Cubosomes: A Smart Nanocarrier For Next-Gen Medicine

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ABSTRACT:

Cubosomes are novel nanostructured lipid carriers having a specific bicontinuous cubic phase structure, generated by the self-assembly of amphiphilic lipids like monoolein or phytantriol in the presence of water and stabilised by surfactants such as poloxamers. This highly complex internal organisation enables the entrapment of hydrophilic and hydrophobic therapeutic drugs, improving their solubility, stability, and bioavailability. Due to high surface area, thermodynamic stability, biocompatibility, and biodegradability, cubosomes form a sophisticated platform for controlled and targeted drug delivery. Formulation optimization, enhanced structural integrity, and increased application areas of cubosomes like oral, topical, transdermal, and intravenous drug delivery have been the focus of recent studies. Site-specific delivery, particularly useful in the treatment of cancer, neurological disorders, and chronic inflammatory diseases, can be achieved by functionalizing with targeting ligands and polymers. Additionally, cubosomes have shown promise in vaccine and gene delivery because they can encapsulate and shield protein and nucleic acid-based substances. In cosmetics and nutraceuticals, cubosomes enable the delivery of sensitive ingredients such as vitamins, antioxidants, and omega-3 fatty acids. Preparation methods involve top-down, bottom-up, spray-drying, and sonication processes, each providing unique benefits depending on application. Quality and performance of formulations are guaranteed by evaluation techniques like cryo-TEM, SAXS, DLS, and HPLC. Although problems such as scalability, long-term stability, and cytotoxicity at high levels of concentration remain, further research helps in improving cubosome technology.

KEYWORDS: Cubosomes, Nanoformulations, XRD, DSC, Bicontinous

INTRODUCTION:

Over the last few years, nanotechnology has transformed pharmaceutical studies with novel answers for efficient medication delivery. Between different nanoformulation systems, cubosomes stand out because they possess exceptional architectural and functional characterizations. Cubosomes are nanoscale aggregates of lyotropic [1] liquid

crystalline particles with an exceptional bicontinuous cubic phase structure. This complex structure consists of two aqueous channels that are separate but continuous with a lipid bilayer in between, forming a highly structured, stable system. Their interesting internal structure allows cubosomes to encapsulate hydrophobic as well as hydrophilic drugs, improving bioavailability and solubility [2].

The idea of cubosomes was developed through research on lipid-based nanomaterials and liquid crystalline phases. During the 1980s, researchers started investigating liquid crystalline phases of amphiphilic lipids and their potential as drug delivery vehicles. These phases have the ability to self-assemble into very ordered structures, such as cubic, hexagonal, and lamellar phases.Scientists found that amphiphilic lipids like monoolein could self-assemble into cubic structures at the nanoscale upon hydration, providing potential drug molecule carriers. Such a phase could be stabilized into nanosized particles to give the foundation for cubosome nanostructures. In the early 1990s, scientists were successful in designing cubosomes, or nanosized particles with a cubic structure phase. The particles were mainly created using amphiphilic lipids such as monoglycerides (e.g., monoolein) blended with water. Cubosomes possessed some benefits over other lipid-derived nanocarriers, such as increased surface area, improved encapsulation properties, and the capacity to encapsulate both hydrophobic and hydrophilic drugs [3,4].

In the 2000s, more applications of cubosome nanofomulations were investigated in the field of nanomedicine [5]. Experiments confirmed their potential for delivering a diverse array of therapeutic agents, including small molecule therapeutics, peptides, proteins, and nucleic acids. Based on their biocompatibility, low toxicity, and drug release control, cubosomes emerged as a promising delivery platform for controlled drug delivery. One of the most important benefits of cubosomes was that they were capable of encapsulating hydrophilic drugs in their aqueous core and hydrophobic drugs in the lipid bilayer. Their use in multiple pharmaceutical formulations was made possible by this feature [6]. Their cubic structure also yielded a high surface-to-volume ratio, which enhanced drug encapsulation efficiency as well as release profiles.

In the 2010s, scientists began to investigate the combination of cubosomes with other newer drug delivery technology to further increase their utility. These included:

- Surface modification: By altering the surface of cubosomes with targeting ligands or polymers, they could be designed to target specific cells or tissues. This rendered them especially valuable in cancer treatment and other precision medicine uses [7].
- Hybrid systems: Cubosomes were frequently blended with other nanomaterials, including nanoparticles, gold particles, and polymeric systems, to form hybrid nanostructures with added properties such as extended circulation times, improved stability, and more controlled drug release [8,9].
- Use in vaccines: Cubosomes have been utilized as adjuvants in vaccine compositions because they can improve the immune response. The cubic structure provides a means to present antigens in a manner that effectively stimulates the immune system [10,11].

Cubosomes are mostly made of amphiphilic lipids like phytantriol or monoolein, along with stabilizing surfactants to ensure nanoparticle stability. The three-dimensional geometry of the cubic phase ensures a high surface area, enabling controlled and extended release of the drug [12]. Cubosomes also have very good biocompatibility and biodegradability, thereby making them prime potential candidates for the pharmaceutical, cosmetic, and food industries. These features have established cubosomes as a potential drug delivery platform for a variety of therapeutic agents, including poorly water-soluble drugs, peptides, proteins, and genetic material. The multifunctionality of cubosome nanoformulations makes them capable of overcoming many limitations of traditional drug delivery systems. For example, their capacity for encapsulation and protection of fragile bioactive molecules improves drug

stability. Additionally, the ability to tune size and surface modification allows for targeted delivery to target tissues, enhancing therapeutic efficacy with reduced side effects [13-14]. Characterization methods are important in determining the stability, structure, and performance of cubosome formulations [15]. Methods like small-angle X-ray scattering (SAXS), cryogenic transmission electron microscopy (Cryo-TEM), and dynamic light scattering (DLS) are usually used to assess the cubic phase structure, particle size, and surface charge. These analyzes are necessary to optimize formulation parameters and improve drug delivery performance [16].

Cubosomes have shown promise in various therapeutic applications. In the treatment of cancer, cubosome-based systems enhance drug penetration within tumors and control drug release, which increases the therapeutic effect [17]. In topical application, cubosomes facilitate increased drug permeation through the skin and are thus beneficial in dermatological therapies [18]. In addition, their ability to deliver hydrophobic antifungal, antibacterial, and antiviral agents have been proven effective in treating infectious diseases [19]. Although they have many benefits, cubosome nanoformulations are hindered by limitations including scalability in manufacturing, long-term stability, and cytotoxicity at high concentrations [20]. Current research seeks to overcome these challenges by refining formulation strategies, investigating alternative lipid materials, and adding biocompatible polymers for enhanced safety profiles [21]. Finally, cubosome nanoformulations are a cutting-edge evolution in the pharmaceutical sciences, providing better drug delivery with increased bioavailability, stability, and targeted release. With ongoing research and technological innovations, cubosomes are poised to make significant contributions to unmet medical needs and to broaden therapeutic horizons in modern medicine [22,23].

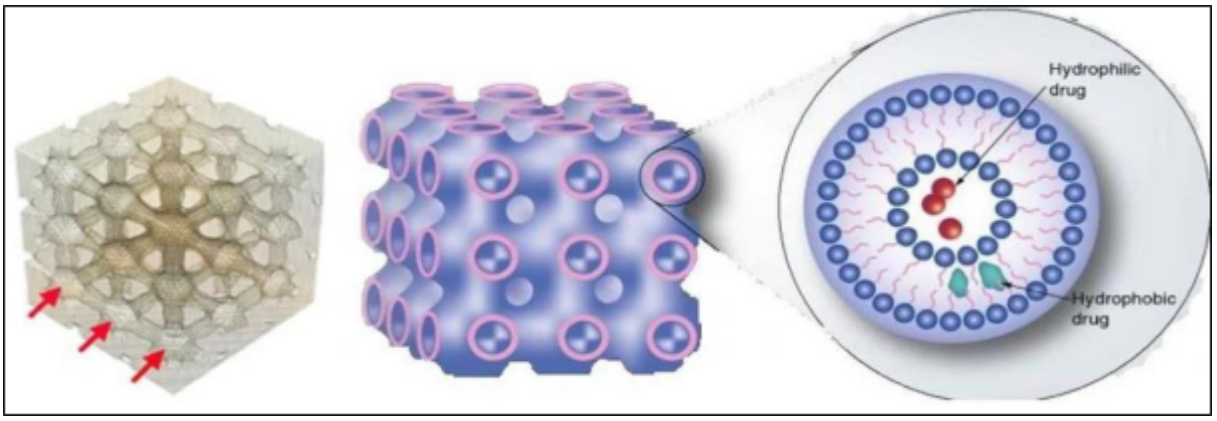
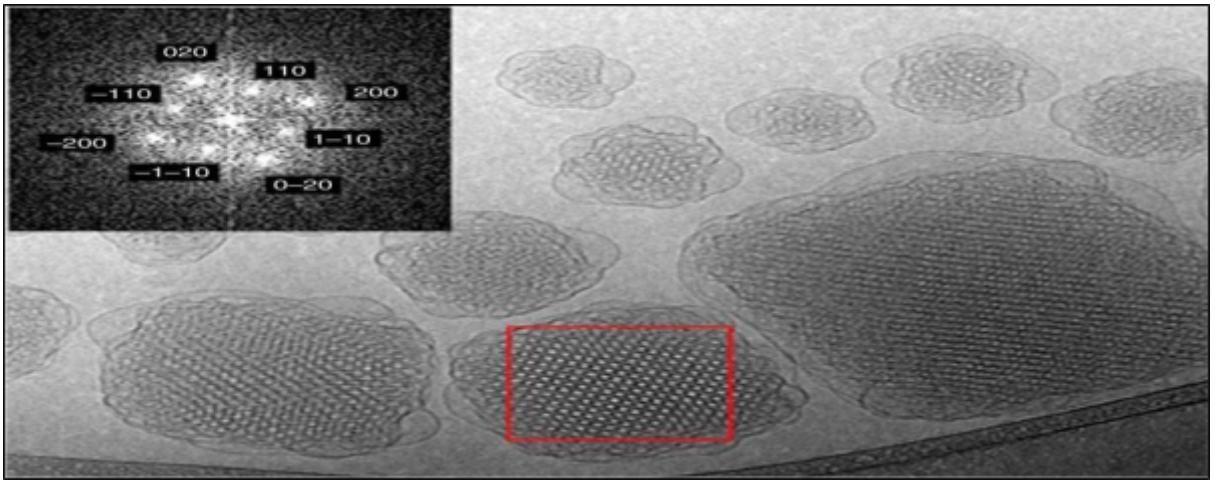


Fig No.1: Structure of cubosome [89]



- **Mechanism of Drug Release from Cubosomes:**

Cubosomes (CBs) have controlled release of drugs as a result of their small pore sizes, which allow tortuous diffusion through the ribosomal structure. The rate of release of trapped drugs is a function of their molecular weight and polarity. Hydrophilic drugs release quickly due to greater contact with the aqueous phase, while hydrophobic drugs release slowly because they are attracted strongly to the hydrophobic part of the cubic phase. The release of drugs from CBs usually fits the Higuchi diffusion-controlled model [24]:

$$Q = (DmCd\ 2A - Cd)\ t^{(1/2)}$$

where Q = amount of drug released, Dm = coefficient of diffusion, Cd = solubility, A = agent concentration in the matrix, and t = time. Alternatively, the Korsmeyer–Peppas equation is employed [25]:

$$F = (Mt/M) = Km\ t^{n*}$$

where F = fraction released, Mt = released drug, M = total drug, Km= kinetic constant, n = release exponent, and t = time. For MXD-CBs, release is Higuchi kinetics with an exponent "n" of 0.6792, which signifies non-Fickian (anomalous) release, which includes both diffusion and erosion-controlled mechanisms [26].

- **Advantages:** [27-32]

1. **Non-toxic and Biodegradable:** Cubosomes are non-toxic, disposable, non-irritating, and non-allergic, hence safe for application.

2. **Easy Production:** Production is easy and inexpensive.

3. **Huge Surface Area:** Huge interior surface area increases their effectiveness in carrying and delivering drugs.

4. **Thermodynamic Stability:** High level of thermodynamic stability, which keeps them stable over long periods of time.

5. **Physicochemical Stability:** They are stable even with excess water present.

6. **Regulated and Targeted Release:** With the use of certain polymers, cubosomes can provide regulated and targeted release of drugs.

7. **Improved Bioavailability:** Their reduced size enhances absorption and bioavailability of drug.

8. **Lower Healthcare Costs:** Infrequent administration decreases total healthcare costs.

9. **Lower Adverse Reactions:** They decrease the adverse reactions that occur with burst release in injections.

10. **Improved Solubilization:** Cubosomes are better than other lipid-based carriers, such as liposomes, for solubilizing capacity.

- **Disadvantage:** [32-36]

1. **Poor Water-Soluble Drug Trapping:** The excess amount of water restricts water- soluble drug trapping.

2. **Manufacturing Challenges:** Due to their high viscosity, large-scale production

3. **Leakage Issues:** Leaks can occur in cubosomes during in vivo transfer or storage.

4. **Particle Size Instability:** With time, the particles of cubosomes can grow in size, impacting their performance.

5. **Phase Transition Sensitivity:** Alteration in the external environment can cause phase transition causes stability.

TYPES:

Cubosomes are one of the classes of nanostructured lipid carriers with a cubic structure when they are hydrated. Cubosomes are most commonly produced using amphiphilic lipids such as monoglycerides and diglycerides, although their structure might differ based on the particular composition of the lipid and the way they are prepared. The major categorization of cubosomes might be according to their structural structures, lipid components, and envisioned applications. This is a detailed description of various types of cubosomes:

Table no.1: Types of cubosomes

Type of Cubosome	Description	Structure
Monoolein-Based Cubosomes [37]	Developed from monoolein (a monoglyceride). Widely used and most versatile for drug delivery.	Inverse cubic phase (QII) with hydrophilic core and hydrophobic lipid bilayer.
Polymer-Modified Cubosomes [38]	Cubosomes with polymers (e.g., PEG) added to improve stability and surface characteristics.	Cubic phase with polymer coating on the surface.

Cholesterol-Containing Cubosomes [39]	Addition of cholesterol to enhance rigidity and stability of the structure.	Rigid cubic phase, more stable than monoolein-only cubosomes.
Ceramide-Based Cubosomes [40]	Derived from ceramide lipids, employed for bioactive and dermal drug delivery.	Cubic phase structure with ceramide lipids, providing rigidity and bioactivity.
Block Copolymer-Modified Cubosomes [41]	Cubosomes modified with block copolymers (e.g., PLGA, PEG-b-PLA) to enhance stability and drug loading.	Hybrid structure of lipids and block copolymers, providing increased drug delivery characteristics.
Peptide-Functionalized Cubosomes [42]	Peptide-functionalized cubosomes for targeted delivery to a particular tissue or cell.	Cubic phase with peptide ligands on the surface for targeting a specific receptor.
Multicomponent Cubosomes [43]	Composed of a blend of various lipids, providing more complex structures and increased functionality.	Blend of lipids creating a more complex cubic phase structure.
Niosomes-Based Cubosomes [44]	Cubosomes prepared by blending niosomes (non-ionic surfactant vesicles) with lipids such as monoolein.	Hybrid of niosome and cubosome structures, stable with surfactant-based system.

APPLICATION:

Cubosomes are new nanostructures through self-assembly of amphiphilic lipids in aqueous systems to create cubic phases that also exhibit potential use as drug carriers in the delivery and targeting of several diseases across diverse

body systems. [45] Owing to their interesting structural features, cubosomes possess extensive surface area for loading hydrophobic as well as hydrophilic drugs and hence have become useful drug carriers for targeted and controlled delivery. Their application in drug delivery systems is especially beneficial for the promotion of bioavailability, stability, and controlled release of drugs. Some of the major applications of cubosomes are listed below:

- **Cancer Therapy:** Anticancer drugs can be delivered to cancer sites using cubosomes. With surface functionalization by targeting ligands (e.g., antibodies or peptides), cubosomes can selectively accumulate in cancer cells with minimal systemic toxicity and enhanced therapeutic efficacy [49]. Cubosomes can be tailored to target the delivery of doxorubicin to cancer sites, increasing the efficacy of the drug with less systemic side effects such as cardiotoxicity. By appending cancer-targeting ligands (e.g., monoclonal antibodies), cubosomes can enhance cancer cell specificity of drug delivery.
- **Cardiovascular Diseases:** For cardiovascular diseases, cubosomes can be filled with blood pressure regulation drugs, cholesterol-lowering drugs, or anticoagulant drugs. Cubosomes' stability and controlled release nature make them a good fit for chronic conditions that need long-lasting drug action. Cubosomes can be used to encapsulate atorvastatin in order to deliver it in a controlled manner for treating high cholesterol levels over long durations, minimizing frequent dosing. The delivery system helps to sustain the drug's therapeutic levels in the blood for longer durations [46].
- **Central Nervous System Disorders:** BBB is a hindrance to the delivery of drugs to the CNS. Cubosomes can be made to penetrate through the BBB for targeted delivery of drugs to address neurological disorders like Alzheimer's or Parkinson's disease. Cubosomes can be engineered to penetrate the blood-brain barrier and release donepezil directly into the brain, where it assists in controlling Alzheimer's disease symptoms. Controlled release provides a constant drug concentration, maximizing its therapeutic effect [48].

- **Anti-inflammatory and Immune System Modulation:** Cubosomes have the capability to load anti-inflammatory drugs and deliver them locally to inflammation sites (e.g., in rheumatoid arthritis) or amplify the immune response in autoimmune diseases. The controlled release of drugs is important for such treatments. Cubosomes are able to deliver prednisolone to the desired inflammatory locations, i.e., rheumatoid arthritis or inflammatory bowel disease [46]. Localized release of the drug by cubosomes reduces systemic side effects like immunosuppression and enhances local therapeutic effects.
- **Oral Drug Delivery:** Cubosomes are applicable in oral drug delivery systems, where they encapsulate drugs to shield them from the stomach's acidic conditions and facilitate controlled release in the intestine, enhancing the absorption and bioavailability of poorly water-soluble drugs. Cubosomes are employed for improving the water solubility of itraconazole, which is badly water-soluble. With the encapsulation of itraconazole by cubosomes, the drug gets protected from stomach acid, hence better absorption from the intestines for better therapeutic effects [47].
- **Topical Drug Delivery:** Cubosomes are also useful in delivering drugs through the skin, such as for the treatment of dermatological diseases like psoriasis or eczema. They provide sustained release at the site of application, enhancing therapeutic effects and reducing side effects.[45] Cubosomes can be employed for targeted delivery of clobetasol for the treatment of dermatological diseases such as psoriasis or eczema. Their sustained release at the point of application lowers the frequency of application and improves the efficacy of the drug in the treatment of inflammation of the skin.
- **Vaccines and Gene Delivery:** Cubosomes may be used as delivery systems for vaccines or gene therapy. Their capacity to encapsulate and shield genetic material or antigens makes them attractive candidates for use in

immunization and gene therapy. Cubosomes have the ability to encapsulate genetic material, like plasmid DNA or mRNA, for gene therapy or vaccine delivery [47]. This is especially useful in cancer immunotherapy or infectious disease vaccines because cubosomes are able to protect the genetic material from degradation while facilitating its effective delivery to target cells.

DRUG SELECTION CRITERIA:

Choosing the best drug candidate for cubosomes—nanocarriers based on lipids with a cubic phase morphology—means considering a number of factors that match the distinctive characteristics of cubosomes. These are:

1. **Poor Water Solubility (Lipophilicity):**

   Cubosomes are more suited to drugs that have poor water solubility. Lipophilic drugs tend to be poorly absorbed because they are poorly soluble in water. [50] Cubosomes are able to solubilize such drugs, enhance their bioavailability, and improve their absorption, particularly in the cases of oral and parenteral administration [51-52].

2. **Controlled Release Requirement:**

   Sustained or controlled-release drugs are a perfect fit for cubosome formulation. The cubosomes' large surface area and encapsulating feature enable the sustained release of the drug, producing extended therapeutic outcomes with less frequency of dosing [53 - 54].

3. **Targeted Drug Delivery:**

   Cubosomes may be designed to release drugs at specific target locations through the surface modification of cubosomes with target ligands, i.e., antibodies or peptides [55]. This property is advantageous for cancer treatments (e.g., doxorubicin), where the drug should be released at cancerous sites to minimize systemic toxicity [56].

4. **Drug Stability:**

   Cubosomes offer a safe environment to unstable drugs to protect them from degradation by light, oxygen, or enzymatic reactions [57]. This is important for hydrolysis- or oxidation-sensitive drugs, including peptides or proteins. Biologic drugs (e.g., monoclonal antibodies) and gene delivery systems (e.g., DNA vaccines), for example can be helped by cubosome encapsulation to preserve their integrity during storage and delivery [58].

5. Penetration of Biological Barriers:

Those drugs that need to deliver to hard-to-reach places, i.e., the blood-brain barrier (BBB), are suitable for cubosome-based delivery. Biocompatible cubosomes can penetrate biological barriers more effectively than traditional formulations and thus are appropriate for drugs acting on the central nervous system (CNS), e.g., donepezil in the case of Alzheimer's disease [59].

6. Toxicity Issues:

Drugs with high systemic toxicity, e.g., chemotherapeutics, stand to gain from the targeted delivery capability of cubosomes. Through the localization of the drug in the site of action (e.g., cancerous tumors) and limiting its exposure to normal tissues, cubosomes minimize systemic drug toxicity as in the case of paclitaxel [56].

7. Molecular Size and Stability:

Small to medium-sized drugs stable in their molecular state are more likely to be successfully loaded into cubosomes. Larger biomolecules (e.g., some proteins or nucleic acids) can still be encapsulated, though, provided that suitable formulation strategies are applied.Small molecule therapeutics such as paclitaxel or ibuprofen are commonly selected for cubosome formulations because they are small and stable in lipophilic conditions [58]. Large molecules, such as gene therapy vectors or peptides, can be employed if the cubosomes are designed particularly for such applications.

COMPONENTS OF CUBOSOMES:

Cubosome formulations are largely made of natural lipids like monoglycerides, glycolipids, phospholipids, and urea-based lipids, which are capable of self-assembly when water is present. Important lipids are monoolein (GMO) and phytantriol, which are capable of creating bicontinuous cubic and reverse hexagonal phases, respectively [60]. GMO is the major precursor for cubosome formulations and comes in two forms: mixed glyceride and distilled monoolein. When distilled, monoolein is ideal in drugs due to its cleanliness and falls into the category "generally recognized as safe" (GRAS) [61]. Poloxamer, at 0%-20% w/w surfactant usage, stabilizes the preparation, though polyvinyl alcohol can replace it. Phytantriol is desirable due to advantages such as penetration enhancement, retardation, and structural stabilization [62]. Phytantriol, compared to GMO, has higher hydrophobicity as well as a branched hydrocarbon structure, whereas GMO is more elastic [63]. The excipient selection should account for drug-polymer compatibility and the melting point of the carrier molecule, which should be greater than 45°C. Cubosomes are one of the nanostructured lipid carriers (NLCs), self-assembled nanocarriers consisting of amphiphilic lipids and water. Nanoparticles are generally utilized for the encapsulation of both hydrophilic and hydrophobic drugs, enhancing drug solubility, stability, and bioavailability. The preparation of cubosomes encompasses a range of excipients, which will be contributing to the characteristic properties and performance of the final product.

1. Lipids (Structural Excipients):

a. **Glyceryl Monooleate (GMO):** GMO is amongst the most universally used lipids in cubosome preparations. GMO acts as a main structural lipid as it possesses the capability of producing a cubic phase when subjected to water contact. It supports the formation of the lipid bilayer and nanoparticles' self-assemble. It enhances the stability of cubosomes and their encapsulation capacity towards drugs. Act to structurally stabilize the cubosome, thereby allowing the organization of the ordered lipid matrix encapsulating the drug [64].

b. **Phospholipids (e.g., Lecithin):** Phospholipids usually serve to control the properties of the cubosome formulation. Phospholipids contribute to the formation of bilayer architecture and improve hydrophobic drug solubility. Phospholipids can also increase the stability of the cubosome in physiological conditions and control drug release behavior.They assist in the establishment of the cubosomal structure, enhance biocompatibility, and affect the drug release kinetics [65].

c. **Cholesterol:** Cholesterol is generally added to enhance the stability and rigidity of the cubosome preparation. It aids in inhibiting excessive leakage of the entrapped drug, thereby improving the controlled release characteristics. Improves membrane stability and can affect the drug release kinetics [66].

2. Surfactants:

a. **Polysorbates:** Polysorbates are non-ionic surfactants, which are frequently employed in cubosome formulations to enhance the dispersion of the lipid nanoparticles in water. They lower surface tension, increasing the stability of the suspension of cubosomes. Polysorbates also prevent aggregation and enhance the general bioavailability of the cubosomes. Stabilize the suspension of cubosomes, prevent aggregation, and increase solubility in water [67].

b. **Polyethylene Glycol:** PEGylation of cubosomes extends their circulation time in the circulatory system, lowering clearance by the reticuloendothelial system (RES). PEG can also enhance the dispersibility of cubosomes and minimize the occurrence of aggregation, enhancing their stability and performance. Enhances stability, lengthens circulation time (by inhibiting opsonization), and can enhance the bioavailability of the encapsulated drug [68].

c. **Span 60:** Span 60 (sorbitan monostearate) is another surfactant frequently employed in cubosome preparations. It may assist in stabilizing the structure of the cubosome through regulating the interaction between the lipid constituents. It is frequently incorporated in association with other surfactants to provide the desired characteristics to the cubosome preparation Stabilizes cubosomes, enhances the structural stability of the lipid matrix [69].

3. Water:

Water serves as the solvent used in cubosome formulations to disperse and dissolve the lipids. The water and lipids interact in such a way that the lipids self-assemble into a cubic phase in the form of lipid nanoparticles. The quality and purity of water utilized are vital in guaranteeing the stability and uniformity of the cubosome formulation. Serves as the vehicle for lipid dispersion and the development of the nanostructure [69].

4. Stabilizers and Protectants:

a. **Tocopherol:** Tocopherol is commonly utilized as an antioxidant in cubosome formulations. It assists in protecting the lipid matrix from oxidation, which can deteriorate the stability and integrity of the cubosomes. Tocopherol also assists in ensuring the structural characteristics of the lipid nanoparticles, enhancing their shelf life [70]. Protects the formulation from oxidation, extends the shelf life of cubosomes.

b. **Citric Acid:** Citric acid is commonly used to adjust the pH of cubosome formulations, especially when the formulation requires a specific pH range for stability or to enhance drug release. It can also prevent precipitation of certain components, ensuring the integrity of the formulation. Adjusts pH and stabilizes the formulation [70].

5. **Active pharmaceutical ingredient**:

The active pharmaceutical ingredient is the drug or therapeutic substance packaged in the cubosome formulation. The choice of drug is very important as it determines the selection of lipids and excipients. The drug can be hydrophilic or hydrophobic, and the cubosome formulation can be modified to support drugs of various kinds. The principal therapeutic ingredient being delivered, whose solubility, stability, and release profile are greatly influenced by the formulation [70].

6. Other Excipients:

a. **Salt:** Salts can be added to modify the osmolality of the formulation, making it suitable for the physiological environment. Salts can also affect the stability and aggregation of cubosomes.Regulates osmolality and prevents aggregation.

b. **Cryoprotectants;** Cryoprotectants find frequent application in cubosome preparations intended for freeze-drying. They preserve the structural stability of cubosomes when freeze-drying, inhibiting ice crystal formation damage and securing long-term product stability. Maintains protection to cubosomes through freeze-drying and storage [70].

MANUFACTURING:

1) Top-Down Method:

One of the most popular methods of preparing cubosomes is the top-down method. In this process, a lipid material like monoolein or phytantriol is initially melted and a stabilizer like Poloxamer 407 added to the molten lipid. After this, water is added to the mixture gradually with constant stirring, resulting in the creation of a bulk cubic phase—a thick gel-like material with a crystalline nanostructure. The viscous cubic gel is then mechanically processed using high-energy devices like high-pressure homogenizers or ultrasonicators. The devices assist in breaking the large cubic phase into nano-dimensions as particles, which are stabilized in the aqueous phase because of the existence of the surfactant, leading to the cubosomes formation [71].

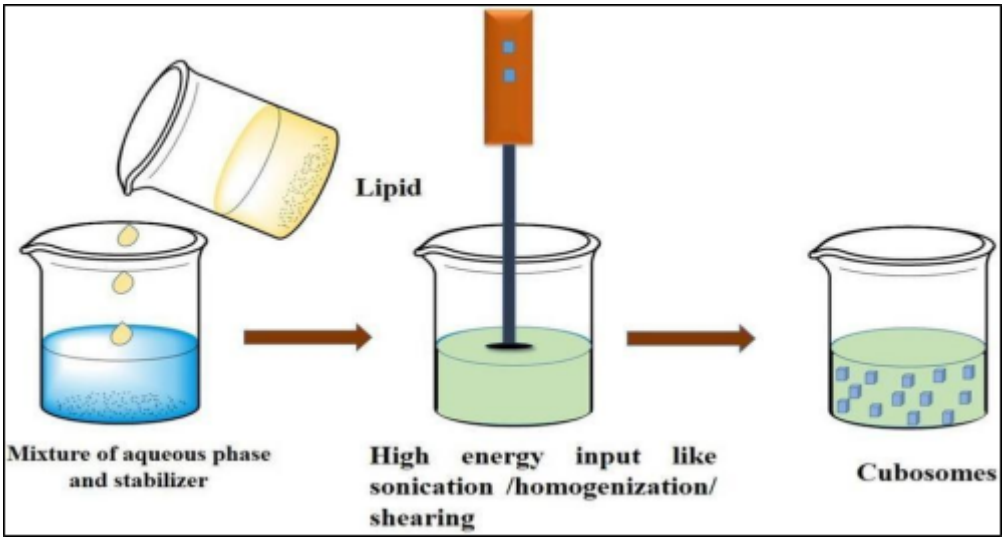


Fig no.3: Diagrammatic representation of top-down method [91]

2) Bottom-Up Method:

The bottom-up method, or the solvent dilution or precursor method, is a low-energy process well-suited for sensitive molecules. Here, the lipid and stabilizer are initially dissolved in an organic solvent that is miscible with water such as ethanol, acetone, or acetonitrile. The solution is then added to an aqueous phase under mild stirring conditions. As the solvent penetrates into

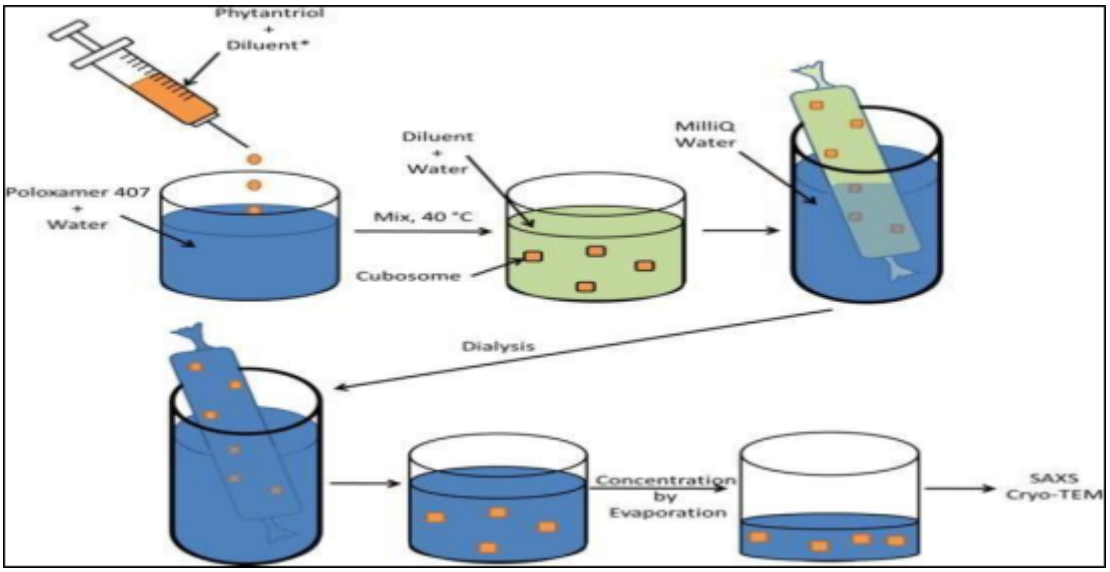


Fig no.3: Diagrammatic representation of Bottom-Up Method [91]

the water, the molecules of the lipid spontaneously self-assemble into a cubic nanostructure, resulting in the formation of cubosomes. At times, the excess solvent is eliminated by evaporation or dialysis to improve the purity of the dispersion. This method has the advantage of not utilizing high temperatures or mechanical energy, which is ideal for labile bioactive agents [72].

3) Spray-Drying Method:

In spray-drying, the aim is to produce a dry powder version of cubosomes that will be easily rehydrated whenever necessary. First, a solution or emulsion is created by dissolving the lipid and stabilizer in a volatile organic solvent like ethanol. The solution is then atomized with a spray dryer, which breaks it up into thin droplets within a hot air chamber. The droplets move

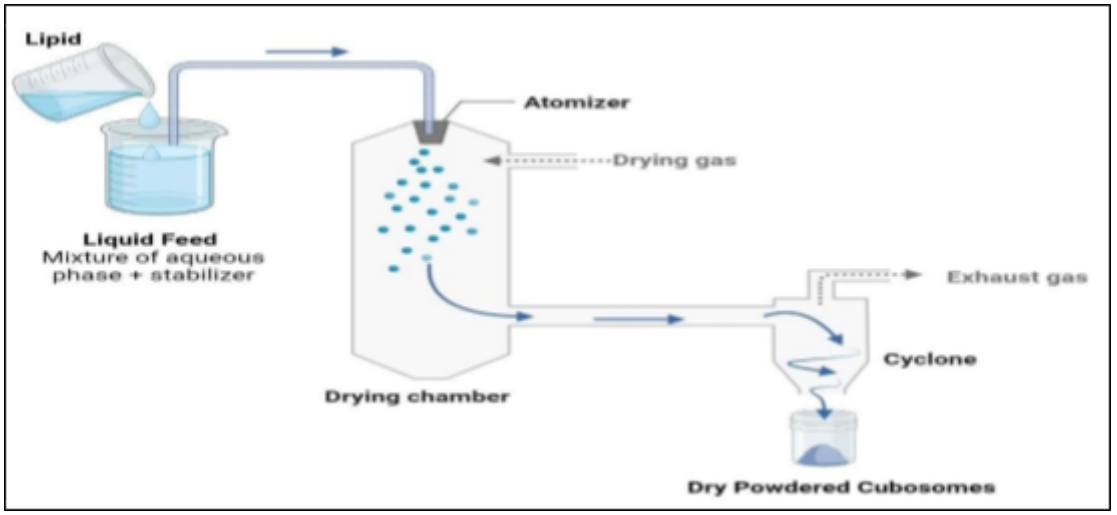


Fig no.4: Diagrammatic representation of bottom-up method [92]

through the chamber as the solvent quickly evaporates because of the heat, leaving solid particles containing the nanostructures of lipids. These dried particles are harvested as a powder, which can be stored and subsequently reconstituted in water to reform cubosomal dispersions [73].

4) Sonication Method:

The sonication method is a straightforward and common laboratory-scale method for the manufacture of cubosomes. It starts with the preparation of a bulk cubic gel by blending lipid with water and a stabilizer. Once it is created as a gel, this gel is subjected to ultrasonic waves via a probe sonicator. Sonication produces the high-frequency sound waves that can shatter the cubic gel into nano-sized particles. A dispersion of cubosomes is thus obtained. While it is easy in small-scale preparations, this approach is generally suited for research because scalability and regulation of particle size uniformity would be a major concern [74].

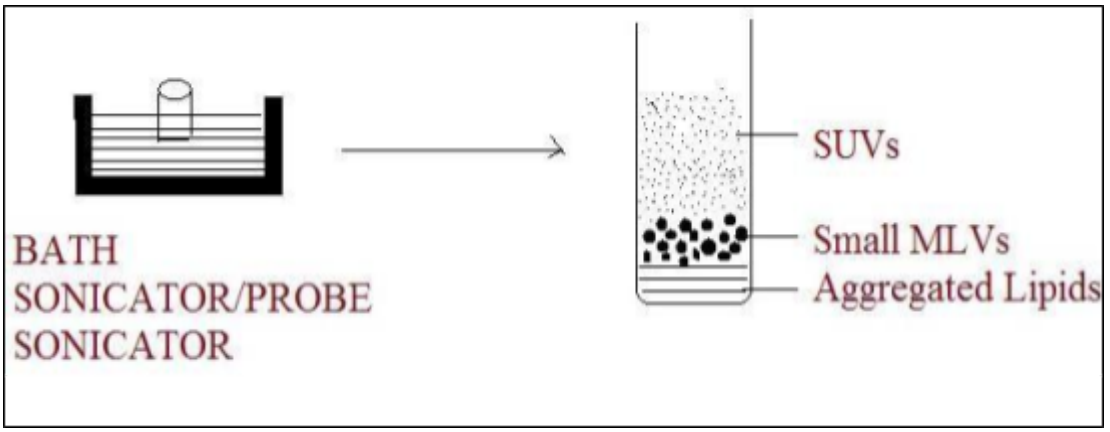


Fig no.5: Diagrammatic representation of spray drying method [93]

EVALUATION:

- **Photon Correlation Spectroscopy:**

Photon Correlation Spectroscopy (PCS) is used to determine the particle size distribution of cubosomes. The technique is based on dynamic light scattering (DLS), where a Zetasizer machine determines the scattering of light by the particles in the dispersal. The sample is diluted with an appropriate solvent to minimize the concentration of the particles, making it possible to get precise readings. The scattering intensity is calibrated to around 300 Hz, and the measurements are taken at a temperature of 25°C, which is controlled. The data obtained are particle size distribution, zeta potential (which is an indicator of the surface charge of the particles), and polydispersity index (PDI), which gives information on the homogeneity of particle sizes in the dispersion. Particle size distribution can also be determined on the basis of average volume-weighted size that provides a truer picture about the characteristics of the dispersion [75].

- **Polarized Light Microscopy:**

The polarized light microscopy is utilized to study optical properties of the cubosomes and, more so, the structural integrity and coatings on the surfaces. Through characterization of the sample under polarized light, anisotropic (structured) materials and isotropic (uniform) materials can be differentiated. Cubosomes, having a highly ordered nanostructure, are capable of being birefringent upon analysis under polarized light. This enables the scientists to identify any crystalline or ordered domains in the structure of the cubosomes, as well as yielding information on the internal architecture and possible surface treatments or coatings [72].

- **High-Performance Liquid Chromatography:**

HPLC is utilized for cubosome formulation analysis, particularly drug quantitation. A validated densitometry procedure is utilized where the sample is scanned on a chromatographic plate. Post separation, the plate is stained using a mobile phase containing cupric sulfate (penta hydrate), phosphoric acid, and water. The staining reaction supports identification and quantification of the compounds present in the cubosomes. The samples are then subjected to a UV light source, and the corresponding absorbance is read at the correct wavelength to calculate the concentration of the analyte. The reproducibility and precision of this method make it appropriate for quality control in cubosome formulations [73].

- **Entrapment Efficiency:**

The entrapment efficacy of cubosomes is the quantity of drug that is effectively entrapped within the nanostructures of the cubosome, compared to the free drug in the dispersion. In order to measure this, 1 mL of cubosome dispersion is initially diluted with 4 mL of deionized water. Subsequently, a further dilution is done by taking 1 mL of the first dilution and adding 4 mL of deionized water. This dilution is then passed through a syringe filter with a 0.1 μm pore size to separate the unentrapped drug. The filtrate is analyzed spectrophotometrically at 250 nm. The concentration of the unentrapped drug is calculated, and the entrapment efficiency is determined by subtracting the free drug concentration (Cf) from the total drug concentration (Ct) and using the following formula:

Entrapment Efficiency % = (Ct - Cf/Ct) × 100

This process guarantees that the efficacy of the formulation in entrapping the drug is properly quantified, and the experiment is conducted three times to guarantee reliability [75].

- **Particle Size Distribution Measurements:**

Particle size distribution is another significant parameter that affects the performance of cubosomes in drug delivery. Laser diffraction is employed to determine the particle size distribution of aqueous dispersions and spray-dried powders of cubosomes. Laser diffraction technique examines how light is scattered by particles in the sample, and the size distribution of the particles can be calculated using this. This technique gives vital information regarding the homogeneity of the dispersion to ensure that the cubosomes fall within the target size range for efficient drug delivery [71].

- **Cryo-Transmission Electron Microscopy:**

Cryo-TEM is a high-resolution imaging technique used to give precise structural information about cubosomes. A thin layer of the cubosome dispersion is applied onto a 600-mesh transmission electron microscopy grid, and the sample is blotted to produce a thin film. The sample is then immediately frozen at liquid ethane at its freezing point to prevent denaturation in its native form. The grid is next left on the transmission electron microscope (TEM) stage, and images are recorded at -180°C. This technique enables visualization of the cubosome internal structure, such as the cubic phase and any possible encapsulated drug. Cryo-TEM offers highly resolved, artifact-free images, with information on the cubosome shape and morphology at the molecular level [72].

- **Pressure Ultra-filtration Method:**

Pressure ultra-filtration is a technique utilized to determine drug release from cubosomes. The technique entails employing an Amicon pressure ultrafiltration cell equipped with a Millipore membrane. The cubosome dispersion is inserted into the cell, and pressure is exerted to push the drug across the membrane while retaining the cubosome particles. The amount of drug released is determined by measurement, offering important information regarding the release profile of the cubosomes. The technique assists in knowing the way in which the cubosomes release the encapsulated drug with respect to time and is critical for determining the therapeutic effectiveness of the formulation [73].

- **Thermal Analysis:**

Thermal properties of the cubosome formulation are analyzed using Differential Scanning Calorimetry (DSC). DSC

assists in identifying the physical state of the drug and other ingredients within the cubosomes, e.g., glycerol monooleate. This is especially critical for phase transition, e.g., melting, crystallization, or degradation. For instance, the melting point range of the ingredients of the cubosomes (approximately 37°C to 56°C) tells us when the formulation materials melt and can plasticize the glycerol monooleate. Also, thermal events between 200°C and 300°C can signal the degradation of glycerol monooleate since no distinct drug melting peak is seen within this temperature range. This thermal profile gives valuable information regarding the stability and integrity of the cubosome formulation [74].

- **Light Microscopy:**

Light microscopy is employed to view the dispersions of cubosomes following dilution in deionized water. Using an optical microscope equipped with a micrometer slide for calibration, scientists can inspect the size, shape, and homogeneity of cubosomes at varying magnifications of 400x and 1000x. This provides for visual examination of the dispersion for any issues that may arise like aggregation, inhomogeneity, or size heterogeneity in the cubosomes [76].

- **Drug Content Analysis:**

The drug content in cubosome dispersions is determined by HPLC following filtration and dilution of the sample in a methanol-water mixture. The use of HPLC ensures accurate measurement of drug concentration, and the data obtained give an indication of the drug loading capacity of the cubosomes. The analysis is important to ensure the product has the desired level of active pharmaceutical ingredient [71].

- **Transmission Electron Microscopy:**

TEM is used to study the shape, size, and internal organization of cubosomes at high resolution. For this technique, cubosome samples are negatively stained with phosphotungstic acid and placed on a carbon-coated grid. After air drying at room temperature, the samples are investigated using an electron microscope. The electron micrographs thus obtained yield information on the morphology and structural characteristics of the cubosomes in terms of any internal compartments or encapsulated drug [74].

- **X-Ray Diffraction:**

ray diffraction is employed to examine the crystallographic structure of cubosomes. This method detects the spatial structure of atoms or molecules in the sample and yields information about the molecular ordering and phase composition of the cubosome formulation. Utilizing an X-ray generator, e.g., the Philips PW 1830, scientists are able to record a diffraction pattern that informs them of whether the cubosomes have a crystalline or amorphous structure, which may affect their stability and drug release characteristics [77].
X-

- **Gel Permeation Chromatography:**

Gel permeation chromatography is employed to determine the entrapment efficiency and drug loading in cubosomes. The method consists of an ultrafiltration process where unentrapped drug is separated from the cubosome formulation. The unentrapped drug content is quantified, and the total drug content added in the formulation is utilized to determine the entrapment efficiency. This approach gives an accurate quantitation of the drug encapsulation in the cubosomes

[78].

- **Viscosity Measurements:**

The viscosity of cubosome formulations is measured using a Brookfield rotary viscometer. The viscometer is operated at different rotational speeds, and the viscosity is measured at 25°C. The measurements are typically conducted using spindle #18 at 20 rpm. The average of three readings is taken to determine the formulation's viscosity. Viscosity is an important parameter as it affects the ease of administration and stability of the cubosome dispersion [72].

- **Visual Examination:**

The formulations of cubosomes are visually examined for attributes like color, turbidity, and homogeneity. The examination is carried out commonly over a span of 6-10 days following preparation to monitor any possible changes. Macroscopic particles or settling that could happen over this time can be identified, and it can be ensured that the formulation is stable and consistent in the long run [87].

- **Stability Studies:**

Stability studies are carried out to evaluate the long-term physical stability of cubosome formulations. Over time, the organoleptic (sensory) and morphological features of the cubosomes are tracked. Physicochemical properties like drug content, particle size distribution, and evidence of physical instability (e.g., phase separation or aggregation) are measured from time to time. Stability studies predict the shelf life and storage conditions necessary to ensure the efficacy and quality of the cubosome formulation [88].

MARKETED FORMULATIONS:

Unfortunately, to date, cubosome-based products are mainly in research and development, and there are no commonly available, branded consumer or pharmaceutical products. However, there are certain companies and products in clinical trials or applications that incorporate cubosome technology. These products may not be easily available in the market.There are some companies which are working on cubosomes and its formulations given in the table no.2.

Table no.2: cubosomes formulations

Brand Name	Company	Strength
Cubosome Gel [78]	Avanti Polar Lipids	Custom (drug dependent)
Cubosomal-Q10 [79]	Phospholipid Research Center	Custom (Coenzyme Q10 delivery, usually 1-5%)
Cubosome Insulin [80]	Nanomi B.V.	Custom (insulin formulation)

RECENT ADVANCES AND RESEARCH:

- **Enhanced Formulation Strategies for Cubosomes:**

In recent research, scientists have been working on optimizing the cubosome formulation to improve their stability and drug delivery characteristics. Cubosomes are generally made up of lipid matrices, with glycerol monooleate (GMO) being the most widely used lipid, along with surfactants, like polysorbates or lecithin, and water. These ingredients self-assemble into a bicontinuous cubic phase, which is responsible for the characteristic structure of cubosomes [81]. Recent attempts have been made to:

1. Stabilize the cubic structure:

Another issue with cubosomes is ensuring the integrity of their structure upon storage and post-administration. Scientists have investigated the use of stabilizers like polymers and surfactants to hinder the phase transition of cubosomes to less stable structures. For instance, the addition of block copolymer stabilizers has been found to improve the stability of cubosomes and shelf-life without affecting their drug delivery efficacy [81,82].

2. Incorporation of hydrophobic and hydrophilic drugs:

The bi-compatibility of cubosomes to incorporate both hydrophobic and hydrophilic compounds has been one of the greatest areas of study. Cubosomes have been shown in recent studies to be able to encapsulate a wide range of drugs such as anti-cancer drugs, antibiotics, and anti-inflammatory agents. For example, scientists have been able to load hydrophobic drugs such as curcumin and hydrophilic drugs such as antibiotics into cubosomes, thus increasing the solubility and bioavailability of such poorly water- soluble drugs [83].

- **Applications in Controlled Drug Delivery**

Cubosomes have been extensively researched for controlled drug delivery, with current developments aimed at enhancing their therapeutic effectiveness [50]. Cubosomes provide controlled and sustained release of entrapped drugs, which can result in decreased frequency of dosing and enhanced patient compliance. Some major developments are:

1. **Targeted drug delivery**:

Current research has also been aimed at functionalizing cubosomes with targeting ligands (e.g., antibodies, peptides, or aptamers) that enable the selective delivery of drugs to a particular tissue or cell. This is especially useful in cancer treatment, where targeted delivery of chemotherapeutic agents using cubosomes can minimize systemic side effects while maximizing the local concentration of the drug at the tumor site [65].

2. **Increased permeability and retention effect**:

Scientists have employed the EPR effect to increase the tumor tissue accumulation of cubosomes. By designing cubosomes with specific surface properties, e.g., by adding PEG (polyethylene glycol) to acquire "stealth" properties, they can be made to evade the immune system and preferentially accumulate in tumor tissues, a major approach for cancer drug delivery [85].

3. **Hydrophilic and lipophilic drug pairs**:

New drug delivery systems of cubosomes have been formulated to co-deliver hydrophilic as well as lipophilic drugs. These systems are particularly favorable in the treatment of sophisticated diseases where more than one drug is needed, e.g., in cancer or multi-drug-resistant infections [71].

- **Cubosomes for Vaccine Delivery**

The other recent research area involves the application of cubosomes in vaccine delivery. Cubosomes can be used as adjuvants to boost the immune response against antigens. The special structure of cubosomes allows them to encapsulate protein and nucleic acid-based vaccines [72].

1. **Antigen presentation and immune activation**:

Cubosomes have been utilized by scientists to deliver antigenic proteins in a way that induces maximum immune system recognition. Cubosomes have been found to facilitate improved antigen presentation to immune cells and hence improve the efficacy of vaccines. For instance, in one recent study, cubosomes were utilized to deliver a malaria vaccine, registering an enhanced immune response as opposed to conventional vaccine formulations [50].

2. DNA and RNA-based vaccines:

The recent success with mRNA-based COVID-19 vaccines has created a renewed interest in delivering genetic material using cubosomes. Research has indicated that cubosomes can safely encapsulate mRNA, providing an encouraging delivery tool for gene therapies and mRNA vaccines. Protecting the genetic material from degradation while ensuring its release in cells is a significant plus in this field [63].

- **Cosmetic and Dermatological Applications:**

There is also growing interest in cubosomes for application in cosmetics and dermatology where penetration enhancement and controlled release are essential. Use of cubosomes in cosmetic creams enables encapsulation of sensitive substances like vitamins, antioxidants, and peptides, with resultant enhanced stability and extended release [83].

1.Anti-aging and anti-inflammatory care:

Studies in recent times have established that cubosomes have the ability to deposit active agents such as retinoids and vitamin C into deeper layers of the skin, making anti-aging products more effective. Cubosomes also improve the stability of such compounds, which otherwise would break down due to exposure to air and light [85].

- **Nutraceutical and Food Applications**

In the nutraceuticals sector, cubosomes are in the process of being created for the increased bioavailability of minerals, vitamins, and omega-3 fatty acids. The majority of such compounds possess limited solubility in water, thereby restricting their body absorption. When such compounds are encapsulated inside cubosomes, scientists are able to enhance their solubility and their body absorption within the gastrointestinal tract [86].

1. **Omega-3 fatty acids:** One of the major areas of research has been the creation of cubosome-based formulations for omega-3 fatty acids like EPA and DHA. These fatty acids are usually poorly absorbed in the body, but cubosomes can greatly improve their bioavailability, rendering them more potent as dietary supplements [86].

2. **Polyphenols and antioxidants**: Polyphenols like green tea or berries have been proven to be healthy but are susceptible to degradation. Cubosome-based delivery systems have been designed by scientists to encapsulate these antioxidants to enhance their stability and absorption within the body [86].

CONCLUSION:

Cubosomes are a pioneering development in nanotechnology-driven drug delivery, providing a promising platform for future medicine. The lipid-based nanocarriers have a distinctive bicontinuous cubic phase morphology, which allows for the entrapment of hydrophilic as well as hydrophobic drugs. Their large surface area, structural stability, biocompatibility, and capacity to allow controlled and targeted release render them better than traditional systems such as liposomes. Cubosomes are especially useful in the treatment of chronic diseases, cancer, neurological diseases, and for the improvement of oral, topical, and vaccine delivery.Their composition includes amphiphilic lipids such as monoolein or phytantriol, stabilizers like poloxamer, and techniques such as top-down, bottom-up, spray-drying, and sonication. Testing methods such as Cryo-TEM, PCS, DSC, and HPLC guarantee the stability and functionality of these systems. Despite limitations such as leakage, scalability, and long-term stability, continuous innovations such as surface modifications, hybrid systems, and targeted ligand attachments are resolving these issues.

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