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CUBOSOMES: A VERSATILE NANOCARRIER FOR ENHANCED TRANSDERMAL APPLICATION

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ABSTRACT
Cubosomes are self assembled liquid crystalline particles that are unique, sub-micron nano structured particles mainly prepared using amphiphilic lipids and an appropriate stabilizer.Amphiphilic lipids can spontaneously self-assemble with proper ratio of water to form a cubic phase. Cubic phases can enclose hydrophilic, hydrophobic and amphiphilic drugs for delivery. Nanostructured cubosomes, prepared by fragmentation of bulk cubic phase gels or lyotropic methods that retain the same inner structure of cubic phase.Their lipid bilayers are arranged in 3D space such that they have an uninterruptedcubicsurface, separated by two interconnected aqueous channels. Thus, they have a large surfacearea and lower viscosity involving numerous internal segments, giving them a definitive advantage over other lamellar vesicles infacilitating the better entrapment efficiency and also sustained release of active therapeutic substances.These unique properties make cubosomes excellent novel delivery systems applicable for oral, mucosal, transdermal, parenteral and even ocular drug delivery.The major limitation for transdermal application is the permeability issue since the drug has to permeate through a major skin barrier, Stratum corneum.The excellent penetrationenhancing property of cubosomes made them use much in delivery of drugs through transdermal route.Their self-assemblingproperties make their production uncomplicated, with two major manufacturing techniques: the top-downand bottom-up techniques.Cubosomes are having wide range of applications in various fields and they can be characterized by various evaluation parameters. So, Cubosomesthat are gaining more attention in pharmaceutical field are discussed in this review.

Key Words: Cubosomes,nanostructure,cubic phase, transdermal

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INTRODUCTION
Transdermal drug delivery system is a painless way of systemic drug delivery by applying the medicated formulation to safe and intact skin. These dosage forms designed to deliver a therapeutically effective amount of drug across patient's skin. The main objective of transdermal drug delivery system is to deliver drugs into systemic circulation through the skin at predetermined rate with minimal inter and intra patient variation. It can provide a non-invasive alternative to parenteral pathways, whileavoiding problems such as needle phobia. Transdermal dosage forms are becoming more popular because of their exclusive advantages. Improved bioavailability, controlled absorption, extra uniform plasma levels, painless and reduced side effects, easy application and

flexibility of terminating drug administration by simply removing the patch from the skin[22].

Overcoming the stratum corneum's barrier effect, delivering the drug to skin tissue, and getting past cellular and vascular tissue to reach the target region are the most challenging aspects of building a transdermal drug delivery system. The problem is that skin tissue can only carry a certain quantity of the drug. To address these issues, numerous novel transdermal drug delivery techniques have been thoroughly investigated and have become preferred modes of administration. Furthermore, this kind of improvement could give an edge over current medication delivery methods in terms of the administered dose, cost-effectiveness, and therapeutic efficacy. These novel transdermal drug delivery systems include liposomes, niosomes, transfersomes, ethosomes, and cubosomes, have the capacity to bypass the skin barriers, and increase drug permeability through the stratum corneum barrier.Among these, cubosomes are lipid based colloidal system that reflects the cubic molecular crystallography and are more effective for enhancing permeation[16].

Cubosomes are discrete, submicron nanostructured particles of bicontinuous cubic liquid crystalline phases. Their diameter ranges from 10 to 500 nm. They resemble square, slightly spherical dots, with each dot denoting the presence of a pore size of 5 to 10 nm. The term "bicontinuous" describes two distinct hydrophilic regions that are continuous but do not intersect, separated by the bilayer. Because of their best features, which include a high drug payload due to their large internal surface area and cubic liquid shape, as well as their ability to encapsulate hydrophobic, hydrophilic, and amphiphilic molecules, cubosomes have significant potential for creating nano-sized particulate systems for topical delivery[24]. Cubosomes are referred to as amphiphilic because they most likely consist of polymers, lipids, and surfactants with both polar and non-polar components. The hydrophobic effect of the polar solvent drives the amphiphilic molecules to spontaneously identify and assemble into a nanometer-sized liquid crystal. Cubosomes, then, are bicontinuous, cubic liquid phases that surround two distinct water zones that are separated by bilayers that are regulated by surfactants. Furthermore, these have optical isotropic, viscous, and solid properties in common with liquid crystalline substances having cubic crystallographic symmetry. Particulate dispersions that are colloidally and thermodynamically stable can be formed by breaking up the cubic phase. Cubosomes, which produce a bicontinuous cubic liquid crystalline phase by hydrating a mixture of monoolein and poloxamer 407, are very important in the development of nanodrugs[10].

Particles with a diameter of a few hundred nm or less that can arise from more unusual membrane phases have drawn considerable attention in recent years. These consist of the discontinuous micellar cubic phase (micellarsomes), the hexagonal phase (hexosomes), and the bicontinuous cubic phase (cubosomes). Because of their larger membrane surface area, capacity to solubilize both hydrophobic and hydrophilic molecules, and balanced nanostructure, they greatly complement vesicles rather than replace them [3].

Advantages

1. Biocompatible and non-toxic.
2. Outstanding bioadhesive qualities.
3. Improves skin penetration.
4. Exhibits thermodynamic stability over time.
5. Encapsulates hydrophilic, hydrophobic, and amphiphilic substances.
6. Allows substantial drug loading due to cubic crystalline structures and large interior surface area[19].

Disadvantages

1. Limited ability to entrap water-soluble medications due to high water content.
2. Difficulty in large-scale production due to high viscosity.
3. Possibility of particle growth with prolonged exposure.
4. Susceptibility to phase change in response to external environment changes [23].

Structure of Cubosome

The basic structure of cubosomes includes honeycombed structures separating the two internal aqueous channels along with large interfacial area. Amphiphilic molecules form bicontinuous water and

oil channels separated by bilayer. In general, the structure preserves the stability and effectiveness of active ingredients like proteins and vitamins. Surfactants generate bilayers within the structure, which are twisted into a three-dimensional, periodic, minimum surface that forms a densely packed structure resembling a "honeycombed" mixture of bicontinuous lipid and water domains. Because it may hold molecules that are amphiphilic; that is both lipid and water soluble, its structure differs from that of liposomes[7].

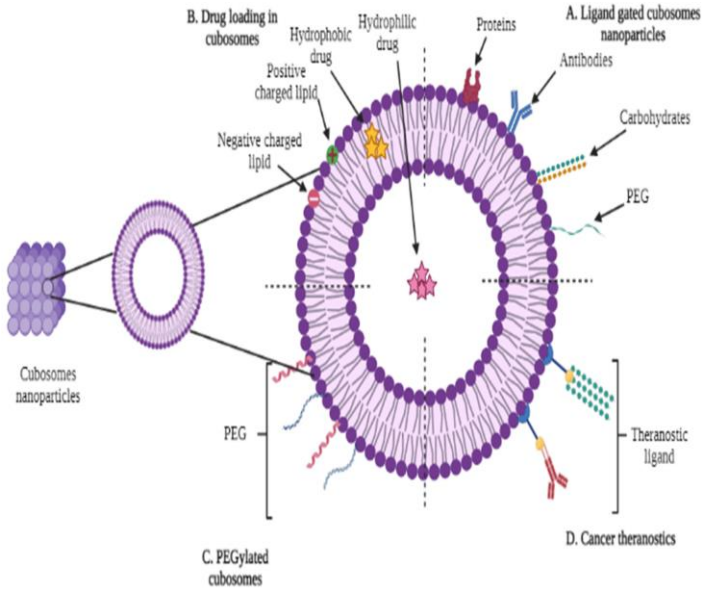

Fig-1Cubosomes exhibiting internal and cubic structures with potential of drug delivery

Mechanism of Cubosome formation

Lipids can be categorized into two primary groups: non-lamellar lipids and lamellar lipids. Planar lipid bilayers are formed by lamellar lipids, but phases like hexagonal and bicontinuous cubic phases can develop due to non-lamellar lipids.In lipid membrane systems, three different types of lipid bicontinuous cubic phases have been observed: the gyroid, the double diamond, and the primitive. Most cubosome structures that have been documented so far have either double diamond or primitive form.

The idea that the lipid mixture, stabilizer, and loaded protein or target molecule self-assemble to produce a lipid bicontinuous cubic phase is the basis for cubosome production. Phytantriol and monoolein are the two lipids that are most frequently utilized in cubosome production. They display Pn3m cubic phase shape under excess water circumstances, with temperatures ranging from room temperature to above 80°C and 43°C, respectively. Both lipids have been approved for *in vivo* use, are biocompatible, and have already undergone extensive bulk format characterization[3].

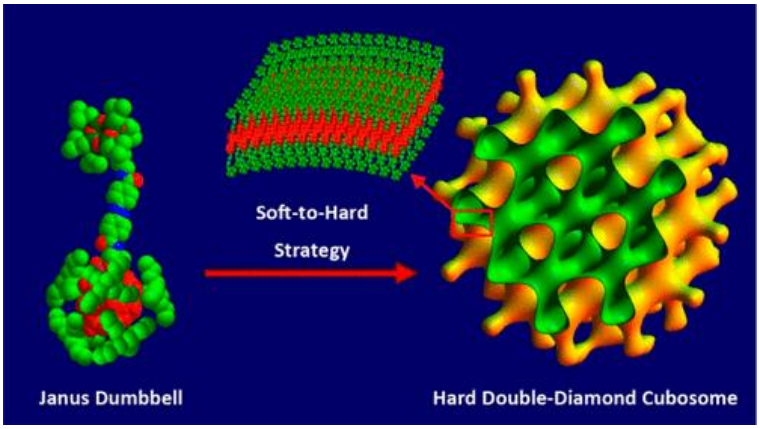

Fig-2 Lipid self-assembly of cubosomes

Types of Cubosomes

1. Liquid cubosome Precursors

In this, nucleation produces the particles, which subsequently increase through saturation. It has been noted that the hydrotrope dilution process results in smaller and more stable cubosomes.Monoolein is dissolved in any hydrotrope to produce this. Subsequently, the combination spontaneously precipitates or crystallizes when diluted. It bulks up the handling of solids and avoids high energy processes. It makes the preparation of cubosomes simple to scale up. They are typically found in mouthwashes and hand washes.

2. Powdered Cubosome Precursors

They are made of dehydrated surfactant that has been polymer-coated. The precursor powders are hydrated to generate the cubosomes. Here, lipids are solids that are waxy and sticky. Spray drying is an appropriate method that works well for this and is appropriate for large-scale production [23].

Structural components of Cubosomes

A. Amphiphilic lipids

The two amphiphilic lipids that are most frequently employed to create cubosomes are:

1. Phytantriol (PHYT).
2. Glyceryl monooleate (GMO), also known as monoolein.

GMOs are made up of a blend of oleic acid and other fatty acid glycerides, with monooleate making up the majority. Monooleate belongs to the class of amphiphilic lipids that can form different types of lyotropic liquid crystals. According to reports, GMOs with hydrocarbon chains between 12 and 22 have a higher propensity to produce cubic phases.GMO is biocompatible and biodegradable material under as generally recognized as safe (GRAS) category by FDA, mainly used as emulsifier in the food industry. PHYT is a material with a phytanyl chain, also exhibit the formation of cubic phases upon increasing the water content. PHYT, chemically 3,7,11,15-tetramethyl-1,2,3-hexadecanetriol, is a frequently used component in cosmetic products. It has been suggested as a viable GMO substitute for cubosome production. PHYT provides an additional benefit over GMO in that it exhibits higher structural stability.

B. Stabilizers

According to the research scientists, surfactants play a crucial role as stabilizers in improving the stability of cubosomes against coalescence into the bulk cubic phase. Poloxamer 407 (P407) is a tri-block copolymer composed of PEO, PPO, and PEO. It is primarily studied as a surfactant in cubosome preparation, with the PPO portions situated either at the cubosome surface or inside the bilayer structure, while the PEO chains are exposed to the surrounding water phase. Typically, P407 is applied up to a 20% w/w concentration, depending on the amount of dispersed phase[7].

METHODS

1. Top-down Approach

The most popular method for manufacturing cubosomes is the top down approach, which consists of two primary phases. To create the bulk viscous cubic aggregates, first combine the lipid that forms the cubosomes with an appropriate stabilizer. Second, the creation of cubosomes is ultimately achieved by dispersing the generated viscous cubic aggregates in aqueous solutions using high energy, such as sonication or high-pressure homogenizer. Fortunately, it has been discovered that cubosomes made via the top-down approach are stable against aggregation for up to a year. Nevertheless, this process has limitations when it comes to large-scale production because it takes a lot of energy to form viscous cubic aggregates that are then dispersed into cubosomes. This can be an issue when temperature-sensitive bioactive agents, like peptides and proteins, are needed[8].

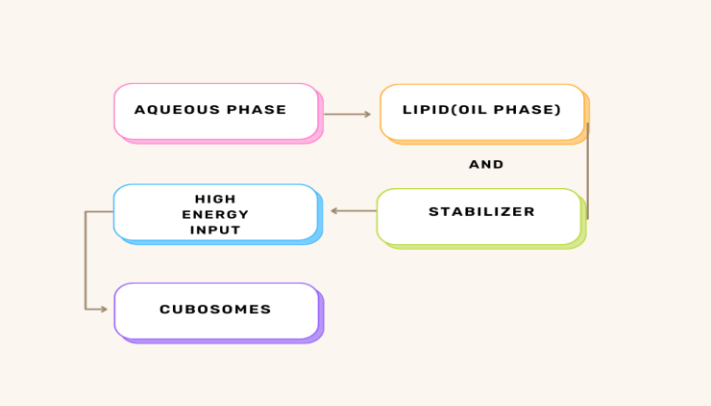

Fig-3 Schematic representation of the top-down approach method

2. Bottom-up Approach

The solvent dilution method, commonly known as the bottom-up approach, entails dispersing a mixture containing cubosome-forming lipids, a stabilizer, and a hydrotrope in excess water with minimal energy input. In this method, the hydrotrope plays a crucial role by dissolving water-insoluble lipids, forming lipid precursors, and preventing the formation of liquid crystals at high concentrations. A hydrotrope is a molecule that enhances the solubility of poorly soluble agents in aqueous media through hydrotropic solubilization, involving the addition of another solute. Commonly used hydrotropes include urea, sodium alginate, and sodium benzoate.The solubilizing mechanism of the hydrotrope includes complex formation with the hydrophobic agent. The bottom-up technique offers advantage over the top-down approach, requiring less energy input. Consequently, it can be safely used for preparing

cubosomes loaded with temperature-sensitive agents. Furthermore, cubosomes produced through this method exhibit long-term stability due to the homogeneous dispersion of stabilizers on the surface of the nanovesicles[21].

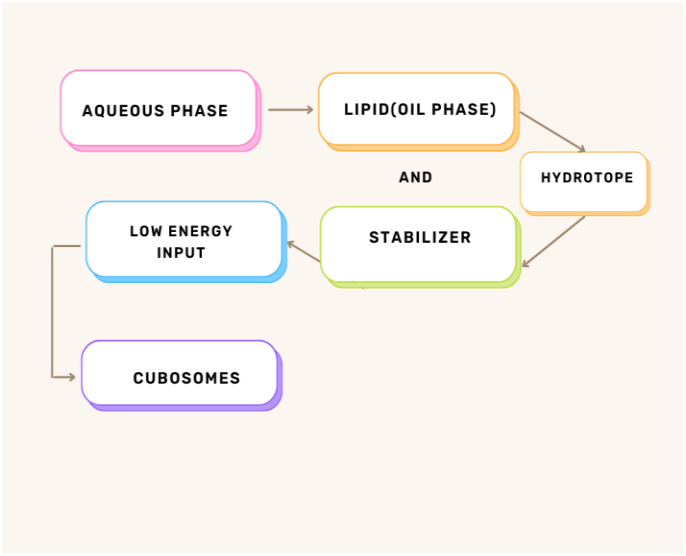

Fig-4 Schematic representation of the bottom-up approach method

CHARACTERIZATION OF CUBOSOMES

1. Drug content

Drug concentration in cubosomal formulation was measured by HPLC. By accurately weighing 5 gm of formulation and dissolving it in 50 ml of purified water, the amount of drug present in the formulation was determined using sonication with phosphate buffer at pH 7.4. Sonication for 15 minutes and heating for 5 minutes. The test was conducted into the triplicate, and the average percentage of drug content was calculated[4].

2. Vesicle morphology

The method used for morphological analysis was Transmission electron microscopy. Through vitrification of the samples in a thin layer suspended between grids covered with polymer, cryogenic-TEM enables direct viewing of samples in their hydrated condition. Due to the difficulties associated with dehydration, traditional (negative staining) transmission electron microscopy (TEM), which involves drying objects on carbon grids before viewing them under a microscope, is not advised. With its ability to directly visualize and validate crystalline symmetry, cryo-TEM is a potent addition to scatter data. Cryo-TEM combined with scattering is the gold standard for characterizing the structure type of non-lamellar liquid-crystalline dispersions[2].

3. Particle size distribution

The particle size distribution of the dispersions was determined using photon correlation spectroscopy.Measurements of Cubosomes using refractive index (RI) were made at 25^0c at an interval of 100 s. Water was added to the samples to change the signal strength. The polydispersity index and average particle size (z-average) were calculated[17].

4. Zeta potential

The Zeta potential magnitude of cubosomes provides an explanation for the strength of electronic repulsion between particles with identical charges. One of the most important measure of the formulation's stability is the zeta potential [20].

5. Stability study

The stability testing is done to guarantee the quality, safety, and effectiveness of the active medication ingredient in dosage forms throughout storage. For a duration of six months in a stability chamber, cubosomal gel formulations were kept in tightly closed, amber-colored glass containers sealed with aluminum foil at three different temperatures: room temperature (25°C±2°C/60%RH±5%), refrigerator temperature (4.0 °C±1. 0 °C), and accelerated temperature (40°C±2°C/75% RH ± 5%). The samples were taken out at the end of the first, second, third, and six months to assess the in-vitro drug release, pH, entrapment efficiency, and drug content[14].

6. pH measurements

The pH of the cubosomes was determined using pH meter. It is done by directly submerging the electrode of a pH meter in cubosome dispersion which is stored under room temperature[1].

7. Entrapment Efficiency

Ultra-filtration, dialysis, small-angle X-ray scattering, and chromatography techniques can all be used to determine the entrapment efficiency and drug loading of cubosomes. Fluorescence correlation spectroscopy,

HPLC analysis, and a UV spectrophotometer can all be used to further analyze the amount of medication that is not entrapped. The concentration of unentrapped drug is calculated and deducted from the overall amount of drug added in the formulation. The drug amount is then analyzed using radioactivity or a spectrophotometer[13].

8. Drug release study

The drug concentration gradient across the cubosomes acts as the driving force behind drug diffusion, which is the basis for the drug release process from cubosomes. Dialysis and pressure ultra-filtration techniques can be used to assess the drug release from cubosomes. Using the dialysis method, the drug release from cubosomes in water at 37°C was assessed. Five mL of the cubosomal sample were put into a dialysis bag, which was then put inside a flask with 250 mL of water. After that, the flask was placed in a rotating incubator shaker set at 37°C and 50 rpm. Periodically, a 5-mL aliquot was removedand replaced with an equal volume ofwater. HPLC used the standard curve as a guide to determine the drug's concentration after it was released. To describe the mechanism of drug release from the cubosomes, several mathematical models of the Higuchi, first-order, zero-order, Weibull, Hixson–Crowell, and Ritger–Peppas equations were utilized[18].

APPLICATIONS OF CUBOSOMES

1. Cubosomes as Transdermal Drug Delivery

Cubosomes, characterized by well-defined morphology and particle size, along with their compatibility with human tissues and cells, exhibit significant potential as drug delivery systems for mucosal and transdermal applications. The structural similarity between cubosomes and epithelial cells, coupled with the favorable permeability of cubosomes, facilitates the easy penetration of drugs through the mucosal and skin epidermis, leading to improved drug bioavailability. In a study by Gan and Han, cubosomes were employed as carriers for dexamethasone and flurbiprofen in ocular treatments. Both *in vitro* and *in vivo* investigations demonstrated a substantial increase in the apparent permeability and bioavailability of dexamethasone and flurbiprofen when delivered through cubosomes, compared to traditional phosphate eye drops[9].Because cubosomes increase skin hydration, which raises formulation viscosity due to molecular restructuring, they also increase permeability and extend the release of active ingredients, improving the formulation in multiple ways. Emulsifiers organized in liquid crystalline formations in the aqueous phase were shown in a study to improve the penetration of active substances via the skin[15].Cubosomes' capacity to modify permeability has drawn attention as a potential topical delivery system. This has uses in the treatment of burns, rheumatoid arthritis, post-operative pain, and ocular delivery, among other diseases. Due to their higher bioadhesive qualities, cubic phases are useful for topical and mucosal deposition as well as the delivery of various medications utilizing the special qualities of liquid crystal, and liquid crystal nanoparticle technology is the foundation of topical delivery methods[12].After researching cubosomes for topical delivery of the antimicrobial peptide (AMP) LL-37, it was discovered that the cubosomes had no potential to cause skin irritation, allowing for topical administration[5].The cubosomes have a penetration-enhancing impact on the skin because of their comparable cubic phase structure to the stratum corneum. This is because the lipid portion of the particles mixes with the lipids of the stratum corneum, causing the stratum corneum to become more fluid. Additionally, cubosomes have the potential to be promising drug carriers for transdermal administration because they are known to be skin-adhesive.

2. Cubosomes for Oral Delivery

It has been suggested that cubosomes would make great oral medicine delivery systems. Generally, cubosomes were investigated as a drug delivery device to improve the oral bioavailability of poorly water-soluble medications. The nanostructure lipid-based liquid crystalline system can also be used to produce regulated sustained release of medication. The prolonged levels of rifampicin lipophilic drug release from cubosomes were documented in an *in vitro* investigation. Amphotericin B, an amphiphilic medication, was found to be released continuously from the cubosome system in a different investigation. The potential of cubosomes as an innovative vaccine delivery technology was recently demonstrated by the

effective development of cubosomes carrying the model protein ovalbumin, which exhibited a high entrapment ratio and gradual release behavior*in vitro*[25].

3. Cubosomes for Parenteral Drug Delivery

Since cubosomes have a distinct solubilization, good encapsulation, prolonged release behavior, and *in vivo* stabilization, they have been designed as appealing drug release system vehicles. Furthermore, cubosomes retain their controlled release characteristic while having a lower viscosity than the liquid crystalline phase. Cervin showed that when somatostatincubosomes were intravenously injected into rats, their terminal half-life was significantly longer than six times longer than that of the corresponding somatostatin solution. Additionally, cubosomes are a preferable alternative to standard microspheres and implants because of their excellent syringeability and minimal solvent consumption during preparation. However, several studies suggested that self-assembled monoglyceride and GMO materials could cause hemolysis *in vivo* when administered intravenously; as a result, parenteral distribution of cubosome-based drug delivery systemswas limited[6].

4. Melanoma Therapy

Cancer is a major health challenge and represents the second leading cause of death according to WHO. Delivering the anticancer drugs is again a major issue facing today. Some os them include route of administration and stability issues. Nanocarriers can be used as novel strategy to deliver these drugs. Few anticancer medications have been observed to spontaneously encapsulate into cubosomes and to exhibit specific physicochemical characteristics recently. The unique structure of this promising nanocarrier suggests its application in this therapy. In preclinical and clinical investigations, many methods have been explored to selectively target nanomedicines to tumor areas in the body, with active and passive targeting of cancer cells [17].

5. Cubosomes for Ophthalmic drug delivery

Because of the blinking, tears, and frequent nasolacrimal discharge, drugs were injected into the eyes as drops. Poor bioavailability of the medications is mostly caused by corneal epithelium, lipophilicity of the drug, and pH.Flubiprofen'stranscorneal permeability can be increased, as demonstrated by an *in vitro* corneal penetration assessment. Dexamethasone is given with cubosomes to improve the eye's ocular bioavailability and pre-ocular retention. When the medication was combined with cubosomes, it increased around 3.5 and 1.8 times more than when dexamethasone eye drops were used alone.

6. Cubosomes in Nasal route

Treatment for illnesses of the central nervous system can be achieved non-invasively and effectively with direct nasal-to-brain delivery of medicines, which bypasses the blood-brain barrier (BBB). Cubosomes containing Gly14-human (S14G–HN) were used to study the potential therapeutic benefit for AD. This finding demonstrated that S14G-HN's effects in AD can be enhanced by the use of odorranalectincubosomes. MayuriAhirrao has conducted research on the use of cubosome particles to give resveratrol via the nasal route with the goal of treating Alzheimer's disease. The probe sonication method is used to create GMO P407 cubosomes. Drug release *in vitro* exhibited a regulated pattern for over twenty-four hours[11].

CONCLUSION

Cubosomes, which produce a bicontinuous cubic liquid crystalline phase has found to be a potential advantage in drug delivery through various routes which includes oral, intravenous, nasal, ophthalmic and transdermal drug delivery systems. One of the most distinctive feature of cubosomes is their bioadhesive nature, which allows them to be used in topical as well as mucosal formulations for the administration of various medications. The ability of cubosomes to include molecules with various physicochemical properties, such as polar and non polar molecules, is the primary reason for the interest in using them in biomedicine that increases a treatment's effectiveness. Furthermore, cubosomes' versatility extends beyond their capacity to encapsulate multiple molecules at once; they have been effectively employed as a theranostic tool. Despite all of cubosomes' advantages, there are still a

few significant challenges that need to be addressed. It entails developing cubosome applications even further and learning more about stabilisers and membrane interactions. It is important to carefully monitor a number of additional cubosome structural parameters *in vitro* and *in vivo*, including encapsulation efficiency, drug release profiles, dosage frequency, and compatibility of the cubosomal components with blood fluids and commonly administered medications in co-morbid patients. Over half of novel medications brought to the market in the next 10 to 20 years are predicted to comprisecubosomes because of their excellent control relese activity. Additional specialized research is necessary to validate this intriguing theory and gain a deeper understanding of how vesicles and cubosomes regulate medication release

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