A Review On: A Potential Drug Delivery Method Using Cubosomes:

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Abstract

Cubosomes, nanoscale particles composed of a bicontinuous cubic liquid crystalline phase, have emerged as promising carriers for drug delivery due to their unique structural properties. These particles, with their large surface area and low viscosity compared to bulk cubic phases, can encapsulate a wide range of drugs, including hydrophobic, hydrophilic, and amphiphilic compounds. Their ability to self-assemble under physiological conditions, shield active ingredients from degradation, and enable controlled drug release makes them suitable for various medical and cosmetic applications. This review discusses the synthesis methods (top-down and bottom-up), applications in cancer treatment, oral and intravenous drug administration, topical delivery, and viral disease management, along with their potential for regulated release behaviors. The review also highlights the characterization techniques used to evaluate cubosome morphology, particle size, entrapment efficiency, and drug release kinetics. Finally, the advantages and disadvantages of cubosome-based drug delivery systems are summarized, emphasizing their potential and challenges for large-scale industrial application.

Keywords: Cubosomes, Drug delivery, Nanoscale particles, Bicontinuous cubic phase, Self-assembly, Controlled release, Cancer treatment

1. Introduction:

Cubosomes are tiny particles that differ in a few important ways from their parent cubic phase while retaining the same architecture. They have a higher specific surface area than the bulk cubic phase because they are nanostructured and usually submicron in size. Their dispersions become less viscous due to the increased surface area, which makes them more fluid and possibly beneficial for a range of uses. Cubosomes are an intriguing topic for research in domains like medication delivery, nanotechnology, and materials science because of their structural and physical characteristics. **(1).** Lipids, surfactants, and certain polymers are examples of amphiphilic substances, which have both polar (hydrophilic) and non-polar (hydrophobic) components. The hydrophobic effect is primarily responsible for the distinctive characteristics that these compounds display at the nanoscale. The bicontinuous cubic liquid crystalline phase, often known as cubosomes, is one prominent structure that results from these interactions. Cubosomes are interesting for a variety of applications in material science and drug delivery systems because of their high degree of molecular orientation and structural symmetry.**(2).** Amphiphilic lipids are capable of self-assembling into highly ordered,

biomembrane-mimetic nanoparticles, such as cubosomes, under physiological conditions. This property makes them promising candidates for use in nanocarriers, where they serve to protect active ingredients from degradation and enable controlled release. The study discusses the formation of different lyotropic liquid crystal phases, including micellar cubic phase (i1), hexagonal phase (h1), lamellar phase (lα), and bicontinuous cubic phase (q1), which can be manipulated by adjusting amphiphile concentration and temperature.**(3).**

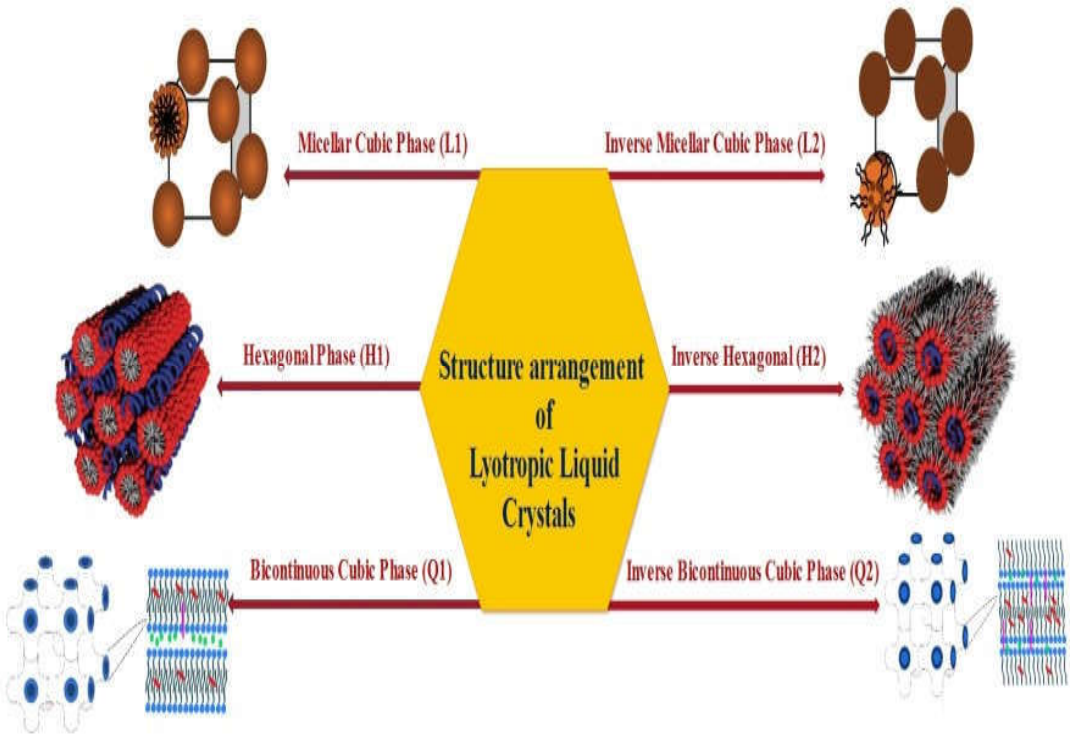

Fig. 1. Phases of lyotropic liquid crystals

The construction of intricate nano-systems with great target selectivity is made possible by nanotechnology. By means of its functionalization, a carrier may be directed to the intended location, hence facilitating its internalization into cells. This feature enables the payload to be delivered into subcellular compartments. **(4,5).** Cubosomes, cubic phase gels, and cubic phase precursors are the three forms of cubic phase structures that are frequently utilized in drug delivery systems and are covered in this study. Although these systems have potential delivery capabilities, their wider applicability is limited by their stiffness and viscosity. Despite these drawbacks, cubic phase gels' capacity to deliver drugs locally and with regulated release has made them useful for transdermal, mucosal, vaginal, and periodontal drug administration.**(6,7).**

2. Applications:

A. According to recent research, a number of anticancer medications have been effectively encapsulated in cubosomes, and their physicochemical characteristics have been comprehensively described. This strategy has the potential to enhance cancer care.**(8).** Because of its distinct structure, this nano carrier has potential for treating melanoma. Numerous tactics have been investigated to target nanomedicines precisely to tumors; preclinical and clinical research has shown promise for both passive and active targeting strategies.

B. Oral drug delivery issues such big molecules, poor absorption, and limited water solubility are addressed with cubosomes. Large proteins have been encapsulated using them, allowing for targeted activity in the digestive tract. **(9).** Cubosome technology is appropriate for drugs with restricted absorption windows because it allows tailored drug release at several absorption sites, such as the upper or lower intestine.

C. The encapsulation, solubilization, and targeted distribution of medications to sick areas are made possible by intravenous drug delivery employing lipid nanoparticles with liquid crystal structures. **(10).** Compared to emulsions and liposomes, cubosomes are better injectable carriers because they can carry larger payloads of peptides, proteins, and insoluble substances.

D. Cubosomes' potent bioadhesive qualities make them perfect for topical and mucosal medication administration. Topical delivery techniques use liquid crystal and nanoparticle technology to deliver drugs to mucosal surfaces, such as the buccal, ocular, and vaginal mucosa, precisely and efficiently.

E. Vehicle for delivering drugs : one typical application for such novel materials is as a drug delivery mechanism. Research conducted in collaboration with l'oreal and nivea, two major cosmetic corporations, is attempting to employ cubosome particles as pollution absorbents and stabilizers for oil-in-water emulsions in cosmetic products. **(11).**

F. Because of its sustained drug release, which is influenced by leftover particles, cubosomes can be used with a wide range of medications with different characteristics. Cubosomes based on monoglycerides show great promise for topical uses such as mucosal and percutaneous administration.

G. Because of their microbicidal qualities, monoglycerides can be utilized to provide intravaginal therapies for stis brought on by viruses like hiv and bacteria like *neisseria genericae* and *chlamydia trachomatis*.**(12).**

3. Technique for preparation:

There are two primary approaches for creating cubosomes: top-down and bottom-up. To stop them from aggregating, both need a stabilizer, such as f127. Stability, biocompatibility, and ideal drug release are some of the variables that influence the preparation method selection.**(13).**

3.1 top-down methodology :

The top-down approach is the most popular method for manufacturing cubosomes. (figure 2). **(14).** The two primary steps in the cubosome production process are first forming viscous cubic aggregates by mixing the lipid with a stabilizer, and then employing high-energy techniques like sonication or high-pressure homogenization to disperse these aggregates in aqueous

fluids.**(13).** Because it takes a lot of energy to create and disperse viscous cubic aggregates, cubosomes made using the top-down method are unstable for up to a year but are difficult to make on a big scale. When adding temperature-sensitive bioactive substances like proteins and peptides, this procedure may provide challenges.**(15)**

3.2 bottom-up methodology:

In a recently discovered cubosome manufacturing process, the nanostructure's building pieces are created first, and then the cubosome is assembled. By dissolving lipids that are insoluble in water, hydrotropes use less energy to stop liquid crystals from forming at high concentrations. By dispersing droplets of the inverse micellar phase into water at 80°c and progressively chilling the mixture, cubosomes are created. By diluting a monoolein-ethanol solution with aqueous poloxamer 407, the emulsification process allows cubosomes to be produced at room temperature. During this process, liquid crystals are formed, as demonstrated by cryo-tem measurements. **(17).**

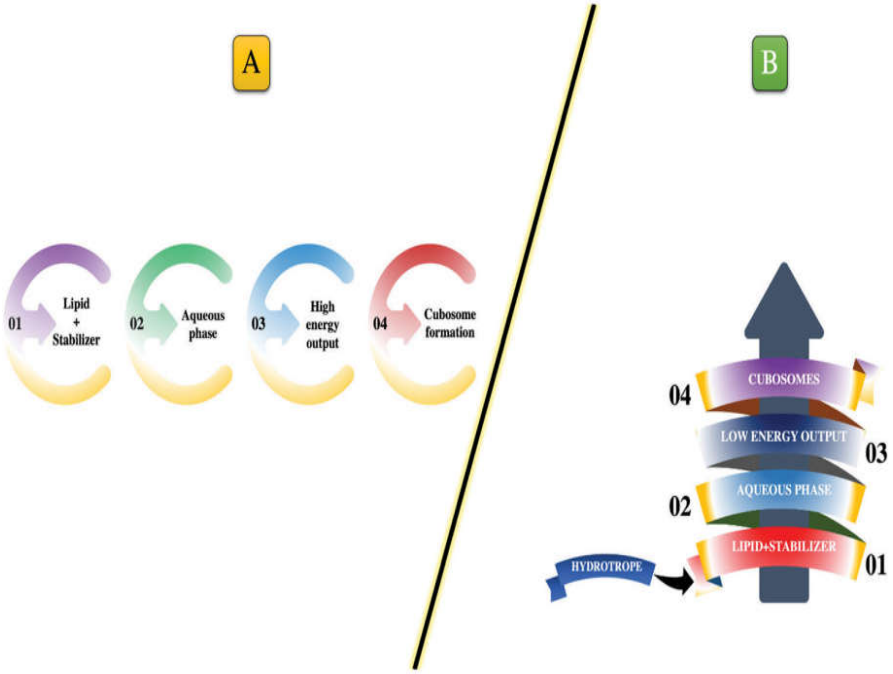

Fig 1: An illustration of the two main processes used to produce cubosomes: (a) top-down methodology, and (b) bottom-up methodology. **(16).**

4. Cubosome types:

The several kinds of cubosomes are as follows**.**

4.1 fluid precursors of cubosomes:

By dissolving monoolein in a hydrotrope, such as ethanol, the hydrotrope dilution process produces smaller, more stable cubosomes. Particle growth is seen during nucleation, crystallization, and precipitation, allowing diluted mixes to either spontaneously crystallize or quickly precipitate cubosomes. By avoiding the manipulation of bulk materials, the "quid precursor" method is the most straightforward.**(18,19)**

4.2 cubosome precursor in powder:

Compared to liquid-phase hydrotropic precursors, powdered cubosome precursors have the advantage of being composed of dehydrated surfactant particles coated with polymers. When these powders are hydrated, cubosomes with an average size of 600 nm are produced, as shown by cryo-tem and light scattering. Lipids provide cubosomes their waxy, sticky texture, while starch coated with waxy lipids helps control particle size and avoid agglomeration. One efficient technique for creating these precursor powders is spray drying.**(20)**

5. Cubosome characterization:

5.1 cubosome morphology:

The morphology of cubosomal nanoparticles was examined using a transmission electron microscope (fei, netherlands, tecani g20 model) running at 60 kv with a lab6 electron source and super twin lens. Using a 1% sodium phosphotungstate solution, samples were prepared on a 200-mesh carbon-coated copper grid with surplus liquid removed. They were then seen at magnifications of up to 1,000,000×.

5.2 analysis of particle size:

The polydispersity index (pdi) and particle size (z-average) of cubosomal dispersions were measured using dynamic light scattering and the zeta sizer nano zs (malvern, uk). The samples were analyzed in triplicate at 25 ± 0.5 °c after being diluted 100 times with deionized water.

5.3 efficiency of entrapment:

Centrifugation ultrafiltration was used to test the effectiveness of drug entrapment.**(21)**. A 5-fu-loaded cubosomal dispersion was diluted to 10 ml with deionized water and centrifuged at 4000 rpm for 15 minutes in an ampicillin ultra 3000 mwco centrifuge tube in order to determine the drug entrapment efficiency (ee). To evaluate adsorption to the ultrafiltration membrane, a known medication solution was filtered. Using spectrophotometry, the amount of free 5-fu in the filtrate was measured at λmax = 266 nm. By deducting the free drug from the total amount of drug in 1 milliliter of dispersion, the amount of entrapped 5-fu was determined. Ee (%) was computed as follows:

Ee(%) = amount of drug entrapped/total amount of drug × 100.

5.4 dsc, or differential scanning calorimetry:

The physical state of 5-fu in cubic gel was examined using differential scanning calorimetry (dsc) with a shimadzu dsc-60 (japan). 5-fu-loaded cubic gel, blank cubic gel, pure 5-fu powder, gmo, and poloxamer 407 samples (5 mg) were cooked at 10 °c/min using nitrogen gas in an aluminum pan. The reference was an identical empty pan.

5.5 diffraction of x-rays:

5-fu-loaded cubic gel, pure 5-fu, gmo, and poloxamer 407 were all subjected to x-ray diffraction (xrd) patterns using an x'pert-pro diffractometer (panalytical, netherlands) equipped with a cu anode. 45 kv, 30 ma, 0.02° step size, 0.5 s/step counting rate, and a scattering angle (2θ) range of 4–50° were the settings used to record the data at room temperature.

5.6 drug release from cubosomes in vitro:

Using dynamic dialysis, the 5-fu release from cubosomes was assessed in vitro.**(22).** The drug release rate was measured by dialyzing a 5-fu-laden cubosomal dispersion against 100 ml of phosphate buffer (ph 7.4) at 15-minute intervals to separate the free drug from the loaded cubosomes in dialysis tubing (10,000 mwco, millipore, usa).**(23).** By sealing 5-fu-loaded cubosomes (equal to 1 mg of medication) or plain drug solution in dialysis bags (10,000 mwco, millipore, usa), the release of 5-fu from cubosomes was investigated. The bags were agitated at 50 rpm while submerged in 100 ml of phosphate buffer (ph 7.4) at 37 ± 0.5 °c. At certain intervals, samples (3 ml) were taken out, examined with a uv spectrophotometer at 266 nm, and then replaced with new buffer. Every experiment was carried out three times.

6. Evaluation study:

6.1 analysis of stability:

over a three-month period, the stability of the cubic gel containing 5-fu was assessed. The samples were kept between 4 and 8 °c in sealed amber glass vials that were covered with aluminum foil. Following storage, materials were vortexed for three minutes to distribute them throughout deionized water. Entrapment efficiency (ee%) and mean particle size of the resultant cubosomal dispersion were measured and presented as the average of three independent experiments.

6.2 in vitro cytotoxicity of cubosomes loaded with 5-fu

The bioassay-cell culture laboratory (national research centre, cairo, egypt) assessed the 5-fu cubosomal dispersion's in vitro cytotoxicity against 5-fu solution. The dispersion contained both free drug and 5-fu-loaded cubosomes. The mtt assay was used to measure cytotoxicity; cell viability was determined by the mitochondrial-dependent conversion of yellow mtt to purple formazan.**(24).** The hepg2 human hepatoma cell line was used for the mtt cell viability test. The cells were incubated at 37 °c with 5% co2 in rpmi 1640 media supplemented with 10% fetal bovine serum, 1% l-glutamine, and 1% antibiotic–antimycotic. Cells were seeded at 1×10^4 cells/well in 96-well plates and incubated for 24 hours following a 10-day culture. After that, the medium was changed, and cells were exposed to different 5-fu doses for 48 hours (100, 50, 25, and 12.5 µg/ml). After adding mtt solution (2.5 µg/ml) and incubating for four hours, the formazan crystals that had developed were dissolved by adding sodium dodecyl sulfate solution. Cell viability was computed by comparing treated and control cells, and absorbance at 595 nm was observed. Using spss 11 software and probit analysis, the ic50—the concentration that inhibits 50% cell growth—was calculated.

6.3 in vivo assessment of cubosomes loaded with 5-fu

The in vivo study was carried out in compliance with ec directive 86/609/eec on animal experiments and approved by the helwan university faculty of pharmacy's animal ethics committee.

6.3.1. Rat liver biodistribution of 5-fu

two sets of nine mature male wistar rats (weighing 160–180 g) were randomly selected. Group 1 was given a subcutaneous dosage of 10 mg/kg plain 5-fu solution in phosphate-buffered saline following a 12-hour fast with unrestricted access to water, while group 2 was given an identical dose of 5-fu cubosomal dispersion that contained both free and loaded 5-fu cubosomes. At 1, 2, and 3 hours after the injection, three rats from each group were cervically dislocated to end their lives. The livers underwent dissection, saline washing, and phosphate-buffered saline (ph 7.4) homogenization. After centrifuging the homogenate, the supernatant was frozen for additional examination.

6.3.2 5-fu concentration analysis

With a few modest modifications, the 5-fu level of the liver homogenate supernatant was determined using a liquid chromatography-tandem mass spectrometry (lc-ms/ms) technique. Following centrifugation, 250 µl of mobile phase was used to dissolve the residue after the organic layer had been evaporated. An lc-ms/ms system including an accela hplc 1200 pumping system, an accela autosampler, and a hypersil gold c18 column was filled with a 10 µl aliquot of the solution. Using a flow rate of 0.25 ml/min, the mobile phase (acetonitrile:0.1% formic acid) was employed for separation. Heated electrospray ionization (hesi) in positive polarity mode was used to perform mass spectrometric analysis on a tsq quantum access ax mass spectrometer. The calibration curve was linear (0.4–10 µg/ml), and the method's lower limit of quantification for 5-fu was 0.1 µg/ml. For data control and analysis, thermo scientific xcalibur 2.1 software was utilized.

6.3.3 histopathological analysis and liver function

Comparing the effects of 5-fu cubosomal formulation and free 5-fu solution on rat liver function and histological alterations was the goal of this experiment. Four sets of three male wistar rats each, weighing between 175 and 190 g, were created. 5-fu cubosomal dispersion and 10 mg/kg free 5-fu aqueous solution were administered subcutaneously to groups 1 and 2 for seven days. Group 3 was given blank cubosomal dispersion as a negative control, while group 4 (normal control) was not given any treatment. Following treatment, a semiautomatic analyzer was used to assess the serum levels of liver enzymes (ast and alt) from blood samples. After the rats were put to sleep, their livers were taken out for pathological examination. After being preserved in 10% formalin saline, liver tissue was washed in xylene, dehydrated with alcohol, and embedded with paraffin. Hematoxylin and eosin were used to stain thin sections (4 µm) for microscopic inspection.**(25).**

6.4 analytical statistics

the results were compared using the student's t-test (spss version 12.0), and stability data was analyzed using the paired t-test. Differences were deemed statistically significant when $p < 0.05$, and results are shown as mean ± standard deviation (sd).**(26).**

6. Advantages and disadvantages: (27)

Advantages

1. Cubosomes are biocompatible, biodegradable, non-irritating, and thermodynamically stable.
2. Drugs that are hydrophilic, lipophilic, or amphiphilic can all be incorporated into cubosomes.

3. Because of their large internal surface area, cubosomes have a high drug-loading capacity.
4. For drug delivery, they employ cubic liquid-crystalline phases with three-dimensional nanostructures that contain hydrophilic and hydrophobic domains.

5. A complex diffusion pathway for the continual release of entrapped drug molecules is provided by the vast interfacial surface.

6. Cubosomes' lipid constituents are biocompatible, bioadhesive, and digested.

Disadvantages

1. Because water-soluble drugs include a lot of water, they are less prone to become stuck in cubosomes.
2. Due to their high viscosity, cubosomes are challenging to create on a wide scale.

3.Leakage could occur during storage or in vivo transmission.

4.Cubosomes may undergo phase alterations in response to environmental stimuli.

5. If cubosomes are left unsupervised for an extended amount of time, particle proliferation may take place.

Conclusion

Cubosomes represent a promising class of nanocarriers with potential applications in drug delivery across a wide range of therapeutic areas, including cancer treatment, viral infections, oral, intravenous, and topical drug administration. Their ability to encapsulate a variety of drug types and release them in a controlled manner offers significant advantages in terms of bioavailability and therapeutic efficacy. However, challenges related to large-scale production, stability, and encapsulation of water-soluble drugs need to be addressed before cubosomes can become a mainstream delivery platform. Continued research and development, particularly in scaling up production methods and enhancing the stability of cubosome formulations, will be essential to realizing their full potential in both medical and cosmetic applications.

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