

RESEARCH PAPER

CUBOSOMES AS EMERGING LIPID-BASED NANOCARRIERS: FORMULATION, CHARACTERIZATION, THERAPEUTIC APPLICATIONS AND FUTURE PERSPECTIVES

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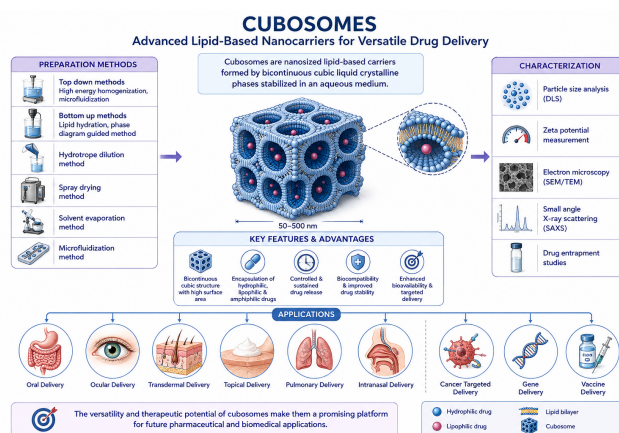
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Abstract:

Cubosomes are nanosized lipid-based carriers formed by bicontinuous cubic liquid crystalline phases stabilized in an aqueous medium. Cubosomes have attracted considerable attention as advanced drug delivery systems due to their unique internal architecture, high surface area, biocompatibility and ability to encapsulate hydrophilic, lipophilic and amphiphilic drugs. They enable controlled and sustained drug release, improve drug stability, bioavailability and targeted delivery. Various preparation methods such as top down, bottom up, hydrotrope dilution, spray drying, solvent evaporation and microfluidization methods have been developed to prepare stable cubosomal dispersions. Cubosomes are typically characterized by particle size analysis, zeta potential measurement, electron microscopy, small angle X-ray scattering and drug entrapment studies. The applications of cubosomes in oral, ocular, transdermal, topical, pulmonary, intranasal, cancer targeted, gene and vaccine delivery systems have exhibited promising results. The versatility and therapeutic potential of cubosomes make them a promising platform for future pharmaceutical and biomedical applications.

Graphical Abstract:



Keywords: Cubosomes; Lipid nanoparticles; Drug delivery; Controlled release; Bioavailability; Targeted delivery; Nanocarriers.

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1. INTRODUCTION

Progresses in nanotechnology have revolutionised drug delivery in the pharmaceutical field, through the design of carrier systems that can improve drug solubility, stability, bioavailability, and therapeutic efficacy. Lipid-based delivery systems are among the many nanocarriers that have attracted significant attention owing to their biocompatibility,

biodegradability and the ability to encapsulate a wide variety of therapeutic agents. Traditional lipid carriers such as liposomes and solid lipid nanoparticles have already proven great success, but some limitations, such as poor physical stability, drug leakage and limited loading capacity have prompted the development of more sophisticated nanostructured

systems. Cubosomes have been identified as a promising next-generation lipid nanocarrier that can overcome many of these limitations.¹

Cubosomes are discrete submicron particles of nanostructured material derived from the bicontinuous cubic liquid crystalline phases formed by the self-assembly of amphiphilic lipids in an aqueous environment. These nanoparticles possess a unique three-dimensional honeycomb-like structure with two non-intersecting aqueous channels separated by a continuous lipid bilayer. The unique interior architecture provides an exceptionally high interfacial surface area that allows the entrapment of hydrophilic, hydrophobic, and amphiphilic molecules in one carrier system. This has led to an increasing interest in cubosomes for controlled, sustained and targeted drug delivery applications.²

The most commonly used lipids for the preparation of cubosomes are glyceryl monooleate (GMO) and phytantriol, which spontaneously form thermodynamically stable cubic phases on hydration. Stabilizers (e.g. Poloxamer 407) are commonly added to prevent particle aggregation and ensure colloidal stability. The cubic phase symmetries of cubosomes (e.g., Pn3m, Im3m and Ia3d structures) can also vary, and this can affect drug loading and release properties, depending on the composition of lipids and the conditions under which they are prepared. These structural features are critical for achieving sustained drug release and improved therapeutic performance.³

Cubosomes have been studied extensively in recent years for various routes of administration such as oral, transdermal, ocular, pulmonary, intranasal and parenteral delivery. Their bioadhesive properties, high drug loading capacity, structural stability and protection of encapsulated drugs from degradation make them highly desirable for delivery of poorly water-soluble compounds, peptides, proteins, anticancer agents and nucleic acid therapeutics. Moreover, the development of surface modification and ligand-mediated targeting approaches has improved their capability for site-specific and tumor-targeted drug delivery.⁴

Recent studies have emphasized the increasing importance of cubosomes in the treatment of cancer, eye diseases, skin disorders, neurological conditions and infectious diseases. Several studies have reported better therapeutic outcomes with improved cellular uptake, controlled drug

release, increased permeability and reduced systemic toxicity. However, challenges related to large-scale manufacturing, regulatory approval, long-term stability, and clinical translation still pose significant barriers to commercialization; therefore, the present review is aimed to provide an extensive overview of cubosomes including their structural features, composition, preparation, characterization, therapeutic applications, recent advances, limitations, and future perspectives. The article also discusses the translational potential of cubosome-based drug delivery systems and their role in future nanomedicine.⁵

2. HISTORY AND EVOLUTION OF CUBOSOMES

The idea of cubosomes emerged from studies of lipid-water systems and liquid crystalline phases in the second half of the twentieth century. Amphiphilic lipids in aqueous media form well-ordered cubic liquid crystalline structures, as demonstrated by initial studies of Luzzati and coworkers. Employing X-ray diffraction methods, these researchers showed that certain lipid molecules are capable of self-assembling into elaborate three-dimensional bicontinuous networks characterized by long-range structural order. These results provide a foundation to understand the cubic phases and their potential applications in biological and pharmaceutical sciences.

The term “**cubosome**” was introduced by Kare Larsson in the 1980s in relation to studies of lipid cubic phases. Larsson recognized that the bulk cubic phases could be dispersed into colloidal nanoparticles while retaining their unusual internal bicontinuous structure. This breakthrough turned cubic phases from a physicochemical curiosity into a practical pharmaceutical carrier system. The particles obtained, called cubosomes, showed properties different from those of traditional vesicular carriers such as liposomes, including larger internal surface area, enhanced structural stability and the capacity to entrap molecules with different physicochemical properties.⁶

The pharmaceutical relevance of cubosomes was further supported by the studies on lipid digestion and biological membranes in the 1990s. Patton and Carey noted the spontaneous formation of dispersed cubic particles in the processes of fat digestion in the gastrointestinal tract. These observations implied the possibility of the existence of cubic phase structures in biological environments in their native state and thus supported their biocompatibility and potential for drug

delivery applications. This structural resemblance to biological membranes further stimulated research interest in therapeutic applications of cubosomes.

One of the major challenges encountered in the early cubosome development was the extremely high viscosity of the bulk cubic phases which complicated large scale processing and formulation. To overcome this problem, the top-down method was designed where the bulk cubic gel was broken into nanoparticles by high-pressure homogenization or sonication. This method was effective, but it required a great deal of energy input and specialized equipment. Later developments resulted in the bottom-up approach (also known as the solvent dilution or precursor method), where cubosomes are formed spontaneously from molecular precursors under controlled conditions. These technological advances greatly increased the feasibility of cubosome production and stimulated research in this field.⁷

The discovery of appropriate amphiphilic lipids furthered the development of cubosomal systems. Among these materials, glyceryl monooleate (GMO) and phytantriol (PHYT) are the most commonly used cubosome-forming lipids due to their ability to self-assemble into stable bicontinuous cubic phases upon hydration. Stabilizers, such as Poloxamer 407, enabled the formation of colloidal stable dispersions, preventing particle aggregation and improving storage stability. These advances have rendered cubosomes versatile nanocarriers for the delivery of hydrophilic, lipophilic and amphiphilic therapeutic agents.

Cubosome research exploded in the first decade of the twenty-first century. The improved characterization techniques, including cryo-transmission electron microscopy (cryo-TEM), small-angle X-ray scattering (SAXS), dynamic light scattering (DLS), and nuclear magnetic resonance (NMR), have given detailed information on the cubosome architecture and internal organization. These analytical advances allowed researchers to correlate structural properties with drug-loading capacity, release kinetics and biological performance, thus enabling rational formulation design.

In the last years, cubosomes have emerged from simple lipid nanocarriers to versatile drug delivery platforms. Surface modification strategies including polymers, ligands, antibodies, peptides, and targeting molecules have been employed for active targeting of diseased tissues, especially tumors. Cubosomes

have shown great promise in oral, ocular, transdermal, pulmonary, intranasal and parenteral drug delivery. Furthermore, DDSs have attracted interest for cancer therapy, gene delivery, vaccine delivery and brain targeted therapeutics due to their ability to improve the solubility of poorly water soluble drugs and to impart controlled drug release.

The evolution of lipid nanocarriers from conventional liposomes to the advanced cubosomal systems indicates the increasing demand for more advanced drug delivery systems to overcome biological barriers and enhance therapeutic outcomes. Current research is directed towards scalable manufacturing, personalized medicine applications, stimuli responsive cubosomes, and clinical translation. Although cubosome-based pharmaceutical products are still not at the stage of widespread commercial success, ongoing advances in formulation science and nanomedicine suggest that cubosomes may find an important place in future drug delivery strategies.⁸

3. CUBOSOMES: STRUCTURE AND COMPOSITION

Cubosomes are of great pharmaceutical interest due to their highly ordered internal nanostructure. Cubosomes are lipid vesicles with a bicontinuous cubic liquid crystalline structure of a lipid bilayer folded into a three dimensional periodic minimal surface, unlike conventional lipid vesicles. This leads to two separate continuous aqueous channels which are interlinked throughout the particle without crossing each other. The lipid bilayer forms a barrier to these aqueous domains, leading to a unique honeycomb-like internal organization with an extraordinarily large surface area. Such a structure allows for the simultaneous incorporation of hydrophilic, hydrophobic and amphiphilic therapeutic molecules in a single carrier system.

Cubosomes differ from liposomes and other lipid nanoparticles because of their bicontinuous. Drugs are usually trapped in liposomes either in the aqueous core or in the lipid bilayer. On the contrary, the complex internal network of cubosomes offers many compartments for drug localization. Hydrophilic drugs can be incorporated in the aqueous channels, lipophilic drugs can partition into the lipid bilayer and amphiphilic molecules can localize at the lipid-water interface. Such structural versatility is important for the high drug-loading capacity and controlled-release characteristics of cubosomal systems.⁹

3.1 CUBOSOMES INTERNAL ARCHITECTURE

Cubosomes are often viewed as a 3D labyrinth of aqueous channels separated by a continuous lipidic membrane. The lipid bilayer can be significantly curved and remains thermodynamically stable. The result is a very ordered nanostructure with pore diameters typically ranging from 5 to 50 nm, which depends on the lipid composition and preparation conditions. The large internal interfacial area is responsible for efficient drug encapsulation and sustained release. Furthermore, the tortuous diffusion pathways in the cubic phase result in prolonged drug release kinetics, thereby making cubosomes particularly promising for controlled drug delivery applications

3.2 FORMS OF THE CUBIC PHASES

Depending on the lipid composition, the degree of hydration, the temperature and the formulation conditions, cubosomes can exhibit different cubic phase symmetries. Three main cubic structures have been widely reported in pharmaceutical studies.¹⁰

A. Primitive Cubic Phase (Im3m)

The primitive cubic phase is made up of a rather open lattice of aqueous channels separated by a lipid bilayer. The water channels in this structure are larger and the membrane curvature is lower than in other cubic phases. The increased volume of water allows for more loading of hydrophilic drugs and biological macromolecules. The Im3m phase is often encountered during phase transitions and in certain hydration conditions.

B. Double Diamond Cubic Phase (Pn3m)

The cubic phase most frequently observed in cubosome formulations is the Pn3m structure. It has two interdigitated aqueous channel systems in a diamond like geometry. This phase exhibits good structural stability and allows balanced accommodation of both hydrophilic and lipophilic drugs. The physicochemical properties of the Pn3m phase make it the most preferred phase for many GMO-based cubosomal systems.

C. Gyroid Cubic Phase (Ia3d)

The gyroid cubic phase has a very complex three-dimensional geometry of continuous curved surfaces. This stage offers a large internal surface and better structural stability. It has been reported that the Ia3d phase may provide improved sustained-release behavior due to the tortuous diffusion pathways. For this reason, gyroid structures are especially

attractive for long-acting drug delivery applications.¹¹

3.3 MAJOR COMPONENTS OF CUBOSOMES

A. Glycerol monooleate (GMO)

The most frequently used lipid for the preparation of cubosomes is glyceryl monooleate (monoolein). GMO is a biodegradable and biocompatible monoglyceride that spontaneously self-assembles into bicontinuous cubic phases upon hydration. The amphiphilic character of the compound allows to form very stable liquid crystalline phases under physiological conditions. Extensive research has been conducted on the use of cubosomes based on GMO for oral, transdermal, ocular and parenteral drug delivery. Moreover, GMO enhances the solubility of poorly water-soluble drugs and facilitates sustained drug release.

B. Phytantriol (PHYT)

Another important lipid to form cubosomes is phytantriol, which has attracted considerable attention owing to its superior chemical stability in comparison to GMO. Phytantriol does not have ester bonds, unlike GMO, and is thus less prone to enzymatic hydrolysis. Phytantriol based cubosomes exhibited excellent structural integrity prolonged stability and controlled release of drug. These properties make phytantriol especially suitable for long-term storage and pharmaceutical applications in which enhanced stability is required.

C. Poloxamer 407

The most commonly used stabilizer in cubosomal formulations is Poloxamer 407. It is a nonionic triblock copolymer of segments of polyethylene oxide and polypropylene oxide. During the preparation of cubosomes, Poloxamer 407 adsorbs onto the particle surface and provides steric stabilization which prevents aggregation and coalescence. The addition of Poloxamer 407 enhances the colloidal stability, dispersibility and shelf-life. The amount of Poloxamer 407 in most of the pharmaceutical cubosome formulations is about 5-15% of the lipid content.

D. Water

Water is a key component of cubosome systems, because the self-assembly of amphiphilic lipids to form cubic phases is driven by hydration. Degree of hydration strongly affects phase behavior, channel dimensions, drug loading characteristics and release profiles. Thus, the proper amount of water is essential for the maintenance of the desired cubic structure and therapeutic performance.¹²

3.4 MECHANISM OF SELF-ASSEMBLY

Cubosomes are formed by spontaneous self-assembly driven by thermodynamic forces. When amphiphilic lipids such as GMO or phytantriol interact with water, hydrophobic interactions drive the lipid tails to minimize contact with the aqueous environment and hydrophilic head groups to orient towards water. This molecular arrangement gives rise to highly curved lipid bilayers, which finally pack into bicontinuous cubic phases. The resulting structures are in a state of minimum free energy and have thus a remarkable thermodynamic stability.

Addition of stabilizers prevents aggregation of cubic phase particles and leads to formation of nanosized dispersions. Depending on the formulation parameters, the particle sizes of cubosomes are generally in the range of 100–500 nm, making them suitable for various routes of administration and targeted drug delivery applications.¹³

3.5 IMPORTANCE OF STRUCTURE IN DRUG DELIVERY

The special structural features of cubosomes directly influence their pharmaceutical performance. The large internal surface area allows for high drug loading, and the tortuous aqueous channels regulate drug diffusion and offer sustained release. Also, the lipophilic nature of cubosomes favors interaction with biological membranes, increasing drug absorption and cellular uptake. The possibility of encapsulation of different therapeutic agents in one carrier system further extends their application in modern drug delivery. These benefits made cubosomes as promising nanocarriers for oral, ocular, transdermal, pulmonary and targeted drug delivery systems.¹⁴

4. CUBOSOME COMPOSITION

The composition of cubosomes is important for their structural organization, physicochemical properties, drug loading capacity, stability and therapeutic efficacy. One of the typical cubosomal formulation comprises of a lipid phase responsible for the formation of the cubic phase, stabilizers to prevent the aggregation of particles, surfactants and co-surfactants for aiding dispersion, hydrotropic agents to enhance the solubility of drug and functional excipients for targeted or stimuli responsive delivery. Selection and optimization of these components are important for the successful development of cubosome based drug delivery systems.

4.1 LIPIDS FOR CUBOSOME FORMULATION

Lipids are the main structural component of cubosomes and upon hydration give rise to bicontinuous cubic liquid crystalline phases. Due to their amphiphilic nature these lipids spontaneously self-assemble into highly ordered nanostructures with an interconnected network of aqueous channels separated by lipid bilayers. Of all the lipids studied, glyceryl monooleate (GMO) and phytantriol are the most studied, because of their excellent cubic phase formation, biocompatibility and pharmaceutical acceptability.

4.1.1 Glyceryl Monooleate (GMO)

Glyceryl monooleate (GMO), also referred to as monoolein, is the most widely used lipid for the formulation of cubosomes. GMO is a monoglyceride, derived from glycerol and oleic acid, a molecule with hydrophilic and lipophilic domains that spontaneously forms bicontinuous cubic phases in the presence of water. Due to its amphiphilic nature, GMO has good self-assembly properties and has become the preferred lipid for the preparation of cubosomal drug delivery systems.

The advantages of GMO are excellent biocompatibility, biodegradability, high drug-loading capacity and the possibility to incorporate molecules with different physicochemical properties. GMO forms lipid matrix that helps in sustained and controlled release of drug. It also increases membrane permeation and drug absorption. In addition, the unusual nanostructure architecture of the GMO offers significant promise for enhancing the dissolution and bioavailability of poorly water-soluble drugs.

The successful incorporation of therapeutic agents such as curcumin, simvastatin, doxorubicin, ketoconazole, amphotericin B and indomethacin in GMO based cubosomes has been reported in many studies. These formulations showed improved therapeutic efficacy, better pharmacokinetic profiles and extended drug release as compared to the conventional dosage forms. Thus, GMO based cubosomes have been extensively studied for oral, ocular, transdermal, pulmonary and parenteral drug delivery.

4.1.2 Phytantriol (PHYT)

Another important lipid widely used in the preparation of cubosomes is phytantriol. Unlike GMO, phytantriol does not have the ester linkages in their molecular structure, which makes it less prone to enzymatic hydrolysis and oxidative degradation. This distinctive

structural feature confers greater chemical stability and better resistance to degradation under physiological and storage conditions.

The capability of phytantriol to form very stable cubic liquid crystalline phases has been a major contributor to its growing use in pharmaceutical nanotechnology. Cubosomes prepared using phytantriol often show improved storage stability, structural integrity and consistent phase behavior compared to GMO based systems. These attributes are especially beneficial for formulations needing long shelf life and sustained therapeutic activity.

Phytantriol based cubosomes are a promising delivery system for anticancer drugs, peptides, proteins and nucleic acids. The capacity of these systems to preserve their cubic nanostructure for long times and to release drugs in a controlled and sustained way has been demonstrated by several studies. In addition, recent studies have been exploring the use of phytantriol cubosomes as carriers for brain-targeted and tumor-targeted drug delivery by improving cellular uptake and therapeutic efficacy.¹⁵

4.2 STABILIZERS

Although the cubic phase itself is thermodynamically stable, cubosomal dispersions are prone to aggregate and particle growth during storage due to large interfacial area and high surface energy. Stabilizers are incorporated into the cubosome formulations to prevent coalescence of particles and to maintain the colloidal stability. These agents are adsorbed on the surface of particles and provide steric or electrostatic repulsion to ensure long term dispersion stability

4.2.1. Poloxamer 407

The most commonly used stabilizer in the cubosome formulation is Poloxamer 407. It is often regarded as the gold standard stabilizing agent. It is a nonionic triblock copolymer made up of polyethylene oxide–polypropylene oxide–polyethylene oxide (PEO–PPO–PEO) segments. The hydrophobic PPO block interacts with the lipid surface and the hydrophilic PEO chains extend into the aqueous phase, creating a steric barrier to avoid particle aggregation.

The inclusion of Poloxamer 407 greatly increases the physical stability of cubosomal dispersions by reducing aggregation, improving dispersibility, increasing shelf life and maintaining a narrow particle size distribution. The concentration of Poloxamer 407 is an important formulation parameter, as high

concentrations may alter the internal cubic structure, while low concentrations may lead to particle instability and aggregation. In most of the reported formulations, Poloxamer 407 is used in concentrations of about 5–15% w/w with respect to the lipid content.¹⁶

4.2.2 Other Stabilizer

Other stabilizers apart from Poloxamer 407 have also been investigated in an effort to improve the performance of cubosomes for particular pharmaceutical applications. Poloxamer 188, a lower molecular weight analogue of Poloxamer 407, has been used to produce smaller particles and to maintain colloidal stability. Tween 80 is a non-ionic surfactant shown to be effective in improving drug solubilization and oral bioavailability. PEGylated stabilizers have been studied to increase systemic circulation time, decrease opsonization, and enable passive tumor targeting through the enhanced permeability and retention effect. Vitamin E TPGS has also attracted interest because of its dual role as a stabilizer and absorption enhancer, especially in the delivery systems of anticancer drugs.

4.3 SURFACTANTS AND CO-SURFACTANTS

Cubosome formulations usually consist of surfactants and co-surfactants to aid lipid dispersion, reduce the interfacial tension and improve the formation of nanoparticles. These agents are useful in stabilizing the lipid-water interface and in controlling particle size and drug encapsulation efficiency.

Tween 80 is one of the most commonly used surfactants in cubosomal systems because it lowers the interfacial tension, improves the particle size distribution and oral drug absorption. The use of Span 80 has also been studied for its potential to improve the organization of lipid phases and increase the entrapment efficiency of drugs. Cremophor RH40 is usually used to increase the solubility of drugs with poor water solubility and to improve drug loading into the cubic-phase matrix. Another important excipient is Labrasol®, a permeation enhancer and an aid to intestinal absorption and bioavailability of encapsulated therapeutics. Therefore, the choice of the right surfactants and co-surfactants is important to get the best performance of the formulation.¹⁷

4.4 HYDROTROPES AND SOLUBILISERS

Hydrotropic agents and solubilizers are often used in cubosome formulations to increase the solubility of poorly water-

soluble drugs and to facilitate cubosome formation via low-energy preparation techniques. These compounds change the solvent properties and increase molecular interactions, making hydrophobic molecules more soluble in water.

Commonly used hydrotropic agents are ethanol, propylene glycol, polyethylene glycol 400 and Transcutol® P. These excipients are particularly useful in the bottom-up approach of cubosome preparation where they assist in the dissolution of lipids and drugs before the formation of nanoparticles. Ethanol-assisted precursor techniques have been widely explored for the fabrication of cubosomes due to their simplicity, low energy input and capacity to produce stable nanostructured dispersions. Proper hydrotropic agents can significantly improve the drug incorporation and formulation homogeneity.¹⁸

4.5 FUNCTIONAL EXCIPIENTS

Recent developments in cubosome technology have been achieved by the incorporation of functional excipients, which provide additional pharmaceutical advantages over traditional drug delivery. These excipients are designed to enhance mucoadhesion, targeting efficiency, cellular uptake and stimuli-responsive drug release. Cubosomes are commonly prepared with mucoadhesive polymers such as chitosan, carbopol and hydroxypropyl methylcellulose (HPMC) to increase the retention time at mucosal surfaces and to improve the drug absorption. These polymers often provide improved contact time, resulting in increased bioavailability and therapeutic efficacy.

Active drug targeting and selective delivery into diseased tissues have also been achieved using targeting ligands. Commonly investigated ligands include folic acid, transferrin, hyaluronic acid and arginine-glycine-aspartic acid (RGD) peptides. These ligands facilitate receptor-mediated uptake into target cells, thereby improving therapeutic efficacy while reducing off-target toxicity.

Stimuli-responsive materials have also been developed as a promising strategy for intelligent drug delivery. Cubosomal formulations can be incorporated with pH-, temperature- or redox-responsive polymers to achieve site-specific drug release triggered by physiological or pathological conditions. Such systems hold great promise for precision medicine and targeted cancer therapy.

In conclusion, a rational selection and optimization of lipids, stabilizers, surfactants, hydrotropic agents and

functional excipients is important for successful design of cubosome based drug delivery systems. The synergistic interactions between these components determine the formation, stability and therapeutic performance of cubosomes and thus influence their efficacy in diverse pharmaceutical applications.¹⁹

Table I. Important Ingredients Used in Cubosome Formulation

COMPONENTS	FUNCTION	EXAMPLES
Lipid	Cubic phase formation	GMO
Stabilizer	Prevent aggregation	Polyxamer 407
Surfactant	Improve dispersion	Tween 80, Span 60
Solubilizer	Increase drug solubility	Ethanol, PEG 400
Mucoadhesive polymer	Enhanced retention	Chitosan, HPMC
Targeting ligand	Active targeting	Folic acid, Hyaluronic acid

5. PREPARATION METHODS OF CUBOSOMES

The preparation method of cubosomes is an important factor affecting their particle size, internal nanostructure, drug-loading capacity, physical stability, and therapeutic performance. Cubosomes are generally prepared from amphiphilic lipids such as glyceryl monooleate (GMO) and phytantriol, which self-assemble into bicontinuous cubic liquid crystalline phases upon hydration. The cubic phase is highly viscous and

therefore several preparation techniques have been developed to allow the production of stable cubosomal dispersions. These methods can be broadly divided into high-energy and low-energy approaches. The most reported techniques are the top-down, bottom-up, hydrotrope dilution, spray drying, solvent evaporation and microfluidization methods. Each has its own advantages and disadvantages that make it more or less suitable for pharmaceutical applications.²⁰

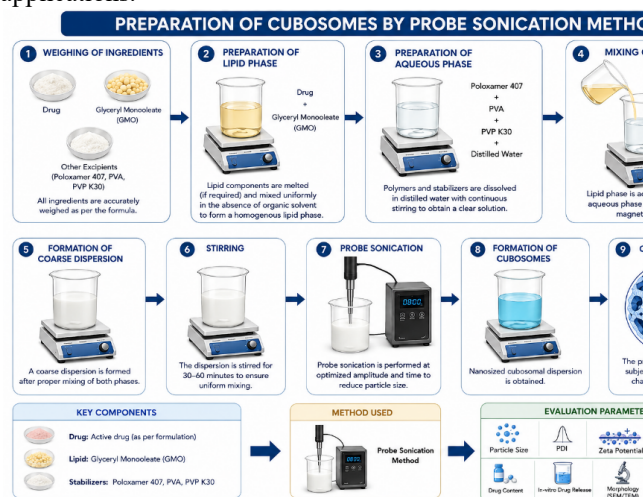


Fig.1

Preparation of Cubosomes

5.1 Top-Down Approach

The most studied and commercially applicable approach for cubosome preparation is top-down approach. In this method, first a bulk cubic phase is prepared by mixing the lipid phase with water under controlled conditions. The cubic gel is highly viscous due to the interconnected three dimensional structure. The bulk phase is then transformed into nano-sized particles by the application of high energy mechanical forces such as high-pressure homogenization, ultrasonication, or milling. The process usually involves the preparation of a homogeneous lipid-water mixture and equilibration to allow complete formation of the cubic phase. Then a stabilizer is added, usually Poloxamer 407, to prevent aggregation of the particles during homogenization. The internal cubic architecture is preserved, but the large shear forces during processing break the bulk cubic phase into discrete cubosome nanoparticles.²¹

Advantages of the top down approach are good control over particle size, high encapsulation efficiency and suitability for hydrophilic or lipophilic drugs. Moreover, the method has demonstrated reproducibility and scalability that makes it attractive for industrial production.

However, the need for high energy input may cause the production of heat that might adversely affect thermolabile drugs and biological molecules. The long processing time and specialized equipment also add to the cost of the manufacturing process. However, the top-down method remains the most used and is the gold standard for cubosome production and has been successfully used for the delivery of anticancer agents, peptides, proteins and transdermal therapeutics.²²

5.2 Bottom Up Approach

The other low energy method of cubosome preparation is the bottom-up method also known as the solvent dilution or the precursor method. Unlike the top-down approach, cubosomes are formed by the spontaneous self-assembly of lipid molecules upon dilution of a molecular solution of lipids and hydrotropic agents. In this method the lipid is first dissolved in a solvent or hydrotrope which is able to avoid the formation of a liquid crystalline phase. The addition of water reduces the concentration of the hydrotrope and the lipid molecules self-assemble into cubosomal nanoparticles. This process is performed without intensive mechanical disruption, which reduces energy consumption and minimises thermal stress.¹⁴⁻¹⁶

The bottom-up approach has attracted much attention because of its simplicity and the suitability for thermosensitive compounds. This method usually generates cubosomes with a narrow particle size distribution and can be modified for encapsulation of proteins, peptides, vaccines and other sensitive bioactive agents. However, residual solvents or hydrotropic compounds may be present in the final formulation, requiring additional purification steps. Moreover, the procedure often demands precise optimization of the solvent composition and dilution conditions to achieve reproducible formation of nanoparticles. However, the bottom-up approach is an attractive strategy for large scale cubosome production because of lower energy and operational simplicity.¹⁷⁻¹⁹

5.3 Hydrotrope Dilution Technique

The hydrotrope dilution method is a special modification of the bottom-up approach and has been widely employed for the preparation of cubosomal dispersions. Hydrotropes are chemicals that increase the solubility of hydrophobic substances in water. In this approach lipids such as monoolein are dissolved in concentrated hydrotropic solutions, which

are often supplemented with ethanol, propylene glycol, sodium benzoate, sodium salicylate or urea. Further dilution with water decreases the concentration of the hydrotrope and results in spontaneous self-assembly of the lipid molecules into cubosomal nanoparticles.^{20–22}

The main advantage of this method is the formation of cubosomes without the need to expose the formulation to high temperature or mechanical stress. Thus it is particularly suitable for heat sensitive drugs and biological molecules. The method is characterized by relatively low production costs and simplified process conditions. However, residual hydrotropes may influence the stability and biocompatibility of the formulation if not sufficiently removed. Moreover, the drug loading efficiency can vary significantly based on the physicochemical characteristics of the active pharmaceutical ingredient and the type of hydrotropic agent utilized.^{2,3–24}

5.4 Method of spray drying

Spray drying has been found to be an appropriate method to produce dry cubosome precursor powders that may be reconstituted into cubosomal dispersions upon hydration. In this method, a dispersion of lipid-stabilizer is atomized into a heated chamber where the rapid evaporation of solvent results in the formation of dry particles. These particles encapsulate the lipid components in a metastable state and form cubosomes upon contact with water. The spray-dried powders have improved storage stability, transportability and suitability for incorporation into solid dosage forms.^{25–26}

The spray drying method offers a number of industrial advantages, including the possibility of continuous operation, scalability, and compatibility with large-scale production. Furthermore the dry powder form facilitates the development of oral, pulmonary and topical formulations. However, thermolabile drugs and biomolecules may degrade during drying at elevated temperatures. To maintain the cubic phase-forming properties of the lipid system, optimization of the inlet temperature, feed rate, and atomization parameters is also required. Despite these limitations, spray drying is a promising technique for commercial development of cubosome-based pharmaceutical products.

5.5 Solvent Evaporation Technique

Solvent evaporation method is another commonly used method for cubosome preparation, especially for lipophilic drugs. In this method, the lipid and drug are dissolved in a volatile organic solvent

such as ethanol, chloroform, or dichloromethane. The resulting organic phase is emulsified into an aqueous stabilizer solution and the solvent is removed by evaporation under reduced pressure or by continuous stirring. As the solvent evaporates, lipids self-assemble and cubosomal nanoparticles are formed.²⁹

This technique provides good control over drug incorporation and is especially advantageous for poorly water soluble compounds. The relatively simple procedure and reproducibility in particle formation makes it attractive for the formulation development at laboratory scale. However, the use of organic solvents raises concerns over residual solvent toxicity, environmental impact and regulatory compliance. Product safety and quality often necessitate extra purification and solvent removal steps. Thus, while solvent evaporation remains a useful approach for research purposes, industry must weigh the challenges associated with solvent use when considering this method.

5.6 Microfluidization Technique

Microfluidization is a novel high-pressure homogenization technique that employs microchannels to produce strong shear forces and particle collisions. During processing the coarse lipid dispersion is forced through precisely engineered interaction chambers at very high pressures. This results in fast particle size reduction and the formation of uniform cubosomal nanoparticles. A repeated passage of the formulation through the microfluidizer ensures a homogeneous distribution of particles and an increased colloidal stability.²²

The microfluidization method has several advantages, such as excellent control of particle size, narrow particle size distribution, and high reproducibility and scalability. These features render the technique particularly suitable for industrial manufacturing of cubosome formulations. In addition, the higher efficiency of homogenization generally leads to better physical stability and less aggregation of particles during storage. However, the high capital cost of microfluidization equipment and the possibility of temperature increases during processing still remain significant limitations. Therefore, suitable cooling systems are often integrated to reduce the thermal degradation of sensitive pharmaceutical ingredients.²⁴

Comparison of Preparation Methods of Cubosomes

The preparation method depends on many factors like drug properties, desired particle size, manufacturing scale and economical considerations. High energy methods like top down processing and microfluidization generally provide better particle size control and formulation stability, though they require specialized equipment and higher operational costs. On the other hand, low-energy techniques such as bottom-up processing and hydrotrope dilution are more suitable for thermosensitive compounds and lead to a reduced manufacturing complexity. Spray drying is a beneficial process for the production of stable dry powders while solvent evaporation is particularly useful for poorly water-soluble drugs. Therefore, the choice of an appropriate preparation method should be based on the intended pharmaceutical application and product specifications.

6. CHARACTERISATION OF CUBOSOMES

Characterization of cubosomes is an important step in the development of cubosome based drug delivery systems as it provides important information regarding particle size, morphology, internal nanostructure, surface charge, drug-loading efficiency, physical stability and release behavior. The unique bicontinuous cubic liquid crystalline architecture of cubosomes necessitates the use of advanced analytical techniques to verify the formation of the cubic phase and assess the quality of the formulation. Comprehensive characterization not only provides reproducibility and stability, but also correlates the physicochemical properties with therapeutic performance. The cubosomes are characterized by different methods such as particle size analysis, zeta potential, transmission electron microscopy, scanning electron microscopy, small angle X-ray scattering, differential scanning calorimetry, Fourier transform infrared spectroscopy, entrapment efficiency studies and in vitro drug release studies.²⁵⁻³⁰

6.1 Particle Size and Polydispersity Index

The size of particles is one of the most important parameters affecting the biological performance of cubosomes. It has direct influence on cellular uptake, drug release, biodistribution, permeability and stability. The particle size of cubosomes is usually determined using Dynamic Light Scattering (DLS), which measures the fluctuations in the intensity of scattered light caused by the Brownian motion of nanoparticles in a liquid. The particle size of most pharmaceutical

cubosomes ranges between 100 and 500 nm depending on the composition of the lipid, the concentration of the stabilizer and the preparation method.

Apart from the mean particle size, polydispersity index (PDI) is also measured to assess the distribution of particle size. A PDI value less than 0.3 generally indicates a uniform population of nanoparticles and high homogeneity of the formulation, while higher values indicate aggregation or a broad size distribution. Smaller cubosomes tend to have better tissue penetration and drug bioavailability and therefore particle size optimization is an important aspect of formulation development.

6.2 Zeta Potential Measurements

Zeta potential is an indicator of colloidal stability and measures the surface charge on cubosome particles. Its value is derived from electrophoretic light scattering techniques that measure the mobility of particles in an electric field. The magnitude of zeta potential is a significant factor in interparticle repulsion and aggregation behavior. Physical stability is generally claimed for formulations with zeta potential values higher than +30 mV or lower than -30 mV due to electrostatic repulsion between the particles being sufficient.

The surface charge of cubosomes can be altered by the addition of charged lipids, surfactants or polymers to the formulation. Zeta potential changes can also affect cellular interactions, biodistribution and mucoadhesive properties. Therefore, zeta potential analysis gives valuable insight into the formulation stability and biological performance.

6.3 Transmission Electron Microscopy (TEM)

Among the most used techniques for the visualization of cubosome morphology and confirmation of nanoparticle formation is Transmission Electron Microscopy (TEM). TEM produces high-resolution images by passing a high-energy electron beam through a thin specimen. With this technique the particle shape, internal structure and size distribution can be directly observed. Cubosomes usually appear as single nanoparticles with a characteristic cubic internal organization.

Cryogenic Transmission Electron Microscopy (Cryo-TEM) has been found to be a particularly valuable tool, since it allows visualisation of cubosomes in their hydrated native state, without structural distortion. Cryo-TEM images provide direct evidence of the bicontinuous cubic

phase and allow a detailed study of the nanoparticle architecture. Thus, TEM is still one of the most reliable methods to confirm the formation of cubosomes.

6.4 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is used to study the external morphology and surface properties of cubosomes. In SEM analysis, a focused electron beam scans the surface of a sample and images are formed based on emitted secondary electrons. SEM gives information on particle shape, surface roughness, aggregation state and structural integrity in detailed. Cubosomes SEM images show mainly spherical or slightly irregular morphologies depending on formulation composition and drying conditions. SEM gives excellent visualisation of surfaces, but sample dehydration and coating procedures may change particle morphology. Therefore, SEM results are often complemented by TEM and Cryo-TEM investigations in order to obtain complete structural information.

6.5 Small-angle X-ray scattering SAXS

SAXS is the gold standard technique for confirming the internal cubic liquid crystalline structure of cubosomes. In the SAXS experiment, the interaction between X-ray and the periodically arranged lipid bilayers in the cubic phase gives rise to characteristic diffraction patterns. The position and ratio of the diffractions peaks give information about lattice parameters and phase identification. Different cubic phases can be distinguished by SAXS, among them the primitive cubic (Im3m), the double diamond cubic (Pn3m) and the gyroid cubic (Ia3d). The technique is very sensitive and can accurately determine the internal nanostructural organization. Thus SAXS is often used to confirm successful formation of cubosomal systems and to monitor structural changes upon storage or drug incorporation.

6.6 DSC Differential Scanning Calorimetry

The thermal behavior of cubosome formulations was studied by differential scanning calorimetry (DSC). The technique determines heat flow related to phase changes occurring in the formulation as a function of the temperature. DSC analysis provides information on the crystallinity of lipids, melting behavior, drug-lipid interactions, and formulation stability. The successful encapsulation and molecular interactions between the drug and the lipid matrix may be indicated by the changes in the melting temperature or enthalpy values after drug

incorporation. DSC is therefore often used to assess the compatibility of the formulation components and the physical state of the encapsulated drugs in cubosomal systems.

6.7 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is an important analytical technique used for identification of functional groups and assessment of possible interactions between drugs and excipients. FTIR spectra provide the information pertaining to chemical compatibility and structural integrity of formulation components. The appearance/disappearance/shift of characteristic absorption peaks may be indicative of hydrogen bonding, molecular interactions or encapsulation effects.²⁴

FTIR studies of cubosome formulations are commonly carried out to confirm that no undesirable chemical reaction occurred between the active pharmaceutical ingredient and the lipid matrix. The technique is simple, fast and widely accepted as a tool for preliminary compatibility assessment during formulation development.²⁵

6.8 Drug Loading and Entrapment Efficiency

Entrapment efficiency (EE %) is the percentage of drug successfully entrapped in the cubosomal structure compared to the total amount originally added to the cubosomal structure. The drug loading capacity is the quantity of drug loaded in a unit weight of cubosomal formulation. These parameters are generally determined by the separation of free drug from entrapped drug by ultracentrifugation, dialysis or membrane filtration techniques followed by quantification by UV-Visible spectrophotometry or high performance liquid chromatography (HPLC). High entrapment efficiency is a desirable factor for increasing therapeutic efficacy and reducing drug wastage. Cubosomes are especially valuable as they can accommodate hydrophilic, lipophilic and amphiphilic drugs in different compartments of their bicontinuous cubic phase. Therefore, cubosomal formulations generally demonstrate higher drug-loading capacities than conventional lipid-based delivery systems.

6.9 In Vitro Drug Release Studies

In-vitro drug release studies are carried out to evaluate the release behavior of encapsulated drug from cubosomes in simulated physiological condition.

Common methods used are dialysis bag diffusion, Franz diffusion cells and membrane diffusion methods. Release studies are usually conducted in phosphate buffered saline or other suitable dissolution media at physiological temperature. Cubosomes have highly ordered internal structure which contributes to controlled and sustained release profiles of drugs. The release mechanism is controlled by drug diffusion through the interconnected aqueous channels of the cubic phase. The release kinetics and mechanisms are often explained by the application of mathematical models, such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas equations. One of the main advantages of cubosome based drug delivery systems is their controlled release properties.

6.10 Stability Studies

Stability studies are important for evaluating the long-term performances and integrity of cubosome formulations. The parameters such as particle size, zeta potential, drug content, entrapment efficiency and visual appearance are monitored at different storage conditions following the International Council for Harmonisation (ICH) guidelines. Stability studies give insight into aggregation, phase transitions, drug leakage and overall robustness of the formulation. The stability of cubosomes is affected by the lipid composition, the concentration of stabilizer, the storage temperature and environmental conditions. Properly optimized formulations often possess excellent physical and chemical stability over long time periods and this makes cubosomes attractive candidates for pharmaceutical commercialization.

7. CUBOSOMES: PHARMACEUTICAL APPLICATIONS

Cubosomes are novel bicontinuous cubic liquid crystalline structures that have attracted significant attention in the area of drug delivery owing to their capacity to simultaneously encapsulate hydrophilic, lipophilic and amphiphilic therapeutic agents. The interconnected aqueous channels and lipid bilayers provide a large internal surface area allowing high drug loading capacity and controlled drug release. Cubosomes have good biocompatibility, bioadhesive properties, structural stability, and the capability to protect encapsulated molecules from degradation. These features have led to the use of cubosomes in different routes of administration like oral, ocular, transdermal, topical, pulmonary,

intranasal and parenteral drug delivery systems. Cubosomes have also been developed in recent years as promising nanocarriers for targeted cancer therapy, gene delivery, vaccine delivery and cosmetic formulations.³⁰⁻⁴⁵

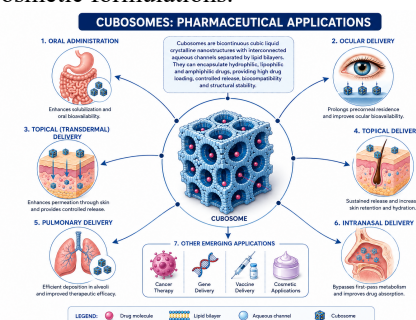


Fig.2 Cubosomes: Pharmaceutical applications

7.1 Oral Administration of Drugs

Oral route is most preferred route for drug delivery due to convenience and patient compliance. However, the oral bioavailability of many drugs is limited by low aqueous solubility, enzymatic degradation and extensive first-pass metabolism. Cubosomes have been promising in overcoming these drawbacks by better solubilization and protection of encapsulated drugs. The bicontinuous cubic phase offers a high interfacial area which is beneficial for the loading of poorly water-soluble drugs and facilitates sustained drug release in the gastrointestinal tract. Furthermore, the lipid constituents of cubosomes may also facilitate lymphatic transport, thereby decreasing hepatic first-pass metabolism and increasing systemic bioavailability. There are some reports of improved oral absorption of some poorly soluble drugs such as amphotericin B, simvastatin and cyclosporine, formulated as cubosomal systems. The cubosomes also have a mucoadhesive character, which leads to an increased gastrointestinal residence time and an increased drug absorption. These advantages make cubosomes attractive carriers for the oral delivery of low bioavailability drugs and drugs with poor dissolution characteristics.

7.2 Drug Delivery to the Eye

Physiological barriers, including tear turnover, nasolacrimal drainage and corneal impermeability, often complicate the treatment of ocular diseases by limiting drug retention and absorption. Cubosomes have been of interest as potential carriers for ocular drug delivery due to their bioadhesive nature, increased precorneal residence time and controlled drug release characteristics. Cubosomes are nanosized particles which can closely interact with the corneal surface and

therefore improve the drug penetration and therapeutic efficiency.

Improved ocular bioavailability of antifungal, antibacterial and anti-inflammatory agents trapped in cubosomal formulations have been reported in several studies. The lipidic nature of cubosomes is also responsible for improved corneal permeation and reduced dosing frequency. Moreover, cubosomal systems showed excellent tolerability and low ocular irritation, which are suitable for the treatment of glaucoma, conjunctivitis, keratitis, and other ocular disorders.

7.3 Topical Drug Administration

Transdermal drug delivery has various advantages such as bypassing first-pass metabolism, sustained drug release and improved patient compliance. However, stratum corneum is a major barrier for drug permeation. Cubosomes have been shown to improve transdermal drug delivery through their interaction with skin lipids and increased membrane fluidity. The bioadhesive properties allow prolonged contact with the surface of the skin and nanoscale dimensions allow deeper penetration into the epidermal layers.

The cubic liquid crystalline structure serves as a reservoir system for sustained release of therapeutic agents. It has been studied that the utilization of cubosomal formulation can enhance the delivery of analgesics, anti-inflammatory drugs, antifungal agents and antihypertensive drugs through the transdermal route. Incorporation of permeation enhancers into cubosomes results in improved drug transport across the skin barrier, making them useful carriers for transdermal therapy.

7.4 Topical Drug Delivery

Topical drug delivery systems are extensively used for treatment of dermatological disorders and localized infections. Cubosomes are of great interest for topical application due to advantages such as high drug loading capacity, controlled release, skin hydration and increased retention at the site of application. This internal cubic structure allows for the sustained release of encapsulated drugs, which can maintain therapeutic concentrations for extended periods.¹⁷

Cubosomal formulations have been successfully investigated for delivery of antifungal drugs, corticosteroids, antibacterial agents and anti-acne medications. The cubosomes' lipid components contribute to the amelioration of the skin hydration and

facilitate the penetration into deeper skin layers. Moreover, their capability to reduce drug irritation and improve therapeutic efficacy has promoted the development of cubosome-based topical formulations for psoriasis, eczema, acne vulgaris and fungal infections.

7.5 Pulmonary Drug Delivery

Pulmonary administration is a non-invasive route for both local and systemic drug delivery. The lungs have a large surface area and are highly vascularized, which facilitates rapid drug absorption. Cubosomes have been the focus as pulmonary carriers for their ability to entrap different therapeutic molecules and deliver them in a controlled way in the respiratory tract. Their nanometric size favors an efficient deposition in the alveolar region and better drug retention in the pulmonary tissues.

Cubosomal formulations have been studied for inhaled delivery of antibiotics, anticancer agents, peptides and proteins. The application of cubosomes in the protection of sensitive biomolecules from degradation enhances their prospects for pulmonary delivery. Moreover, sustained drug release from cubosomes can reduce dosing frequency and enhance treatment outcomes in respiratory diseases.

7.6 Intranasal Drug Delivery Route

Intranasal administration has become an attractive route for systemic and brain-targeted drug delivery. The nasal cavity has a highly vascularized mucosal surface which provides rapid drug absorption and bypasses first-pass metabolism. The mucoadhesive property of cubosomes is very strong and can result in a longer residence time in the nasal cavity with increased drug absorption.

Of special interest is the ability of the cubosomes to facilitate direct nose to brain delivery via olfactory and trigeminal neural routes. This property has attracted significant interest in the design of cubosome-based formulations for neurological diseases, such as Parkinson's disease, Alzheimer's disease, epilepsy, and brain tumours. Encapsulation of the drug also protects it from enzymatic degradation which further improves the therapeutic efficacy.

7.7 Targeted Drug Delivery in Cancer

Cancer therapy remains one of the most studied applications of cubosomal drug delivery systems. Conventional chemotherapy often suffers from severe systemic toxicity and poor selectivity for tumor tissues. Cubosomes have many advantages for cancer therapy such as enhanced drug solubilization, controlled release, prolonged circulation and passive

tumor targeting by enhanced permeability and retention (EPR) effect.

Several anticancer drugs such as doxorubicin, paclitaxel, cisplatin, curcumin and docetaxel have been successfully loaded in cubosomal formulations. Cubosomes have been surface-modified with targeting ligands such as folic acid, antibodies, peptides and transferrin to further improve tumor-specific drug delivery. These targeted systems can lead to drug accumulation in malignant tissues and minimize their exposure to normal cells, hence reducing systemic toxicity and enhancing therapeutic outcomes.

7.8 Applications for Gene Transfer

Gene therapy is a promising approach for the treatment of inherited disorders, cancer and infectious diseases. However, efficient delivery of nucleic acids remains a major challenge because of their susceptibility to enzymatic degradation and poor cellular uptake. The unique internal structure and biocompatibility of cubosomes have shown promise as non-viral vectors for gene delivery.

Cubosomes have lipid bilayers which facilitate complexation of DNA, RNA, siRNA and plasmids and aqueous channels which provide protection from degradation. In addition, cubosomes enhance transfection efficiency by improving cellular uptake and intracellular release of genetic material. Recently, they have also been shown to hold promise for gene silencing, cancer gene therapy and nucleic acid vaccine delivery.

7.9 Vaccine Delivery Applications

Developing efficient vaccine delivery systems is critical for boosting immune responses and maintaining vaccine stability. The inherent adjuvant like properties of cubosomes are due to their lipid composition and nanostructured architecture. Their capacity to capture antigens and immunostimulatory molecules enables a controlled release of antigens and extended interaction with immune cells.

Several studies showed enhanced humoral and cellular immune responses following the administration of cubosome-based vaccines. The immunological efficacy of cubosomes is partly ascribed to their ability to protect the antigens from degradation and promote uptake by antigen presenting cells. Thus, the use of cubosomes for the delivery of protein-based vaccines, peptide vaccines and nucleic acid vaccines is the subject of active research.

7.10 Cosmeceutical and cosmetic uses

Besides pharmaceuticals, cubosomes have been extensively studied for cosmetic and cosmeceutical applications.³⁵ They are suitable carriers for active cosmetic ingredients such as vitamins, antioxidants, moisturizers, essential oils and anti-aging compounds owing to their nanometric size, high load capacity and controlled release properties.

Cubosomes increase skin hydration by forming bioadhesive films on the skin surface and also increase the penetration of active ingredients into deeper layers. Sustained release of the active ingredients prolongs the cosmetic effects and reduces the frequency of application. Hence, cubosomal systems have been included in moisturizing creams, sunscreens, anti-aging products, hair care formulations and skin rejuvenation preparations. These applications demonstrate the versatility of cubosomes beyond the traditional use in drug delivery systems.

8. CONCLUSION

The versatility of cubosomes has led to successful use in a range of drug delivery routes and therapeutic areas. Their unique nanostructure has significant advantages such as high drug loading capacity, controlled release behaviour, improved bioavailability and protection of the encapsulated agents. Cancer targeted drug delivery, gene therapy, vaccine delivery and brain targeted delivery are some of the promising areas of research among the various applications. Further progress in cubosome engineering and surface modification approaches will likely further expand their application in current pharmaceutical and biomedical sciences.

9. Conflict of Interest

Nil

10. References

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