocular, in vivo	Higher corneal permeation; good tolerance [99]
Nose-to-brain	S14G-humanin (peptide)	Odorranalectin-modified cubosomes	Intranasal; rats	Enhanced brain uptake [100]
Brain	Phenytoin	Selachyl alcohol / Tween 80	Intravenous; rats	Cubosomes outperformed hexosomes [42]
Peptide	Insulin	Self-assembled 'nanocubicles'	Oral; diabetic rats	Sustained hypoglycemic effect [101]
Peptide	Antimicrobial peptides	GMO cubosomes	In vitro	Proteolytic protection of LL-37 [102]
Gene	siRNA	Cationic gyroid cubic lipid complexes	Cell culture	Efficient silencing at low toxicity [54]
Cosmeceutical	Alpha-lipoic acid	GMO / Poloxamer 407 dispersions in gel	Clinical, topical	Anti-wrinkle efficacy [103]

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Theranostic	Docetaxel + photosensitizer	Photosensitizer-copolymer-stabilized cubosomes	Cancer cells	Synergistic chemo-photodynamic effect [104]
Imaging	NIR dye and MRI agent	Cubosomes and hexosomes	In vivo	Dual-modality biodistribution imaging [105]

➢ *Oncology and Targeted Chemotherapy*

Cancer therapy is the most extensively investigated application of cubosomes, because many anticancer agents are poorly soluble, toxic to healthy tissues and subject to multidrug resistance [34, 35]. Cubosomes have been loaded with a wide range of cytotoxic agents, including paclitaxel, docetaxel, doxorubicin, 5-fluorouracil and cisplatin. Nasr and co-workers prepared GMO cubosomes containing the hydrophilic drug 5-fluorouracil and observed a markedly higher hepatic drug concentration than with drug solution after administration to rats; however, greater hepatocellular damage was also noted, highlighting that improved targeting must be balanced against local toxicity through dose optimization [92]. Oral administration of doxorubicin-loaded bicontinuous cubic nanoparticles improved bioavailability and therapeutic efficacy while reducing the cardiotoxicity associated with the free drug, illustrating that the lipid matrix can alter tissue exposure profiles [93]. Co-loading of cisplatin and metformin in cubosomes was used to target the metabolism of colorectal cancer cells [106], and AT101-loaded cubosomes showed promise against glioblastoma cells [95]. Docetaxel cubosomes incorporated into a thermoresponsive in situ gel have been proposed as an injectable depot for localized, sustained chemotherapy [91].

Active targeting has been achieved by decorating cubosomes with ligands. Paclitaxel-loaded cubic and sponge-phase nanoparticles stabilized by Poloxamer 407 and a PEG-lipid, with or without functionalization by epidermal growth factor receptor antibody fragments, reduced tumor burden and extended survival in an ovarian cancer xenograft model compared with free drug [94], while cubosomes bearing Affimer binding proteins selectively targeted carcinoembryonic antigen-expressing colorectal cancer cells in vitro and in vivo [55]. Click chemistry has been used to attach targeting moieties without antibodies [107], and poly-ε-lysine-coated cubosomes co-loaded with cisplatin and paclitaxel reduced burst release and were proposed as theranostic probes against hepatoma cells [108]. Cubosomes can also carry photosensitizers for photodynamic therapy, alone or in combination with chemotherapy, to achieve synergistic tumor cell killing [104, 109]. Despite these encouraging results, most oncology studies remain preclinical, and the in vivo fate of the internal structure after intravenous administration requires more systematic evaluation [25, 110].

➢ *Dermatological and Transdermal Applications*

The skin is a particularly suitable target for cubosomes because GMO and phytantriol are bioadhesive and act as penetration enhancers by interacting with stratum corneum lipids. Cubosome dispersions of indomethacin were among the first to be evaluated as vehicles for percutaneous administration [111]. Capsaicin-loaded phytantriol and GMO cubosomes with an Im3m structure provided sustained release and markedly higher retention of the drug in the stratum corneum than a conventional cream, with only light irritation on repeated application to mouse skin [96]. In photodynamic therapy, lipid cubic phases enhanced the skin penetration of aminolevulinic acid derivatives [97]. In a comparison of liposomes, transfersomes, ethosomes and cubosomes for transcutaneous delivery of a peptide vaccine, cubosomes and ethosomes showed superior skin retention and deeper peptide penetration in vitro [112]. Related non-lamellar dispersions, such as reverse hexagonal monoolein–oleic acid systems, have been used for topical peptide delivery [113]. The viscous cubic gel formed on hydration can also act as a film-forming reservoir on the skin or wound surface, prolonging contact time.

➢ *Ocular, Nasal, and Mucosal Delivery*

Topical ocular delivery is limited by rapid precorneal clearance, tear turnover and the barrier properties of the cornea. Cubosomes address these limitations through bioadhesion and corneal penetration enhancement. Dexamethasone-loaded liquid crystalline nanoparticles improved preocular retention and ocular bioavailability [98], and flurbiprofen cubosomes showed low irritancy and higher bioavailability than conventional eye drops [99]. Similar benefits have been reported for pilocarpine nitrate [114], timolol maleate [115] and the antifungal sertaconazole nitrate [116], supporting cubosomes as a versatile ocular platform.

Intranasal administration offers a non-invasive route to the brain that bypasses the blood–brain barrier through olfactory and trigeminal pathways. Odorranalectin-modified cubosomes enhanced brain delivery of a peptide after intranasal administration and improved pharmacodynamic outcomes in a rat model of amyloid-β-induced neurotoxicity [100]. For systemic brain targeting, a direct in vivo comparison of selachyl alcohol cubosomes and hexosomes after intravenous administration showed that cubosomes delivered more phenytoin to the brain [42], and polysorbate 80-stabilized phytantriol cubosomes have been designed as a step towards blood–brain barrier targeting [47]. Piperine-loaded Tween-integrated GMO cubosomes have been developed as brain-targeted oral nanomedicine for Alzheimer's disease [117]. In the gastrointestinal tract, cubosomes improve the solubility and oral bioavailability of poorly water-soluble drugs such as simvastatin and amphotericin B [118–120], partly because digestion products of GMO can form mixed micelles and new liquid crystalline structures that sustain drug solubilization.

➢ *Peptide, Protein, and Gene Delivery*

The aqueous channels of cubosomes are comparable in size to small proteins, and the membrane-mimetic environment can preserve the conformation and activity of labile biologics [64, 121]. Early work showed that self-assembled "nanocubicle" particles could serve as carriers for oral insulin delivery and produce a hypoglycemic effect in animals [101]. Phytantriol cubosomes prepared from liquid precursors entrapped the model antigen ovalbumin and released it in a sustained manner, supporting their investigation as vaccine delivery systems [75]. Antimicrobial peptides post-loaded into

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cubosomes showed peptide-dependent effects: activity was preserved for some peptides, and LL-37 was protected from proteolytic degradation [102]. Multicompartment cubic nanoparticles with high protein loading have also been described [79].

For nucleic acids, cationic lipids can be incorporated into the cubic phase to enable complexation. Leal and co-workers showed that siRNA embedded in a cationic gyroid cubic lipid matrix achieved highly efficient gene silencing, attributed to the fusogenic character of the cubic phase [54]. The same group described "cuboplexes", in which siRNA is confined within the water channels of cubic phase particles, as topologically active delivery systems [89]. Studies of cubosome fusion with model membranes have shown that cubosomes can fuse with lipid bilayers and transfer their contents, providing a mechanistic basis for intracellular delivery [66]. These findings position cubosomes as potential alternatives or complements to lamellar lipid nanoparticles for RNA therapeutics, although efficiency and safety comparisons with clinically approved ionisable lipid nanoparticles are still lacking [8, 24].

➢ *Cosmeceutical and Nutraceutical Applications*

Cubosomes were first commercialized in cosmetics, where phytantriol and GMO are established ingredients and liquid crystalline structures provide hydration, stability and controlled delivery of actives. A clinical study showed that liquid crystalline nanostructured dispersions of alpha-lipoic acid reduced facial wrinkles, illustrating their cosmeceutical potential [103]. In foods and nutraceuticals, cubosomes and hexosomes have been investigated for encapsulation of bioactive compounds such as curcumin, carotenoids, vitamins and polyphenols, improving their solubility, protection from oxidation and bioaccessibility [36]. A direct comparison of cubosomes and liposomes for curcumin delivery indicated that the choice of carrier influences encapsulation and cellular delivery [122]. Cubosome-encapsulated metal nanocrystals have shown antimicrobial activity, extending applications to anti-infective and hygiene products [123].

➢ *Theranostic and Imaging Applications*

Theranostic systems combine diagnosis and therapy in a single carrier. The amphiphilic matrix of cubosomes can accommodate imaging probes in the bilayer or water channels while simultaneously carrying drugs [30]. Nitroxide-containing lipids were used to produce metal-free cubosomes visible by magnetic resonance imaging [124]. Fluorescent cubosomes loaded with drugs were proposed as theranostic nanoparticles [125], and cubosomes have been used for in vivo fluorescence lifetime imaging [126]. Dual-modality near-infrared fluorescence and magnetic resonance imaging cubosomes and hexosomes were developed using high-throughput formulation, and their in vivo biodistribution was characterized [105]. Polymer-free cubosomes have been used for simultaneous bioimaging and photodynamic action in melanoma cells [109], and clickable cubosomes enabled targeting and imaging without antibodies [107]. Cubosomes have also been explored as carriers for anti-infective drugs against intracellular pathogens such as Mycobacterium tuberculosis [127]. These studies demonstrate that cubosomes can serve as multifunctional platforms that link drug delivery with diagnostic monitoring [128, 129].

VI. CHALLENGES, LIMITATIONS, AND FUTURE PERSPECTIVES

Although cubosomes have been studied for more than a quarter of a century, no cubosome dispersion has yet been approved as a medicinal product. The main barriers and the strategies proposed to overcome them are summarized in Table 5 and discussed below.

Table 5 Challenges in Cubosome Translation and Proposed Mitigation Strategies

Challenge	Underlying cause	Consequence	Mitigation strategies
Phase transformation	Drug loading, stabilizer, ions, pH, proteins, lipolysis	Loss of cubic structure and release control	Robust lipids; optimized stabilizer; testing in biorelevant media [44, 87, 110]
Chemical instability	Ester hydrolysis and oxidation of GMO	Altered phase behavior; impurities	Phytantriol; antioxidants; lyophilization; powder precursors [20, 38]
Scale-up	High gel viscosity; energy-intensive dispersion	Batch variability in size and structure	Hydrotrope, microfluidic and continuous processing; QbD [19, 77, 81]
Sterilization	Heat, irradiation and filtration sensitivity	Phase change, oxidation, material loss	Aseptic processing; sterile filtration of small particles [23]
Toxicity	Membrane disruption; hemolysis; complement activation; stabilizer effects	Safety concerns for parenteral use	GMO over phytantriol; citrem, PEG-lipid or polyphosphoester stabilizers [40, 50, 51]
Regulatory	Nanomaterial-specific expectations; limited long-term data	Slow clinical translation	Standardized characterization; safety studies; liquid crystal depot precedents [43, 130, 131]

➢ *Stability and Phase Transformation Issues*

The internal structure of cubosomes is a thermodynamic state that depends on composition, temperature and the surrounding medium, so it can change during formulation, storage or administration [13, 59]. Loading of drugs, particularly amphiphilic or lipophilic molecules at high concentrations, can alter the curvature of the bilayer and convert the cubic phase into hexagonal, sponge or micellar phases [82, 122]. Excess stabilizer may induce Pn3m–Im3m transitions and vesicle formation [44], while ions, pH changes and proteins in biological fluids can trigger further transitions [87]. Once in contact with plasma, GMO cubosomes interact

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with lipoproteins and albumin, undergo lipolysis and may disintegrate into remnant particles [110]. In the gastrointestinal tract, digestion of GMO produces fatty acids and monoglycerides that self-assemble into new structures [38]. Strategies to improve structural robustness include the use of hydrolysis-resistant lipids such as phytantriol, addition of structure-stabilizing lipids, optimization of stabilizer type and concentration, and solidification by spray drying or freeze drying [20, 45]. Importantly, the stability of the internal nanostructure should be verified in biorelevant media, not only in water, because a carrier that loses its cubic structure loses many of the advantages for which it was chosen.

➢ *Scale-Up and Sterilization Challenges*

Laboratory preparation by probe sonication or small-scale homogenization does not readily translate to industrial production. High viscosity of the bulk cubic gel complicates mixing and pumping, energy input must be carefully controlled to avoid degradation and phase changes, and reproducibility of particle size and internal structure across batches requires robust process control [71, 73]. Low-energy hydrotrope and microfluidic methods are attractive for scale-up but require control of residual solvent and consistent mixing [19, 77]. Sterilization is a further challenge: terminal heat sterilization may induce phase transitions or chemical degradation of GMO, while gamma irradiation can promote lipid oxidation. Sterile filtration through 0.22 μm membranes is feasible only for small, deformable particles and can cause losses of material. Aseptic manufacturing is therefore often necessary, which increases cost. Quality-by-design approaches, high-throughput formulation screening and in-line process analytical technologies could help to define the design space and support regulatory submissions [80, 81].

➢ *Toxicity Considerations and Regulatory Aspects*

GMO and phytantriol are widely used in foods and cosmetics, but parenteral or ocular exposure to nanoparticles raises different safety questions. In vitro studies have shown that phytantriol cubosomes are considerably more cytotoxic and hemolytic than GMO cubosomes, apparently because of greater membrane disruption and oxidative stress [40]. Lyotropic liquid crystalline dispersions can induce hemolysis and complement activation after intravenous administration, which may be reduced by alternative stabilizers such as citrem or by phospholipid-rich formulations [51, 132]. Poloxamer 407 itself has been associated with hyperlipidemia in animal studies at high doses, and replacing it with PEG-lipids or polyphosphoester analogues has reduced cytotoxicity [48, 50]. Systematic long-term toxicity, biodistribution, immunogenicity and elimination studies are still scarce [25, 40].

From a regulatory perspective, cubosome products would be evaluated under general frameworks for nanomaterial-containing drug products, which require detailed characterization of the material, demonstration of physical and chemical stability, control of manufacturing and assessment of biological interactions [130]. The clinical progress of non-lamellar lipid liquid crystal technology provides useful precedents. Lipid liquid crystalline depots formed in situ from phospholipid and glycerol dioleate mixtures have demonstrated sustained octreotide release in healthy volunteers [43], and weekly and monthly subcutaneous buprenorphine depots based on the same principle were non-inferior to daily sublingual buprenorphine with naloxone in a randomized trial for opioid use disorder [131]. These examples show that regulators can accept liquid crystalline lipid systems when quality, safety and efficacy are adequately demonstrated, and they offer a template for future cubosome development.

➢ *Emerging Research Directions*

Several research directions are expected to shape the next generation of cubosomes. High-throughput formulation coupled with synchrotron SAXS and machine learning enables rapid mapping of composition–structure–function relationships and the prediction of mesophase behavior [80, 81]. Biomimetic cubic phases incorporating membrane lipids, cholesterol and charged lipids aim to improve fusion with cell membranes and to mimic natural cubic membranes [26, 66]. Hybrid systems in which cubosomes are combined with metal nanocrystals, polymers or inorganic particles expand their functionality to antimicrobial, photothermal and imaging applications [123, 133]. Cubosomes for intracellular infections, vaccines and antimicrobial peptides respond to the growing threat of antimicrobial resistance [102, 127]. Finally, better understanding of how internal structure influences protein corona formation, cellular uptake and endosomal escape is needed to design carriers rationally [24, 89].

➢ *Stimuli-Responsive and Targeted Cubosomes*

The sensitivity of the cubic phase to environmental conditions, a drawback for stability, can be turned into an advantage for responsive delivery. Phytantriol systems containing vitamin E acetate switch between cubic and inverse hexagonal phases close to body temperature, enabling on-demand release in vitro and in vivo in response to local heating or cooling [53]. Incorporation of gold nanorods allows reversible, near-infrared light-triggered switching of nanostructure [133]. Inclusion of ionisable lipids such as fatty acids or pyridinyl derivatives produces pH-responsive mesophases that release drugs selectively in particular regions of the gastrointestinal tract or in acidic tumor and endosomal environments [52, 134]. Enzyme-responsive cubosomes that change structure in the presence of phospholipases associated with disease have also been designed [135]. Targeting is achieved by attaching antibodies, antibody fragments, Affimers, peptides, lectins or small-molecule ligands to the stabilizer or to functional lipids, often using click chemistry [55, 94, 100, 107]. Combining responsiveness with targeting may yield carriers that accumulate at the target site and release their payload only when triggered.

➢ *Translation to Personalized Medicine*

Personalized medicine requires carriers that can be adapted to the individual patient's disease, genotype and treatment needs. Cubosomes are well suited to this goal because their composition can be adjusted modularly: the lipid determines the mesophase and release rate, the stabilizer determines circulation and targeting, and the payload can be a small molecule, protein, nucleic acid or imaging agent [3, 25]. Theranostic cubosomes could allow non-invasive imaging of biodistribution in each patient before or during therapy, enabling dose adjustment and patient selection [105, 128]. The

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capacity to co-deliver drug combinations and to load RNA suggests possibilities for patient-specific combination therapy and gene silencing [54, 106]. Realizing this vision will require standardized characterization protocols, robust manufacturing at small and large scale, predictive in vitro–in vivo correlations and clinical studies. Collaboration between colloid scientists, pharmaceutical technologists, clinicians and regulators will be essential to move cubosomes from promising laboratory nanostructures to approved, patient-centerd medicines.

VII. CONCLUSION

Cubosomes are nanoparticles of bicontinuous cubic liquid crystalline phases that combine a large internal surface area, compartments for hydrophilic and lipophilic molecules, biocompatible and often biodegradable lipids, and a nanostructure that can be tuned and triggered by environmental conditions. Monoolein and phytantriol remain the principal structure-forming lipids, and Poloxamer 407 the principal stabilizer, although newer lipids and stabilizers are improving biocompatibility and functionality. Top-down and bottom-up preparation methods are well established at laboratory scale, and microfluidic and continuous processes promise better control and scalability. Reliable evaluation requires a combination of DLS, cryo-TEM, SAXS, zeta potential, entrapment, release, rheology and stability testing, with particular attention to the persistence of the cubic structure in biological media. Preclinical studies have shown benefits in cancer therapy, dermal and transdermal delivery, ocular and nasal delivery, oral bioavailability enhancement, delivery of peptides, proteins and nucleic acids, cosmeceuticals, nutraceuticals and theranostics. The main obstacles to clinical translation are phase instability in vivo, scale-up and sterilization, stabilizer-associated toxicity and the need for comprehensive safety data. Addressing these challenges through rational, data-driven design, responsive and targeted formulations and close engagement with regulatory science will determine whether cubosomes become the next generation of clinically used lipid nanocarriers.

- *Conflict of Interest*

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