Cubosomes as Novel Drug Delivery System: Comprehensive Insights and Applications

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Abstract

Similar to liposomes and other vesicular systems, cubosomes are lipid vesicles. Certain amphiphilic lipids combined with an appropriate stabiliser enable the formation of cubosomes. Self-assembled cubosomes have garnered a lot of interest and attention since their discovery and identification as active drug delivery vehicles. Cubosomes are versatile lipid vesicles that find application across multiple administration routes, including oral, ophthalmic, transdermal, and chemotherapeutic delivery methods. Their distinctive cubic structure offers several advantages, such as enhanced drug dispersal, a large surface area, and a straightforward manufacturing process. Additionally, cubosomes demonstrate biodegradability and the ability to encapsulate various types of compounds, including hydrophobic, hydrophilic, and amphiphilic substances. These characteristics make them highly promising candidates for drug nano-formulations, particularly in the field of cancer therapeutics. The primary method for preparing cubosomes involves the emulsification of a monoglyceride with a polymer, followed by homogenization and sonication. This process can be classified into two distinct techniques: top-down and bottom-up approaches. review aims to conduct a comprehensive analysis of the cubosomes composition, preparation methods, drug encapsulating strategies, components, and applications.

Keywords: *Cubosomes, top-down and bottom-up approaches, preparation,stratagies.*

1. Introduction

Cubosomes are distinctive nanostructured particles with a sub-micron size, recognized for their bicontinuous cubic liquid crystalline structure. Coined by Larsson, the term "Cubosomes" emphasizes their Molecular crystallography in cubic structures and resemblance to liposomes. These nanoparticles are formed through the self-assembly of specific surfactants with an appropriate ratio of water to microstructure. In contrast to conventional solid nanoparticles, cubosomes are Liquid crystal nano-colloids that self-organize and exhibit rheological properties similar to solids, offering unique and highly valuable characteristics [1]. They exhibit a similar extensive surface area microstructure and dispersions with lower viscosity than the standard cubic phase [2]. Cubosomes likely consist of lipids, polymers and surfactants containing both non-polar & polar components, which makes them amphiphilic. The non-polar effect drives amphiphilic molecules into a polar solvent, where they naturally recognize and organize themselves into a nanometer-sized liquid crystal structure. As a result, cubosomes form a bicontinuous cubic liquid crystalline phase, featuring two separate water regions delineated by bilayers controlled by surfactants. Additionally, they resemble liquid crystal matrices, showing three-dimensional crystal symmetry along with optical isotropy, viscosity, and solidity. The cubic phase possesses the capacity to fracture, leading to the formation of colloidally and thermodynamically stable particulate dispersions. Cubosomes are integral in nanodrug development, serving as structures of the cubic bicontinuous liquid crystal phase formed through the hydration of a combination of monoolein and poloxamer 407 [3].

Cubosomes play a crucial role in drug delivery systems based on nanotechnology [4]. In recent times, there has been a growing interest in pharmaceuticals focusing on particles ranging from a few hundred nanometer to 10-500nm in diameter [5]. The drug-to-polymer ratio typically ranges from 1:2 to 1:1, with variations depending on the specific substance. Successful formulations of cubosomes have been achieved for certain anticancer drugs. The challenge in large-scale production of cubosomes stems from their viscosity and phase behaviour. The cubic phase spontaneously forms when water is combined with particular surfactants [6]. The microstructure of cubosomes is identical to that of the original cubic phase, and the viscosity of cubosome dispersions is significantly lower than that of the overall cubic phase [7]. Cubosomes demonstrate a larger surface area compared to the original cubic phase [8]. They originate from the self-assembly of molecules with surfactant-like or amphiphilic characteristics [9]. Cubosomes are commonly produced by dispersing the bulk cubic phase using high-energy methods [10]. Polymeric surfactants contribute to the colloidal stabilization of cubosomes [11]. The release of cubosomal formulations can take place through either diffusion or absorption [12].

The majority of liquid crystalline systems transform into micelles when highly diluted, yet cubosomes maintain stability across all dilution levels. This is because of the low solubility of the lipid-based cubic phase in water. Controlled drug release involves administering medication in a predetermined manner. A drug delivery system is a mechanism that transports a drug component to a particular site of the body or specific tissue to attain an effective concentration at the target site.

Several benefits of controlled drug release include enhancing therapeutic advantages, minimizing adverse side effects, decreasing the necessity for frequent dosing, improving patient adherence, lowering expenses, reduce cost, etc [13-15]. When drugs are encapsulated within cubosomal vesicles, they convey them to the intended site of action, including high molecular weight drugs. It improves drug transport across skin by acting as a penetration enhancer [16].

Cubosomes are part of the vesicular drug delivery system, first identified in 1980, and they have important applications in nanotechnology. [17]. The three types of cubic phase are cubosomes, cubic gel phase, and cubic phase precursor. A prospective delivery approach for a range of substances, encompassing proteins, peptides, amino acids, low molecular weight compounds, nucleic acids, etc, is the use of cubosomes [18-19]. As a delivery vehicle is the mechanism of a cubic phase. They were greater stability in comparison to the liposomes [20-22]. Even in excess of water, cubic liquid crystals are transparent and stable. Binary systems have the form of cubosomes [23]. Cubosomes have a wide range of applications, including the delivery of antibiotics, analgesics, enzymes, antimuscarinic medicines, and peptides [24]. Cubosomes are made up of highly twisted lipid bilayers and have a large surface area (approximately 400 m2 /g) [25]. The diameter of cubosomes ranges from 10 to 50 nm. Due to advancements in drug formulation stability, enhanced drug loading capacity, controlled drug release, and optimal particle size, cubosomal systems surpass other emerging delivery technologies. The drug within a cubosome diffuses through a channel within the cubic phase [26].

The polymers employed in producing cubosomes play a crucial role in ensuring both stability and controlled release characteristics. These polymers may include block copolymers as well as PEG moieties [27]. Cubosomes have recently been investigated by researchers for cancer therapy, cosmetic creation, topical applicability, and other drug delivery systems. In practice, there are very few anticancer medications being developed [28].

2. History of cubosomes

Luzzati and Husson, [29] along with Luzzati et al., were pioneers in identifying cubic matrices in lipid/water systems through X-ray scattering analysis. Fontell et al. [30] they both independently reached similar observations on cubic phase behaviour in ternary systems containing oils, water, & amphiphiles, although they appeared to be unaware of the lipid-related studies. Concurrently, Lutton extensively investigated the H2O phase dynamics of monoglycerides, which are polar lipids with restricted H2O solubility. The observed behaviour of monoglycerides in aqueous phases reflects their structural resemblance to non-ionic surfactants [31].

Landh and Larsson secured a patent for colloidal mixtures housing non-lamellar lyotropic crystalline states, which they named "cubosomes"[32]. Historically, cubosomes have been manufactured through time-consuming processes that require significant energy input. For example, Gustafsson et al., examined the structure and production of H2O dispersions containing lyotropic liquid crystalline phases derived from lipids.

These formulations were prepared using mixtures of glyceryl monooleate with either sunflower oil or retinyl palmitate, along with a non-ionic triblock polymer (Poloxamer 407), all dispersed in H2O. The process involved adding a lipid and Poloxamer melt to water, followed by size reduction through high-pressure homogenization at 80 °C. In a recent study, Siekmann and co-authors explored the development and analysis of dispersions made up of monoglycerides rich in monoolein, with or without purified soya phospholipids [33]. Their approach included achieving equilibrium of the monoglyceride/ H2O /phospholipid cubic phase, fragmenting it using a Poloxamer 407 solution, pre-dispersing through probe sonication, and ultimately, subjecting it to high-pressure homogenization.

Various researchers have developed experimental methods for producing cubosomes utilizing organic solvents. For example, Spicer and Hyden introduced a technique that relied on diluting an ethanol-based solution of monoolein with an aqueous poloxamer soln. Ethanol served as a hydrotrope, resulting in the spontaneous generation of cubosomes upon dilution. Similarly, Nakano et al. proposed another approach for cubosome production, which involved hydrating a dry film of monoolein/poloxamer using an aqueous buffer [34].

3. Advantages of cubosomes

1. They exhibit biocompatibility, biodegradability, and non-irritating properties [35].

2. Due to their expansive interior surface area, cubosomes possess a significant drug-loading capability. Additionally, they maintain thermodynamic stability over an extended duration [36].

3. Utilizing a particular polymer enables controlled and targeted release of bioactive substances.

4. They reduce the adverse impacts of injections attributed to sudden releases.

5. Due to a decrease in repeated administrations, the overall healthcare expenses are lowered.

6. The ratio of particle volume to bilayer area is greater than that of liposomes [35].

7. The preparation method is straightforward [37].

8. They are capable of encapsulating both hydrophobic/lipophilic (cinnarizine) and hydrophilic (cyclosporine) molecules [38][39].

9. The preparation techniques for cubosomes, such as shearing and homogenization techniques, do not necessitate the use of non-polar solvents [40][41].

4. Disadvantages of cubosomes.

1. Manufacturing cubosomes on a large scale is challenging because of its elevated viscosity [42].

2. Cubosomes, containing a significant water content, are less prone to entrap water-soluble drugs [43].

3. Over an extended period, particles may undergo growth when undisturbed. Cubosomes, in response to changes in the external environment, have the capability to undergo a phase change (dynamics) [32].

4. The absence of a particular polymer prevents controlled drug delivery, increasing the risk of potential leakage during storage or in vivo transmission [44].

5. Structure of cubosome

The fundamental structure of cubosomes consists of honeycomb-like arrangements forming dual internal aqueous channels, alongside a substantial interfacial area. These nanoparticles are best described as nanostructured particles with liquid crystalline phases, demonstrating cubic crystallographic symmetry. Cubosomes form through the self-assembly of molecules with amphiphilic characteristics or surfactant-like attributes.

Within cubosomes, cubic phases display an exceptional solid-like viscosity, a distinctive feature arising from their intriguing bicontinuous structures. These structures enclose two separate water regions, meticulously divided by a controlled bilayer of surfactant. Amphiphilic molecules play a pivotal role in forming these bicontinuous H2O and oil channels. In this context, "bicontinuous" denotes the existence of two continuous but separate polar regions, divided by the bilayer. The interconnected structure of the cubosome results in a visibly clear, viscous gel that resembles cross-linked polymer hydrogels both in appearance and rheology [45].

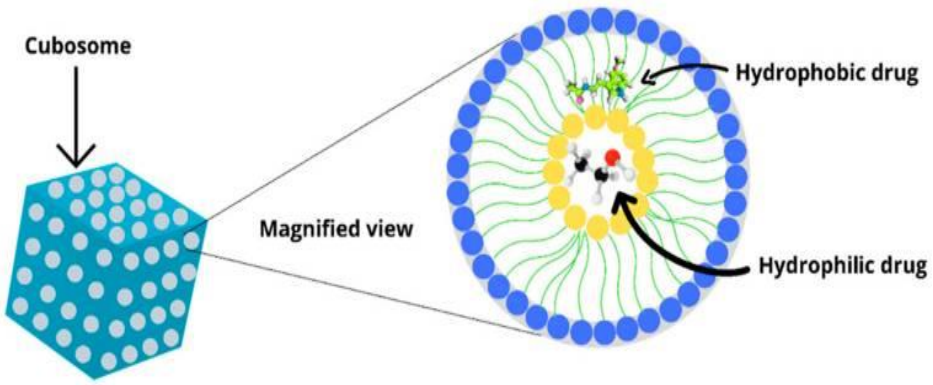

Figure 1. Structure of cubosome

6. Cubosomes and its types:

Amphiphilic BCPs (Bicontinuous Cubic Phases) assemble in specific solvents, resulting in various structures like vesicles (polymersomes), spheres, films, cylinders, fibers, ribbons, multi-geometry nanoparticles and tubules. These formations occur due to minimizing energetically unfavourable interactions between segments and solvents. Bicontinuous meso-structures are characterized by their three-dimensional interconnected phase structure. While bicontinuous phase structures are recognized, they exhibit a limited stability range. Low molecular weight surfactants are frequently employed to produce spread synthetic particles containing nanostructured bicontinuous cubic liquid crystals, commonly referred to as cubosomes [46]. Cubosomes represent an adaptable and pioneering method for delivering drugs, offering a concentrated and prolonged release of active components, along with a range of physico-chemical attributes. These nanostructured materials display cubic crystal symmetry akin to the initial phase but have a significantly increased surface area and reduced viscosity. This dispersion of nanoparticles holds promise as a potential solution to address the main challenges associated with the cubic phase [47]. Cubosomes are formed from Hydrophobic-hydrophilic lipids such as GMO (glycerol monooleate), which possess the capability to spontaneously organize themselves in liquid environments. They exhibit a three-dimensional honeycomb-like structure, typically ranging in size from 100 to 500 nm [48]. Luzzati et al., x-ray scattering techniques were employed to identify the presence of cubic phases within the lipid-H2O system [49]. Fontell et al., arrived at comparable findings concerning the cubic phase in ternary systems comprising of oils, H2O and amphiphiles, despite conducting their research without knowledge of the lipid studies [50]. At a similar period, Lutton extensively investigated the behaviour of monoglycerides in the aqueous phase. Monoglycerides, which have low solubility in water, are polar lipids that demonstrate behaviour in aqueous environments due to their structural similarity to non-ionic surfactants [51]. During the 1980s, Kre Larsson published a review discussing lipid/H2O phases characterized by a cubic structure, expanding upon the studies conducted by Patton and Carey. These researchers had investigated the development of bicontinuous cubic arrangements arising from lipid degradation [52]. Landh and Larsson patented a method for producing colloidal mixtures comprising non-lamellar lyotropic crystal phases, and they introduced the term "cubosomes" to describe these particles. Larsson, a prominent researcher in cubic structures, discovered that cubosomes can originate from larger cubic structures upon dispersion in water. This leads to the creation of submicron particles having an internal structure mirroring that of the original bulk structure [53].

Cubosome particles are formed through mechanical agitation of the cubic lipid-H2O phase found in a three-phase region, along with a dissemination of liposomes. They are named cubosomes to differentiate them from liposomes. Cubosomes possess a distinctive structure enabling them to concurrently accommodate amphiphilic molecules, lipid-soluble, and water-soluble, setting them apart from liposomes. Cubosomes exhibit thermodynamic stability and can persist indefinitely. Cubosomes have the potential for controlled release of active substances, as the intricate diffusion of actives occurs through the "regular" channel structure of the cubic phase.

These formations arise in aqueous surfactant environments with elevated levels of amphiphiles, displaying adequate molecular alignment to be identified by geometric symmetry [48]. Using principles from differential geometry, we can establish definitions for open and closed cubosome structures. In an open cubosome, both water channels are linked to the external environment, whereas in a closed cubosome, one water channel is exposed externally while the other is isolated, as illustrated in Figure 1. Cubosomes are classified into primitive, diamond, or gyroid types, and they retain cubic symmetry similar to their larger bulk parent phase [46][54][55].

6.1. Theories on Cubic Phase Structure:

Cubosomes, also known as bicontinuous cubic phase liquid crystals, display numerous fascinating characteristics as a versatile drug delivery system. These formations create bilayers within the surfactant and assume a minimal surface arrangement, periodic, three-dimensional, and leading to a densely packed structure. The substance generates a transparent, highly viscous bicontinuous cubic liquid-crystalline phase with a distinctive nano-scale arrangement. The process of producing cubosomes is relatively simple, and their improved lipid penetration and emulsification abilities allow them to encapsulate amphiphilic, hydrophobic, and hydrophilic substances. This encapsulation procedure guarantees the precise and regulated discharge of bioactive compounds [56]. Cubosome synthesis generally includes three primary stages related to the cubic structure: the bulk gel, precursor, and particle dispersion phases. The precursor phase represents a solid or semi-solid substance that changes into the cubic phase when triggered by stimuli, such as interaction with a liquid. Conversely, the bulk gel-cubic phase is distinguished by its stiffness, uniformity, and the capability to transform into cubosomes. The concluding phase entails dispersing the solid-like material into smaller particles, leading to the creation of cubosomes [57].

1. Fontell and Drew theory: Cubic phases are found in three-component systems containing oil, water, & amphiphiles, which also encompass various monoglycerides. Monoglycerides, known for their poor solubility in water, display aqueous phase behaviour resembling that of non-ionic surfactants. Lutton's study indicates that monoglycerides with hydrocarbon chains spanning from C-12 to C-22, such as monoolein or C-18 monoglycerides, are significant, demonstrate a larger surface area within the cubic phase. Monoolein, identified as an unsaturated fatty acid, falls within this category [50][58].

2. Gustafson et al. theory: Cubosomes are identified by singular crystal structures where single-layered vesicles are observable, and particles of the lamellar liquid-crystalline phase are uniformly dispersed. Increase the ratio of polymer to monoolein promotes the formation of larger vesicles. [50]. Subjecting bulk cubic phases to ultrasonication initiates gradual transport processes, resulting in highly viscous crystalline structures that require significant energy for fragmentation. Over time, these structures transform predominantly into vesicles through membrane fusion.

This inherent metastability is one of the distinctive features of cubosome systems, specifically in the context of bulk cubic phases. Additionally, cubosomes exhibit colloidal stability through the presence of vesicles [59].

3. System Forming Theory: Cubosomes may manifest in both binary and ternary systems when there is a significant difference in miscibility b/w the cubic phase and the solvent. The addition of poloxamer 407 effectively prevents cubosome aggregation and flocculation, ensuring their colloidal stability. Lamellar bilayer caps can enclose these cubosomes, effectively sealing the gap in the cubic bilayer caused by fragmentation. This encapsulation aids in preserving colloidal stability by obstructing the interaction between hydrocarbon chains and water. Cubosomes covered by a solid crystalline bilayer exhibit improved colloidal stability, whereas those with liquid-crystalline lamellar coatings are comparatively more rigid. Additionally, sponge phase coatings have been suggested as a potential method to stabilize cubosomes. Another promising candidate for cubosome development is phytonadione [59].

4. Schwarz, Jacob and Anderson Theory: Cubic phases are commonly detected in systems of non-ionic surfactants, positioned b/w lamellar and hexagonal liquid-crystalline phases. Notably, the monoolein-H2O system stands out for its expansive cubic phase region across various compositions and temperatures. Surfactant packing concepts are converging, particularly considering that monoolein, with its polar headgroup and nonpolar tail, can generate inversed cubic phase or reversed cubic phase, signifying aqueous phases. Describing cubic phase structures involves concepts from differential geometry and periodic minimal surfaces, often clarified by likening them to soap films. Three classifications of minimal surfaces are investigated in cubic phases, distinguished by their curvatures. In the monoolein-water system, the D-surface arises when water levels are high, while the G-surface is observed when water levels are lower. The p-surface emerges in the presence of a third component, such as caseins or amphiphilic molecules, and is further influenced by the incorporation of block copolymers. X-ray scattering techniques are utilized to determine the existence of cubic phases, while cubosomes are visualized through transmission electron microscopy (TEM) and freeze-fracture electron microscopy [59].

5. Components of Cubosomes: The primary constituents of cubosomes are amphiphilic lipids and stabilizers of diverse qualities, as indicated in Table 1. The selection of lipids for formulating cubic crystals is based on their amphiphilic characteristics, encompassing monoglycerides, glycolipids, phospholipids, and alkyl glycerates. The self-assembly of these amphiphilic lipids into a variety of nanostructures with unique physico-chemical properties and geometries is determined by factors like lipid composition, molecular structure, electrostatic interactions, as well as pressure and temperature conditions [60].

Table 1. Lipid Components and Stabilizing Compounds used in Cubosome Production.

Lipids	Stabilizer	References
Monoolein	Pluronic F127	[61]
Phytantriol	Pluronic F127	[62]
Monoelaidin	Pluronic F127	[63]
β-XP (1-O-phytanyl-β-d-xyloside)	Pluronic F127	[64]
Monoolein or phytantriol	Pluronic F108	[65]
Phytantriol	Myrj 59	[66]
Monoolein	Modified starch	[67]
Sodium octyl sulfate (SCS)	Arginine-based cationicsurfactant	[68]
Monoolein	Laponite XLG	[69]

Glyceryl monooleate (GMO), commonly known as monoolein, stands out as the most prevalent and extensively employed amphiphilic lipid in the creation of cubosomes. The properties and attributes of GMO, crucial for synthesizing cubosomes, encompass the polarity of the unsaturated monoglyceride, which possesses a melting point ranging from 35 to 37°C, a recommended storage temperature between 20 and 30°C, and an HLB (Hydrophilic-Lipophilic Balance) value of three. Typically, GMO is colourless and transparent in nature. To generate bi-continuous cubic structures, the synthesis of GMO involves a blend of glycerides and esters derived from oleic and other fatty acids, predominantly consisting of mono-oleate [70].

The OH groups present in the head segment of the GMO structure play a critical role in establishing hydrogen bonds with both H_2O molecules and alkyl chains in the tail. This characteristic renders GMO an amphiphile, possessing both polar and non-polar traits (Refer to figure 1). Initially proposed in 1984, GMO was identified as a biocompatible material suitable for encapsulation. Its non-toxic, biocompatible, and degradable properties make it a widely employed emulsifier in the food industry and various pharmaceutical formulations [71].

Figure 2. Molecular structure of glyceryl mono-oleate (GMO).

Phytantriol, along with GMO, is employed in cubosome formulation due to its improved permeation abilities and emulsification abilities. Phytantriol, commercially available at a high purity of 95%, has surpassed monoglycerides in recent interest due to variations in purity arising from synthesis from different sources. The liquid crystalline matrices based on Phytantriol are recognized as an exceptional drug delivery system, particularly for sustained release of drugs, especially hydrophilic compounds [62][72].

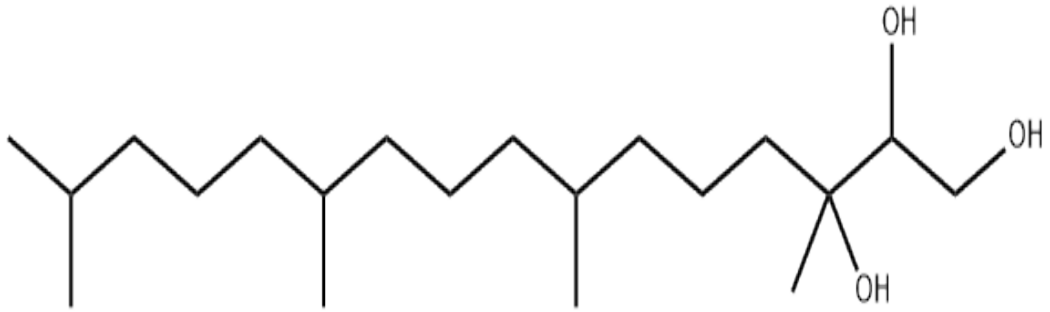

Figure 3. Structure of Phytantriol

Cubosomes consist of large cubic formations that exhibit stability from a thermodynamic perspective, yet their kinetic stability is undermined by the interaction between the non-polar component and external polar H_2O media. Improving the stability of the formulation necessitates the addition of a stabilizing agent to prevent the clustering of dispersed particles and maintain the integrity of the initial bulk cubic structure. This stabilizer forms an electrically charged barrier separating particles, facilitating their dispersion and preventing re-coalescence [73].

The stabilizer impacts the configurations of dispersed particles and governs their phase properties. Particularly, an optimal concentration of P407 ensures the existence of the P-type cubic phase, which is essential for establishing a persistent colloidal mixture. The interactions between P407 and bulk liquid crystal matrices depend on the internal structure and chemical composition of the liquid crystal matrices, influencing their nature and frequency. For instance, P407 integrates into the liquid crystalline structure within the GMO cubic phase rather than being absorbed upon the surface of the PHYT cubic structure [74].

Pluronics, especially F127, also known as "Poloxamer 407," are extensively utilized as Stabilizers in the production of cubosomes, with F127 being recognized as the standard choice. These copolymers, Consisting of polypropylene (PPO) & polyethylene oxide (PEO), dissolve readily in water. The polar and non-polar characteristics of Pluronics are derived from their polyethylene oxide & polypropylene components. The stabilizer concentration may differ based on the dispersed particles and the lipid varieties employed in the formulation. In formulations consisting of GMO as a lipid, Pluronics are added in concentrations of up-to 20% w/w relative to the cumulative weight of the dispersion, and the stabilizer concentration ranges from 2.5% to 10% (w/w) [75].

In recent times, there has been a shift towards utilizing non-ionic molecules as stabilizers rather than F127. Researchers have found that the stabilizing capability of the stearate class of poly (ethylene oxide) surpasses that of F127 when formulating phytantriol cubosomes. Specifically, Myrj59®, a product containing 100 poly (ethylene oxide) units, showed greater effectiveness in stabilizing phytantriol cubosomes compared to the standard F127. However, the mechanism and rationale behind this increased stability remain unclear. Similarly, polyvinyl alcohol (PVA) demonstrated notable effectiveness in stabilizing the dispersion and is suitable for preparing cubosomes [60].

7. Methods of Preparation for Cubosomes:

7.1 High-Pressure Homogenization:

This method is optimal for preparing cubosomes that exhibit extended shelf life and exceptional stability, particularly when undergoing the high-pressure homogenization process [76][77]. There are three steps to it:

7.1.1 Gel Preparation:

In this phase, the amphiphilic surfactants & lipids are mixed in a volatile organic compound and meticulously mixed to achieve a uniform blend. Subsequently, the organic solvent is removed with a rotary evaporator to produce a gel phase in the formulation.

7.1.2 High-Pressure Homogenization:

This method is suitable for sample systems with substantial volumes (30 ml), but it is not suitable for smaller volumes. The temperature chosen in this stage depends on the lipid's characteristics as the process is sensitive to temperature. The dispersion produced is homogenized using a high-pressure homogenizer. It's important to note that this technique is capable of handling only one sample at a time.

7.1.3 Shearing:

In this phase, the formed gel undergoes shearing. The utilization of aqueous solvents results in the formation of a micro-dispersion. This step is essential in the cubosome manufacturing process and occurs just before the homogenization stage.

7.1.4 Automated Cubosome Preparation:

This method can be employed for the production of a significant quantity of cubosomes. The procedure involves the utilization of robotic equipment and a probe sonicator, resembling the probe sonication technique with minimal modifications. Gels are prepared using a 96-well plate with a solvent capacity of 600 µl, and sonication is performed by an automated system. This method streamlines the evaluation of physicochemical properties [78].

7.1.5 Probe Ultra Sonication:

The rapid preparation of small-volume samples is facilitated by this method, capable of dispersing even 600 µl samples, depending on the probe size. Stabilizers are incorporated in this process for gel preparation. Subsequently, a cubic phase is generated through solvent equilibration. Following this, the cubic phase undergoes ultrasonication. It is crucial to carefully control the frequency and amplitude to regulate pulsing and prevent sample overheating [79].

7.2 Special Techniques:

7.2.1 Top-Down Technique: This method is commonly used in the preparation of cubosomes, especially when employing glyceryl monooleate (GMO) as a lipid polymer. The process involves the application of high energy levels to achieve a fine dispersion of cubosomes. The process begins by initially forming bulk cubic aggregates by adding a suitable stabilizer to a lipid, utilizing a homogenizer to apply significant energy for the creation of the dispersion. Cubosomes produced using this method fortunately demonstrate stability for up-to 1 year. However, a significant limitation arises during the preparation of largescale manufacturing batches, where the use of very high energy becomes impractical, particularly when incorporating thermolabile bioactive compounds such as proteins and peptides [80].

7.2.2 Bottom-Up Technique: It is another method for the preparation of cubosomes. Frequently employed in the formulation of cubosomes that incorporate phytantriol as the lipid component. This method, also called the solvent dilution technique, demands less energy input in comparison to the top-down approach. The primary aim of this method is to create cubosomes including a stabilizer & hydrotrope in an abundance of water, while minimizing energy input. Hydrotrope is the key component of this technique, which plays a vital role in controlling the solubilization process for dissolving hydrophobic lipids. Additionally, it inhibits the formation of liquid crystals when concentrations are high. Acting as an emulsifier, the hydrotrope can poorly solubilize in H_2O phase through hydrotropic agents. The bottom-up approach is preferable to the top-down method due to its lower energy requirements, making it a safe option for preparing cubosomes that incorporate temperature-sensitive agents. The even distribution of stabilizing agent on the surface of the resulting small particles contributes to the formulation's long-term stability [81][82].

8. Evaluation and characterization ofcubosomes:

8.1 Visual Inspection Studies: The external characteristics of the cubosomes are evaluated, which involve examining their morphology, turbidity, color, consistency, and the presence of particles [83].

8.2 Transmission Electron Microscopy (TEM): The examination of cubosome morphology can be conducted using TEM, which imparts shape to cubosomal particles through the emission of electrons. Observational microphotography with electron release yields high-resolution images, facilitating effective visualization. In contrast to a light microscope, TEM offers superior resolution, making it an outstanding instrument for characterizing soft matter dispersions. Importantly, it addresses typical drawbacks of electron microscopy, including the need for a vacuum environment, subpar image quality, and the potential for inducing structural changes in the cubic phase [83].

8.3 Particle size distribution: are mainly evaluated using dynamic laser light scattering with a Zeta sizer, employing Photon correlation spectroscopy. The sample is sufficiently diluted with a solvent, tuned to a light scattering intensity of around 300 Hz, and subsequently evaluated in triplicate at a temperature of 25 °C. Typically, the gathered data is presented using the mean volume-weighted size. Additionally, the polydispersity index & zeta potential can be observed [84].

8.4 Zeta potential: The strength of the zeta-potential indicates the level of electron-electron interaction among appropriately aligned, similarly polarized particles. The zeta potential stands as a pivotal indicator of the stability of the formulation [85].

8.5 Viscosity: The measurement of viscosity can be conducted using a viscometer, specifically the Rotational Brookfield Viscometer [83].

8.6 Entrapment efficiency: Can be assessed through ultrafiltration techniques [86]. This technique involves measuring the concentration of the drug that remains unentrapped, which is then subtracted from the total amount of drug added. The drug quantity is subsequently analyzed by using UV spectrophotometer.

8.7 Determination of drug elution: The elution of drugs from cubosomes can be achieved through the pressure-driven ultrafiltration [86]. This method follows the procedure introduced by Magenheim et al., employing an Amicon pressure-driven ultrafiltration cell fitted with a Millipore membrane at ambient temperature (21±3) °C.

8.8 Polarized Light Microscopy: The examination of surface coatings on cubosomes, characterized by optical properties such as birefringence or vesicular structures, can be potentially conducted using polarized light microscopy. This method also allows for distinguishing between anisotropic and isotropic characteristics [87]. Changes in cubic phases can be observed, providing insights into the potential coexistence of layered structures with cross or striated patterns, as well as hexagonal liquid crystals [88].

8.9 Stability studies: The examination of organoleptic and morphological aspects over time allows for the study of physical stability. Furthermore, alterations in particle size distribution and drug content can examined at different time points to evaluate potential changes over the time [89].

9. Applications of Cubosomes:

1. Oral drug delivery system: Cubosomes face obstacles in orally delivering promising compounds due to factors like insufficient Insoluble in water, minimal absorption, and substantial molecular size.Alternatively, large proteins have been enclosed to exert targeted activity within the GIT. To complement this approach, carriers utilizing liquid crystal-based nanoparticle technology can be combined, providingtargeting capabilities& controlled release. These nanoparticles are engineered to generate on-site at a controlled rate, thereby enhancing their effectiveness in distributing drugs in vivo. Additionally, cubosome carriers can be strategically deployed at specific absorption sites, such as the lower or upper intestine, which is crucial for drugs with a constrained site-specific absorption region [90].

2. IV drug delivery system: Lipid nanoparticles with internal liquid crystal matrices, formed by curved lipid membranes, are utilized to dissolve, enclose, and transport medications to specific areas in the body affected by disease.In comparison to emulsions and liposomes, cubosomes nanoparticles exhibit enhanced capacities for carrying peptides, proteins, and numerous poorly soluble small molecules.They are particularly well-suited as carriers for injection due to their ability to accommodate increased payloads [91].

3. Topical drug delivery system: Cubosomes exhibit heightened bioadhesiveness, making them well-suited for applications in local applications and mucosal distributions, including drug delivery. The distinctive attributes ofliquid crystal nanoparticle& liquid crystal technologies are utilized in the development of topical drug delivery systems. These systems stand out by spontaneously generating bioadhesive LC systems at the application site, facilitating controlled and efficient drug delivery to mucosal surfaces like ophthalmic,buccal, vaginal, and others. This intriguing system produces a thin layer on mucosal membrane, consisting of a liquid crystal structure.The nanostructure of this matrix can be manipulated to achieve an optimal delivery profile, offering effective temporary protection for irritated and susceptible skin [92].

4. Therapeutic carrier: These innovative materials are frequently utilized in drug delivery systems. Collaborative research with prominent cosmetic companies such asNivea& L'Oreal is investigating the potential use of cubosome particles to stabilize O/W emulsions and to absorb pollutants in cosmetic products [93].

5. Prolonged Drug Release Behaviours: In recent times, there has been a notable increase in patent activity related to the utilization of cubosomes in personal care products, encompassing skincare, haircare, cosmetics, and antiperspirants. Despite recent efforts, there is still considerable work ahead. Practical aspects such as material scalability, manufacturing scalability, and customization, crucial for establishing a leading position, are currently lacking. Formulators should seriously consider incorporating cubosomes into commercially available products. The cubic phase has been successfully proven to function as a carrier in various in vivo experiments, including depot, transdermal,

ophthalmic adhesion, and muco-adhesion delivery methods. This is attributed to the fusogenic property of Monoolein in cubosomes, enhancing macromolecular penetration. Additionally, it contributes to the improvement of macromolecule penetration. Drugs with diverse physicochemical features have been successfully integrated into cubosomes, and their sustained release behaviour has been thoroughly explored. Cubosome residual particles play a crucial role in the long-term activity of cubosomes. Notably, the topical application of monoglyceride-based cubosome dispersion, for percutaneous or mucosal applications, is viable [78].

6. **Controlled-Release Drug Delivery:** Cubosome researchers predominantly focus on the controlled release of solubilized actives, conducting thorough assessments of various delivery applications and pharmaceutical actives solubilized in both bulk cubic phase and cubosomes. The minute pore size (5–10 nm), ability to solubilize hydrophobic, hydrophilic, and amphiphilic compounds, and biodegradability through simple enzyme activity make cubic phase highly attractive for controlled release. The strong bioadhesive nature of cubic phase suggests its potential as a skin penetration enhancer, indicating excellent compatibility with topical and mucosal deposition, as well as the administration of active ingredients.

Recent research has identified similarities between the bicontinuous structures formed in the layers of human skin and those present in cubic phases, offering promising prospects for an improved understanding and treatment of skin transport. The intricate structure of the cubic phase proves advantageous for slowing down the diffusive release of solubilized active ingredients. According to theoretical considerations, the free solution diffusivity of a solute is expected to decrease by 33% in the cubic phase. Experimental measurements indicate that the diffusivity of small molecules in cubic phases is in the order of 10(10) m2/sec. Currently, aside from a treatment for periodontal disease involving combinations of triglyceride and monoolein with the antibiotic metronidazole, there are no known commercial applications for cubic phase delivery vehicles. In the case of this treatment, when the lipid–drug mixture is applied to the gums and interacts with saliva, it undergoes hydration to form a bulk cubic phase, which subsequently facilitates the distribution of the drug to the gums. Despite the potential of bulk cubic phase as a delivery vehicle, several applications necessitate the use of cubosomes due to the exceptionally high viscosity of bulk cubic phase. It's important to note that while the previously mentioned controlled-release limitations are applicable to small molecule solutes and unmodified cubosomes, there may be distinct controlled-release pathways for cubosomes. For instance, when large poly(amidoamine) dendrimer molecules are trapped in cubic phases, their free diffusivity is reduced by 100% [94].

7. **For cancer treatment:** Recently, certain anti-cancer medications have been adequately enclosed within cubosomes, and their physicochemical properties have been thoroughly examined. The distinctive structure of this promising nanocarrier indicates its potential utility in the treatment of melanoma. Various strategies have been considered to precisely direct nanomedicines to tumors, with both passive and active targeting of cancer cells proving to be viable approaches in both preclinical and clinical investigations [95].

8. For viral treatment: Because of the microbicidal properties of monoglycerides, they could be utilized in developing intravaginal therapies for sexually transmitted diseases caused by viruses (such as HSV, HIV) or bacteria such as Chlamydia trachomatis and Neisseria gonorrhoeae [96].

9. For the treatment of Hair, Skin, & Body Tissue: Cubosomes are formed through the combination of biocompatible H_2O & lipids, rendering them suitable for treating hair, skin, and various bodily tissues. These cubosomes typically consist of monoglycerides or mono-olein. Monoglycerides possess microbicidal properties, and cubosomes also incorporate ethanol, known for its skin-disruptive effects. The heightened lipid fluidity in cubosomes facilitates increased permeation through the skin. This characteristic enables drugs to penetrate the skin, adhere to skin lipids, and subsequently release into the deeper layers of the skin [97].

CONCLUSION

Cubosomes represent a cutting-edge platform in drug delivery with distinct advantages and a wide array of applications. Their cubic lipid phase offers exceptional stability, biocompatibility, and versatility, making them promising candidates for therapeutic interventions. Cubosomes demonstrate advantages over conventional lipid-based nanoparticles, including higher encapsulation efficiency, tunable release kinetics, and improved drug loading capacities. Cubosomes find applications across diverse fields, including drug delivery, imaging, diagnostics, and vaccine delivery. Their ability to encapsulate hydrophilic and hydrophobic drugs, protect them from degradation, and facilitate targeted delivery to specific sites within the body holds significant promise. Additionally, the potential for sustained and controlled release enhances therapeutic efficacy while minimizing side effects and improving patient compliance.

Overall, cubosomes represent a frontier in nanomedicine, poised to revolutionize drug delivery and therapeutic interventions. Continued research efforts aimed at addressing manufacturing challenges and optimizing cubosome properties will likely unlock further opportunities for their widespread adoption in pharmaceutical and biomedical applications, ultimately benefiting patients by improving the efficacy and safety of drug delivery systems.

Acknowledgments

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