Cubosomes Unveiled: A Comprehensive Review of Structure, Function and Emerging Applications

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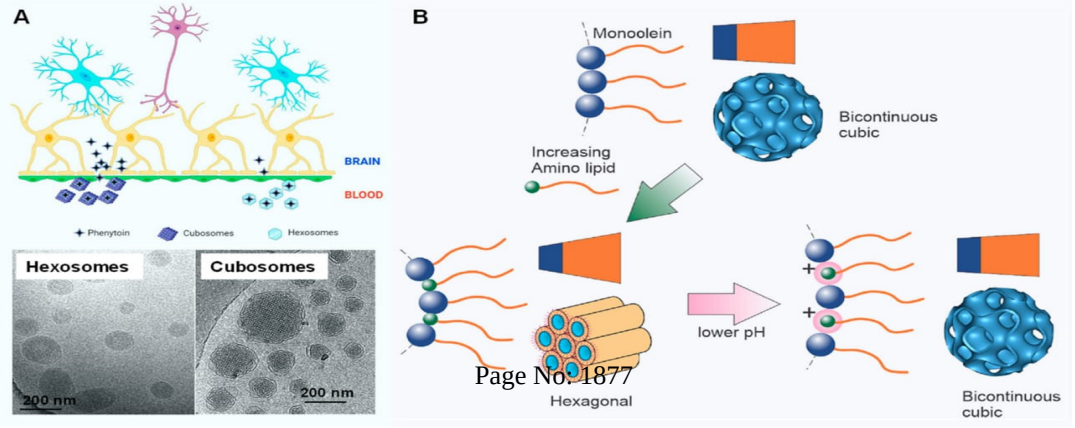
Abstract:

Cubosomes are biodegradable, non-toxic, and have the ability to solubilize hydrophobic, hydrophilic, and amphiphilic compounds. They are thermodynamically stable, so they last endlessly. Cubosomes stabilize colloidal dispersions by addition of polymers. They possess the potential for the controlled delivery of active drug, where diffusion is governed by the tortuous diffusion of the active through the regular channel structure of the cubic phase. Due to their nano pore sizes, cubosomes facilitate the controlled release of drugs and they are vital for maintaining the stability and efficacy of biologically active compounds, such as proteins and vitamins. Compared with other liquid crystalline drug delivery systems, cubosomes have lower viscosity and exist at any dilution in water and further cubic phases.

Keywords: Nanopores, monoolein, cancer therapy, immunotherapy, chemotherapy etc.

1. Introduction:

The word ‘Cubosomes’ is derived by their structure. Cubosomes have cubic crystal lattice. Cubosomes are bi-continuous cubic liquid phase crystals which have non-intersecting hydrophilic regions separated by a hydrophobic bilayer that is twisted into a periodic minimal surface with zero curvature, hence they are called as ‘viscous isotropic phases’ [1]. Larsson has examined the structure of aqueous monoglyceride cubic phases by using X-ray diffraction, and NMR and he found that cubosomes have continuous regions of both hydrophobic and hydrophilic nature, which leads to a conclusion that the cubic phase structures explained by the concept of differential geometry and periodic minimal surfaces[2]. Cubosomes are nanoparticles which are more accurately nanostructured particles and self-assembled liquid crystalline particles with solid-like rheology[3]. Abnormal cell development, undifferentiating cells and tissues, and the capacity to expand into surrounding tissues and metastasis are the main characteristics of cancer. The removal of tumor and possibly neighboring tissues, chemo- and immunotherapy, radiation treatment or combination of these are all options for treatment. However, some treatments lead to serious side effects. As a result,



one of the most active fields of cancer research is the development of systems that might selectively deliver medication molecules to the diseased site without spreading the tumor to healthy parts of the organism[4]. A current trend in Nano medicine and drug delivery research studies is improving the specificity of the treatment to increase efficacy and avoid side effects. Nanoparticles have played a large role in this trend because they can accumulate in target tissues either passively (via the so-called enhanced permeation and retention effect [5]) or actively (using surface-conjugated targeting ligands [6]).

The structure entirely distinguishes cubosomes from hexosomes, which contains hexagons due to its inverse hexagonal phase. The cubosome assemblies present further advantages because the transformations between the different liquid crystalline organizations, e.g., Pn3m, Im3m, and Ia3d, in addition to the inverted hexagonal phases, can be tuned and controlled by changes in the temperature, ionic strength, or pH of the environment of the targeted application sites [7,8]. The cubic structure of cubosomes has the ability to entrap the moiety and then release agents based on different molecular weights and polarities, which follows the laws of Higuchi-diffusion-controlled kinetics [9,10].

Q = [DmCd (2A−Cd) t] ½

According to equation, the release (diffusion) of agents from the matrix depends on the square root of time. Q is the quantity of the agents released per unit area of the matrix, where Dm is the diffusion coefficient of the agents in the cubic matrix, Cd is the solubility of the agents in matrix, A is the primary quantity of the drug per unit volume of the matrix, and t is the time. From that equation, the quantity and rate of drug release can be estimated.

Advantages:

- It is economic.
- It is non-toxic and biocompatible.
- Method of preparation is simple.
- For longer time they are thermodynamically stable. The preparation process is simple. They are good solubilizers when compared to other lipid-based carriers. Increase the bioavailability of water-soluble peptides.
- Targeted release and controlled release of bioactive agents.

Disadvantages:

- Large-scale production is sometimes difficult because of high viscosity nature of cubosomes.
- Due to presence of large amounts of water inside cubosomes there is low entrapment of water soluble drugs.
- Potential to leak during the storage or in vivo transmission.

2. Types of Cubosomes

Cubosomes are of two types liquid and powdered cubosomes depending upon the method used for formulation. Cubosomes can be formed by the dilution of monoolein with a hydrotropic solvent, such as ethanol. The quid precursor technique is used for faster cubosome preparation and this method is applicable to produce cubosomes insitu[11].

3. Components of Cubosomes:

The main components of cubosomes are amphiphilic lipids and stabilizers. Here, lipids are used to formulate the cubic crystals based on their amphiphilic profile and these include monoglycerides and alkyl glycerates. Depending upon the composition, lipid molecular structure, temperature, pressures

these amphiphilic liquids can self assemble and form a nanostructures with different physicochemical properties and geometrics [12].

4. Methods of Preparations of Cubosomes:

Techniques to form cubosomes include the bottom-up, top-down, spray-drying and solvent evaporation methods. Mostly used methods are the top-down & bottom-up. Those both techniques require a stabilizer, such as F127 to prevent aggregation. The selection of suitable method is to ensure or enhance the stability, biocompatibility, and optimal drug release through the cubic matrix of the cubosomes[13].

a. Top-Down Approach:

This method is mostly preferable method for the synthesis of cubosomes specifically when usage of glycerylmonooleate as a lipid polymer. This technique requires the high energy levels for preparation of fine dispersion of cubosomes. The steps involved in this method are, firstly form the cubic aggregates by adding of suitable stabilizer to the lipid and then apply high energy through a homogenizer in order to form the dispersion. The cubosomes prepared by this method are stable up to one year which is the advantage of this method. The main drawback of this method is that when preparing of large scale batches, the application of high levels of energy is not viable when temperature-sensitive bioactive agents, especially peptides and proteins, are incorporated[14].

b. Bottom-Up Approach:

This method is widely used for the preparation of cubosomes especially when preparing cubosomes containing phytantriol as the lipid. It is also called as solvent dilution method. This method requires low energy compared with the top-down approach.This approach focused on forming cubosomes with a hydro trope and stabilizer in an excess of water by applying minimal energy. Here, the hydro trope is the key factor, as it involves in solubilisation process to dissolve the water-insoluble lipids. Hydrotrope act as surface active agents and able to solubilize poorly soluble agents in aqueous media by hydrotropic solubilization. The bottom-up approach is having more advantages than top-down approach because it requires low energy when compared with the top-down approach. One of the advantage of this method is it is stable with temperature sensitive agents and shows long-term formulation stability [15,16].

c. Spray-Drying Method:

Spray-drying method is used in order to formulate powder precursors of cubosomes, and it involves the use of organic solvents that evaporate upon the application of air. The lipid–surfactant solvent mixture is atomized with a wave of hot air, resulting in rapid solvent evaporation and the formation of the dry powder form of the cubosome precursor. Initially mixing the lipid with the stabilizer and then dissolving them into ethanol. A separate mixture of aqueous phase, consisting of a hydrophilic solid carrier, i.e., sorbitol or dextran, is prepared, which is then combined with the previously prepared hydrophobic mixture. Both mixtures are combined with continuous stirring, which leads to the formation of a low viscosity emulsion. This method is simple, cost effective, and normally easy to scale-up [17].

d. Solvent Evaporation Method:

Solvent evaporation is another method of producing powder cubosomes by using a homogenizer or ultra-sonicator. The process is also more similar to the spray-drying method, except for the use of a high-energy sonicator. In this method, first an organic solvent such as ethanol or chloroform is used to dissolve the lipids, which are then added drop wise to the other mixture containing a stabilizer, such as Pluronics in aqueous phase. The mixture is maintained at an elevated temperature under

magnetic stirring. The drug can be dispersed in the lipid or the aqueous surfactant solution. Stirring under elevated temperatures removes the volatile organic solvent, and the mixture is homogenized by ultra-sonication or homogenizer, resulting in the formation of cubosomes. Illustrates the benefits and drawbacks of these four techniques, which are usually used in the formation of cubosomes [18].

- **Physical- Chemical Characterisations of Cubosomes:** The physical and chemical characterizations of cubosomes include the evaluation of morphology using scanning electron or transmission electron microscopes. The particle size of a Nano carrier can be tested using dynamic light scattering (DLS). It also includes the evaluation of the zeta potential of the Nano carrier, and the polydispersity index (PDI) and drug encapsulation efficiency (EE %) of cubosomes.

5. Cubosomes as Tumor-Targeted Drug Delivery: Cancer is a huge threat to human life. Even after years of research, the standard conventional treatment is not always effective in treating the disease. Only lymphocytic leukemia and Hodgkin's lymphoma can be successfully treated with conventional therapy. The main drawback of conventional chemotherapy is that it delivers the toxic anticancer agent indiscriminately to tumors and normal organs and tissues. So, standard treatment is also harmful to normal cells and tissues in the body. Therefore, there is a need to formulate a therapy that only targets the cancerous site, and does not affect the normal healthy tissues. This can only be established by delivering the antitumor drugs selectively to affected areas, thereby protecting the normal tissues. Exploiting the structural and pathophysiological anomalies of tumor tissues, particularly the tumor vasculature, and employing the increased permeability and retention (EPR) effect, is one of the most successful drug delivery techniques [19].Targeted drug delivery, also known as "drug targeting", is used frequently and is quite different from "targeted therapy", which is used in drug discovery [20]. Drug targeting can be defined as the accumulation of certain amounts of drugs at the specific or targeted site regardless of the route and method of drug administration.

- **Skin Cancer Therapy:** Paclitaxel (PTX), a model medication, was encapsulated in this study using monoolein-based cubosomes stabilized by Pluronic F127 and polyethylene glycol polymers. The study was carried out to evaluate the in vivo tumour growth inhibition by paclitaxel (PTX)-loaded cubosomes (CB), and for that purpose, A431 tumour-bearing mice were randomly divided into three groups (five mice per group): Group 1—PBS control; Group 2—PTX control; and Group 3—PTX-CB [21]. Another study revealed there was an active targeting of cancer cells by incorporating paclitaxel (PX) to cubosomes that were designed to be monoolein-based Nano carriers [22].
- **GlioblastomaMultiforme Therapy:** Utilizing a top-down method, glycerylmonooleate (GMO) and the surfactant Pluronic F-127 were used to create cubosomes that were loaded with AT101. Because of its capacity to accelerate apoptosis in tumour cells by autophagic cell death, AT101, the R-(-)-enantiomer of the cotton-seed-derived polyphenol gossypol, is a potential medication in glioblastomamultiforme (GBM) therapy. The main drawback of the drug is its low solubility in aqueous solutions and its low bioavailability, which hinder its response during treatment [23].
- **Lung Cancer Treatment:** Inhalation medication delivery is extremely advantageous for treating non-small-cell lung cancer (NSCLC) because it requires less dosage and reduces systemic toxicity. An FDA-approved antituberculosis medicine called bedaquiline (BQ) has previously demonstrated outstanding anticancer activity. However, its transport via the lungs is constrained by weak water solubility. BQ-loaded cubosome (BQLC) Nano carriers were formulated that can be inhaled to treat NSCLC [24].
- **Colorectal Cancer Therapy:** Cisplatin- and metformin-loaded cubosomes were prepared using GMO, F-127, and polyvinyl alcohol and tested against colorectal cancer. Colorectal cancer (CRC) is a dreadful tumour and remains a leading cause of death worldwide. Cisplatin has been found to be successful in treating cancer, although it is associated with serious side

effects and drug resistance. Combining anticancer drug treatment with effective drug carriers is needed. That is why cisplatin and metformin are incorporated in the cubosomes formulation. When compared with unformulated cisplatin, cubosomal formulations had a stronger cytotoxic effect [25,26].

- **Liver Cancer Treatment:** In the presence of Poloxamer 407 as a stabilizer, a cubic gel phase of monoolein and water was disrupted to create cubosomal dispersions. The goal of the study was to encapsulate the anticancer medication 5-fluorouracil (5-FU) in order to combat liver cancer. The monoolein and water cubosomal dispersions were made with Poloxamer 407 as a stabilizer. On formulations containing 5-FU, in vitro and in vivo experiments were conducted. The entrapment efficiency was determined to be 31.21% for the drug when compared with a 5-FU solution, revealing nanometer-sized particles with a narrow particle-size distribution [27].
- **Ovarian Cancer Treatment:** Icariin was loaded on cubosomes comprising GMO and P407 as a stabilizer. The potential of icariin (ICA)-loaded cubosomes in treating ovarian cancer was explored. The Box–Behnken statistical design was used to optimize the cubosome formulation. The drug entrapment efficiency of the formulations ranged from 78.3 to 97.3%, with particle sizes ranging from 73 to 183 nm. Hy926 endothelial cells. Due to enhanced drug solubility and cellular permeability, it was concluded that ICA loaded cubosomes have the potential to suppress ovarian cancer cell development [28].
- **Cervical Carcinoma:** Temozolomide and doxorubicin were mounted on cubosomes carrying miR-7-5p and comprising monoolein and F108. The effects of drug- and miRNA-loaded vehicles were investigated using molecular biology techniques, such as quantitative real-time PCR, anMTS-based cell proliferation test, flow cytometry, and spheroids formation assay. The anticancer effects were tested using cervical carcinoma-derived (HeLa) cells. MiR-7-5p enhances cell sensitivity to medicine, and nanoparticles containing both miRNA and pharmaceuticals have a better antitumor effect than drug treatment alone [29].
- **Hepatocellular Carcinoma Therapy:** For the sustained release of anticancer drugs, such as cisplatin and paclitaxel, cubosomes coated with a layer of poly-ε-lysine were used. The dispersions of these formulations were investigated by using differential scanning calorimetry and X-ray diffractograms, and they were photographed using a transmission electron microscope. The medicine is completely disseminated in cubosomes according to in vitro tests. In addition, the zeta potential, in vitro release, and entrapment efficiency were assessed. Cubosomes have been discovered to be beneficial in avoiding an initial burst of anticancer medications followed by a steady release of anticancer chemicals. Using the human hepatoma HepG2 cell line, the coated, uncoated, and blank cubosomes were determined to be non-toxic [30,31].
- **Brain TumourTherapy:** Monoolein cubosomes were used to incorporate the anticancer agent doxorubicin (DOX). T98G glioblastoma cells and MTS cell proliferation assay were used to investigate the in vitro cytotoxicity of DOX-loaded cubosomes [32].
- **Breast Cancer Therapy:** The toxicity of blank cubosomes and cubosomes modified with folic acid is examined using MCF-7 cells. The etoposide-loaded blank cubosomes' tumour-targeting ability in this study was primarily a result of their EPR effects, whereas the folic-acid-modified cubosomes actively targeted the tumour through an interaction between the foliate and its receptor, which overexpressed on the surface of MCF-7 breast cancer cells, resulting in a high level of tumour uptake [33].

6. Conclusion:

Cubosome nanoparticles hold promise in the field of drug delivery and sustained drug release, but further optimization is still required, depending on the route of administration, frequency of dosing and the mode of drug release, before such Nano-carriers can truly realize their therapeutic potential in many diseases. They are also attractive Nano-vehicles for loading and delivery of proteins and

peptides but the reported studies are still on a fundamental level and different aspects in terms of structural and morphological characteristics of these soft Nano-carriers, loading capacity of bio macromolecules and their release should be addressed. Future development of cubosome based intravenous Nano-medicines should address blood compatibility at early stages of formulation development. Further, little information is also still available on their stability in biological fluids and biological factors controlling drug release from cubosomes, structural transformation upon contact with biological fluids such as plasma, interactions with cell membranes, and infusion-related reactions to name a few. The application of cubosomes for intravenous drug delivery is an ambitious one; however, these Nano carriers may find accelerated applications for oral, ocular and topical delivery of poorly water soluble drugs, there by offering an alternative, yet, a cost effective opportunity in formulation science.

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