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A REVIEW ON CUBOSOMES AND THEIR NOVEL APPROACHES

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ABSTRACT: Cubosomes are nanoparticles that primarily consist of specific amphiphilic lipids in a definite proportion and are stabilised by a polymer. They are known as bicontinuous cubic phase liquid crystals. cubosomes have tightly packed, twisted three-dimensional bilayers that resemble "honeycombed" formations and are thermodynamically stable. This honeycombed structure separates a large interfacial area and two internal aqueous channels. The term bicontinuous refers to the breakup of two continuous regions by lipid layer but non intersecting aqueous regions that is wicked into space filling structure. Cubosomes are formed by hydrating a surfactant or polar lipid that produces a cubic phase and then dispersing it into smaller particles. Cubosomes have high drug loading ability because of complex structure. They exhibit rheology like a solid. They have the capacity to encapsulate hydrophobic, hydrophilic, and amphiphilic materials. It enhances the solubility of poorly soluble drugs. These are Bio adhesive and biocompatible. They have various drug loading techniques and high internal surfaces. Other abilities of cubosomes are target and controlled release of bioactive agents. cubosome are versatile systems, administrable by different ways such as orally, percutaneously and parenterally, because of their properties. They are prepared by a simple method. cubosomes have high breaking resistance than liposomes. This article gives an overview on structure, preparation methods, evaluation and applications of cubosomes.

Keywords: Cubosomes, Honey combed structures, Bicontinuous cubic phase, Liquid crystals

1.INTRODUCTION: cubosomes are distinct, sub-micron and bicontinuous cubic liquid crystalline nano sized particles [1]. They have the same high surface area microstructure and dispersions that are less viscous than the basic cubic phase. [2] These are mostly made up of polar and non-polar polymers, lipids, and surfactants, hence it is considered to be amphiphilic. The hydrophobic effect pushes the amphiphilic molecules into polar solvent they recognise and combine to form a liquid crystal that is nanometre in size. Thus, cubosomes are bicontinuous cubic liquid phases that are split into two distinct water-filled zones by surfactant-controlled bilayers. They are viscous, solid, and parallel to a liquid crystal material with cubic crystallographic symmetry. When the cubic phase breaks, thermodynamically stable particle dispersions can form and play an important role in the formulation of nanodrugs.[3]

1.1. Advantages:

1. It is economic.
2. It is both biocompatible and non-toxic.
3. The preparation process is easy.
4. It has outstanding bio adhesive qualities.
5. It improves skin permeability.
6. They are thermodynamically stable for a longer period of time.
7. The ability to encapsulate compounds that are amphiphilic, hydrophobic, and hydrophilic.
8. Controlled and targeted release of bioactive substances.
9. High drug loading is caused by increased internal surface area and cubic crystalline formations.

1.2. Disadvantages:

1. Entrapment of water-soluble drugs is less due to of the presence of a large amount of water inside cubosomes. [4]

2. Due to high viscosity, large-scale production of cubosome is difficult.

3 Without a specific polymer, controlled drug delivery is not possible. They may lead to leakage during storage.[5]

2.STRUCTURE OF CUBOSOME:

Structure of cubosome is honeycombed. They are viscous, like a solid. They resemble dots, and each dot indicates the presence of a pore. There are two internal aqueous channels and a large interfacial region between them. Cubosomes are simply the liquid crystalline phases nanoparticles or nanostructure particles.[6]

They possess crystallographic symmetry and self-assembled with amphiphilic substances. The surfactant which separates bicontinuous region of water. Cubosomes similar to hydrogel because of viscous gel due to presence of interconnected property. Water and lipid in cubosomes make them biocompatible.[7]

3.METHODS OF PREPARATION OF CUBOSOMES:

3.1 High-Pressure Homogenization

3.2 Automated Cubosome Preparation

3.3 Probe Ultrasonication

3.1. High-Pressure Homogenization:

It is the best method to the cubosome preparations that have a long shelf life and are particularly stable during the high-pressure homogenization procedure. [8,9] There are three steps to it

3.1.1. Gel Preparation:

In this phase, the lipid and amphiphilic surfactants are dissolved in an organic solvent and then thoroughly mixed to homogeneous mixture. Here, the organic solvent is evaporated using a rotary evaporator to create a gel phase of formulation.

3.1.2. Shearing:

The created gel is being sheared in this stage. The use of aqueous solvents creates a micro-dispersion. It is the crucial stage before homogenization in the process of cubosomes.

3.1.3. High-Pressure Homogenization:

This approach is appropriate for large volume sample systems (30 ml), but not for small volume sample systems. The temperature is selected in this phase based on the characteristics of the lipid because this approach is temperature sensitive. This involves homogenising the produced dispersion in a high-pressure homogenizer. This technique could only handle one sample at a time.

3.2. Automated Cubosome Preparation:

Procedure could be used to produce a large number of cubosomes. This preparation technique utilizes use of robotic equipment and a probe sonicator. The gels It is very similar to the probe sonication technique with few changes. This are prepared using this method by 96-well plate with a 600-µl solvent capacity. A robot then performs the sonication. Here, using this method, it is simple to evaluate the physicochemical properties.[10]

3.3. Probe Ultra Sonication:

The preparation of small volume samples using this method is fast. It can disperse samples, even it is 600 µl in size. Depending on the probe size. Stabilizers are used in this procedure to prepare the gels. After that, a cubic phase is formed by the equilibration of the solvent. The cubic phase is then transferred for ultrasonication after that.[11] Frequency and amplitude should be carefully maintained in order to regulate the pulsing frequency and prevent samples from overheating.

3.4. Special Techniques:

3.4.1 Top-Down Technique

3.4.2 Bottom-Up Technique

3.4.1. Top-Down Technique:

It is the technique that Cubosomes are prepared using the most frequently. Ljusberg-Wahren reported it in 1996. There are two of them. The first stage involves combining the lipid(s) and stabiliser to form the bulk cubic phase (s). The second stage involves dispersing the previous step's resultant mixtures into an aqueous media using high energy techniques like high-pressure homogenization or sonication in order to create cubosomes. Bulk cubic phase resembles a clear rigid gel formed by water swollen cross-linked polymer chains. As shear forces are applied, bilayers are broken, resulting permits cubic phases to rupture in a direction parallel to the shear direction, the number of tubular network branches that rupture required energy is directly proportional. Cubosomes made using this top-down technique are consistently and roughly a year stable against aggregation. Coexistence of vesicles with dispersed nanoparticles of Liquid crystals with lamellae or vesicle-like structures. However, a problem with large-scale production is restricting the inclusion of proteins and other temperature-sensitive compounds and peptides since they require a huge energy required to disperse the cubic phase into cubosomes. [12,13]

3.4.2. Bottom-Up Technique:

It is also known as a solvent dilution method or a liquid precursor. The building blocks of nanostructures is first formed using a bottom-up technique, which are then assembled into finished material. Dispersion of a mixture results in the formation of nano structures containing a polymer, liquid crystal forming lipid hydrotrope with water. Hydrotrope facilitates dissolves water insoluble lipids in liquid precursors. Cubosomes are generated through crystallisation from precursors. This method issue for the large-scale production of cubosomes and have less energy than a top-down strategy.

In comparing these two methods, it was found that using a bottom-up method is more efficient than top-down technique, in creating smaller, high encapsulation efficiency cubosomes, and release slower rates. Additional benefits of this technique generating cubosomes with long-term stability and permits to work with materials that are temperature-sensitive.[14]

3.5. Other Methods:

3.5.1. Emulsification

3.5.2. High Shear Homogenization Technique

3.5.1. Emulsification:

This technique utilizes poloxamer 407 to form cubosomes by diluting the monoolein-ethanol solution [15].

3.5.2. High Shear Homogenization Technique:

Stabilizers are used in this technique to prevent particle aggregation during the shelf-life. (It is a good procedure, but it also has certain limitations due to the high shear application.) [16]

4. EVALUATION AND CHARACTERIZATION OF CUBOSOMES:

4.1. Visual Inspection Studies:

In this cubosome external characteristics are examined appearance factors as colour, turbidity, morphology, homogeneity and particle presence.

4.2. Transmission Electron Microscopy (TEM):

Cubosome morphology could be evaluated by using TEM. It gives cubosomal particles shape. It may release an electron Observational microphotography provides an image with high resolution. Consequently, visualising possible. As compared to light microscope, it gives high resolution. It is excellent tool for soft matter dispersions character determination. It overcomes all the regular electron microscopy demerits (vacuum environment, poor images, inducing structural changes in cubic phase).

4.3. Zeta Potential:

The preparation stability could be evaluated by zeta potential magnitude. It shows a high degree of repulsion.

4.4. Viscosity:

Rotational Brookfield Viscometer could be used to evaluate viscosity.

4.5. Particle Size Analysis:

The samples in this are diluted with an appropriate solvent and exposed to the frequency of light scattering at 300 Hz at 25 ºC.[17] By using dynamic laser light, it is measured utilising a Zeta sizer. In this the PDI and zeta potential can be measured. It gives information about average weight, volume, and size. Determination of particle size by Malvern zeta sizer, there is a need samples be diluted in water 100 times.

4.6. Polarized Light Microscopy:

The analysis of the cubosomal surface coatings, which are optically brief ringent or vesicular, could be possibly evaluated by polarized light microscopy. The differentiation of anisotropic and isotropic could also be provided by this method.[18] The cubic phases alteration could be observed. It gives the information about the possible co-existence of layered (cross or striated pattern) and hexagonal liquid crystals.[19]

4.7. Differential Scanning Calorimetry:

whether the phase transition occurs or not evaluated by DSC. endothermic and exothermic processes are responsible for phase transitions. The liquid crystals are thermodynamic equilibrium systems, as the endothermic and exothermic processes.

4.8. Small Angle X-ray Scattering:

Spatial arrangement, various groups in sample and the small-angle X-ray scattering could be determined. It also gives information about shape of particles, pore sizes, partially ordered substance distance. It measures the molecules which are 5 to 25 nm size structural information. It determines the 3-D arrangement of various groups present in the formulation**.**

4.9. Entrapment Efficiency:

The entrapment efficiency was determined by using 1 ml from each, 4 ml of deionized water was used to dilute the dispersions. The diluted dispersion is again taken, and 1 ml is further diluted, adding 4 ml of deionized water. Dispersion was formed, filtered with a 0.1 µm pore size syringe. At 250 nm, the filtrate undergoes spectrophotometrically analysed. Assuming the dilution factor, the concentration that was attained was multiplied by the whole volume of the produced dispersion. This gives the drug free concentration (C_f) which when reduced from the drug total concentration (C_t) gives the amount of drug entrapped in the cubosomes to get more accurately, each experiment was repeated 3 times.

Entrapment efficiency % of cubosomes = $(C_t - C_f / C_t) \times 100$ ---------Equation-1

Where,

(C_f)=Drug free Concentration

(C_t)=Drug total Concentration

4.10. Drug-loading Determination:

It could be based on the utilisation of ultrafiltration techniques, chromatography using gel permeation. Then, it could be analysis with High Pressure Liquid Chromatography.[20]

4.11. Drug Release Measurement:

Pressure ultrafiltration can be used to determine drug release from cubosomes.[21] It is based on that reported by Magenheim et al. using an Amicon pressure ultrafiltration cell fitted with a Millipore membrane at room temperature (22±2°C).

4.12. Stability Studies:

The physical stability investigations can be carried out by examining the organoleptic and morphological properties with respect to time.[22] Over time, it is possible to evaluate the particle size distribution and drug content.

5.Applications:

5.1. For the Controlled and Sustained-release behaviour:

Due to the unique behaviour of cubosomal particles, or the leftovers, this is possible. It is the most popular use of cubosomes. Due to its small (5–10 nm) pore size, the cubic phase is the most suitable for controlled release. [23,24] Cubosomes can encapsulate a variety of ingredients or APIs with various physicochemical properties. Due to the advantage of biodegradability of cubosomal material by enzymes, controlled and prolonged drug release is possible, and they are not accumulating inside the body.

5.2. Brain targeting:

The BBB prevents the drug delivery to the brain for the treatment of CNS diseases. Both large and small molecule administration is considerable challenge for this barrier. Lipid-based nanoparticle, cubosomes have been considered for their potential to improve brain drug loading. One of the illustrations the use of cubosomes to improve resveratrol trans nasal transport to the brain. These were prepared by utilising probe sonication, Lutrol® F 127, and glycerol monooleate lipid. For the formation of in situ gel for nasal use, it was dispersed into Poloxamer 407 polymer, after obtaining optimized cubosomal dispersion. It showed greater trans nasal permeation and better distribution than drug solution. [25]

5.3. Increasing the corneal permeability:

Ocular drug delivery faces different challenges due to low corneal permeability and bioavailability. In a study, glycerol monooleate and Poloxamer 407 were utilized to produce a cubosome drug delivery system for the treatment of glaucoma. It was observed that the penetration of TM cubosomes have higher than that of marketed available eye drops. [26]

5.4. In cancer cell targeting:

Cubosomes have been effectively used to encapsulate several cancer drugs. Resveratrol is one of the examples, which suffers from substantial first pass metabolism, poor water solubility and isomerization to the inactive cis-isomer upon exposed to light. By formulating this anticancer drug into cubosomes, the cellular uptake was enhanced. [27]

5.5. Transdermal drug delivery systems:

The cubosomes have a penetration-enhancing impact on the skin because cubosome and stratum corneum cubic phase structures are similar, As the cubosomes lipid particles mixes with the lipids of the stratum corneum. However, cubosomes are known to be skin-adhesive, allowing them to be effective drug carriers for topical applications. [28,29]

5.6. Cosmetics:

Cubosomes have been developed as antiperspirants, antiseptics, hair care and other cosmetic products. A fatty acid found in mitochondria called alpha-lipoic acid (ALA) has strong antioxidant properties. This ALA in cubosome dispersions improves the texture and colour of the skin while decreasing facial wrinkles. [30,31]

6.Recent advances:

Sl.No.	Drug	Method	Purpose
1.	Beclomethasone Dipropionate [32]	Top-Down technique	Treat Uveitis and enhance Ocular Bioavailability od BDP
2.	Flurbiprofen [33]	Hot and High-Pressure Homogenization	Ophthalmic delivery enhances Bioavailability and low irritancy
3	Brimonidine tartrate [34]	Emulsification Technique	Treat Glaucoma with sustained release manner
4	Amphotericin B [35]	High Pressure Homogenization	Enhances Oral Bioavailability
5	Norfloxacin [36]	Emulsification	Treat Otitis externa

7.Future prospects:

The cubosome nanoparticles show promise in the field of sustained drug release and drug delivery but additional optimization is needed, depends on the route of administration, dosing frequency and the drug release mode, before such nanocarriers achieve their therapeutic potential in treating many diseases.[37] These are nano vehicles for proteins and peptides loading and delivery but the reported studies are still at a basic level, and various issues regarding the structural and morphological properties of these soft nanocarriers, the ability to load bio macromolecules, and the release of those loaded macromolecules should be addressed.[38] The early stages of formulation development for cubosome-based intravenous nanomedicines should address blood compatibility. with cell membranes, and infusion-related reactions gives structural transformation.[39] Little information known about their stability in biological fluids and biological factors, cubosomes controlling drug release, when contact with biological fluids such as plasma, interactions with cell membrane and infusion related reactions gives structural transformation. Although it is ambitious to utilize cubosomes to deliver drugs intravenously the use of cubosomes for intravenous drug delivery is ambitious, these nanocarriers may find accelerated applications for the oral, ophthalmic, and topical delivery of drugs with low water solubility, providing a different but more practical opportunity in formulation science.

CONCLUSION:

Cubosomes are nanoparticles, but they are self-assembled liquid crystalline particles rather than solid particles. They have ability to encapsulate hydrophilic and lipophilic drugs and exhibit sustained and targeted drug delivery. cubosomes formulated using either high pressure homogenization or ultrasonication. Easily produced by top down and bottom-up techniques. Cubosomes are useful for a variety of immunological substances, proteins, therapeutic prospects, and cosmetics. The cubosomal formulations may be widely used as targeted drug delivery systems for ophthalmic, diabetic, and anticancer therapy due to the possible site specificity. The cubosome technology is relatively new, has a high output, and offers a lot of potential for research into creating novel formulations with commercial and industrial viability.

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