**REVIEW PAPER**

# Cubosomes: A Potential Targeted Drug Delivery System for an Anticancer Therapy

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***Received: 16th Dec, 2025; Revised: 8th Feb 2026; Accepted: 24th Feb, 2026; Available Online: 30th March, 2026***

**ABSTRACT**
Cancer remainsone of the leading causes of mortality worldwide, making the need for advanced drug delivery systems (DDS) to enhance therapeutic efficiency while reducing systemic toxicity more prominent. The clinical efficacy of conventional chemotherapy is limited due to low bioavailability, non-specific distribution, and significant adverse effects. In this regard, cubosomes are a novel nanostructured lipid carrier and represent a good choice for the development of optimal targeted anticancer therapy. Cubosomes are nanoparticles of the bicontinuous cubic phase, which are formed by self-assembly of amphiphilic lipids, that are characterized by unique structures including a greater internal surface area and the presence of dual hydrophilic and hydrophobic domains. These features facilitate the encapsulation of various therapeutic agents with different properties, such as hydrophilic, lipophilic, and amphiphilic drugs. In addition, cubosomes allow controlled and sustained drug release, enhanced stability, and better tumor site targeting either passively, actively, or in a stimuli-responsive manner. Cubosome systems have been successfully loaded with different anticancer drugs such as paclitaxel, doxorubicin, and cisplatin, showing enhanced therapeutic efficiencies. This review highlights the true cubosome potential as a simple and flexible platform for innovative target cancer treatment strategies by analyzing the structural characteristics, preparation techniques, physicochemical properties, targeting strategies, and applications of cubosomes in anticancer therapy.

**Keywords**: *Cubosomes; Anticancer therapy; Nanocarriers; Controlled drug release; Drug targeting; Drug delivery systems*
**How to cite this article:** Kakade PS, Chivate AN, Chivate ND, Cubosomes: A Potential Targeted Drug Delivery System for an Anticancer Therapy. Int J Drug Deliv Technol. 2026;16(25s): 572-588. DOI: 10.25258/ijddt.16.25s.70
**Source of support:** Nil.
**Conflict of interest:** None

## INTRODUCTION

Cancer is responsible for high incidence rates and is an important clinical challenge worldwide. According to GLO-BOCAN 2020 estimates, 19.3 million new cancer cases and 10 million deaths from cancer were diagnosed in 2020[1]. Because of this, cancer is the leading cause of mortality worldwide and a significant barrier to longer life expectancies.The treatment of cancer, despite the numerous advances in medical science, still poses a global challenge owing to the challenges of drug resistance, non-selective drug distribution, and the severe side effects associated with traditional chemotherapy[2]. Because traditional medication delivery methods cannot function precisely at the target spot, they harm healthy tissues and organs, thereby reducing the therapeutic efficiency. As a result, there is an increasing demand for novel drug delivery systems that enable improved drug targeting, increased bioavailability, and decreased systemic toxicity[3].

In recent years,nanotechnology-based drug delivery systems have attracted the wide interest of researchers since they canovercome the limitations of conventional therapies[4]. Additionally, cubosomes have recently been established as a new class of lipid-based nanocarriers with significant potential for selective delivery of anticancer therapies. Cubosomes are self-assembled nanostructured particles from amphiphilic lipids (e.g., glyceryl monooleate, phytantriol) dispersed in water in the presence of a stabilizer[5]. With two intercalated water channels surrounded by a lipid bilayer, they exhibit a clear bicontinuous cubic liquid crystalline phase. It has a high internal surface area and can incorporate diverse therapeutic agents.

One of the most practical benefits of cubosomes has been their ability to encapsulate a hydrophilic and lipophilic drug concurrently.

Furthermore, due to their nanoscale size, they benefit from improved EPR effect for passive targeting of tumor

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tissues[6]. To improve delivery efficiency during tumor insertion, cubosomes can also be coupled with specific ligands for active targeting. In addition, their controlled and sustained release behavior helps to maintain therapeutic concentrations of drugs for a prolonged period, which decreases the frequency of dosing and adverse effects[5].

In recent studies, Many anticancer drugs, such as paclitaxel, doxorubicin, and cisplatin, have been loaded into cubosomes with favorable therapeutic responses. This review describes the types of cubosomes, their composition, characterization, preparation methods, and targeting ability in anticancer drug delivery, emphasizing cubosomes' potential as a novel and effective platform in cancer therapy.

## 1. CUBOSOMES: AN OVERVIEW

### 2.1 Structure of Cubosomes

Cubosomes are distinguished by their honeycombed structure, which divides the two inner aqueous channels, and they exhibit a large interfacial surface (Fig.1). Cubosomes are nanoparticles, formed through the self-assembly of amphiphilic or surfactant-like molecules in a liquid crystalline phase of cubic crystallographic symmetry.The cubic stages have excessive, solid-like viscosity due to the special Bicontinuous arrangement, which places two distinct zones of water in the same volume and separates them with a controlled bilayer of surfactant application. Amphiphilic molecules create two hydrophilic domains that are divided by the bilayer; as a result, they create bicontinuous water and oil channels, where bicontinuous indicates two distinct hydrophilic regions.The structure's connections result in a clear, viscous gel that resembles cross-linked polymer hydrogels in both appearance and rheology. Cubosomes are tiny particles that are square or slightly circular in shape. The pores in the lipid water system that are filled with aqueous phase cubic phases are represented by horizontal dots. Luzzati and Husson used the X-ray scattering method to identify them years ago[7].

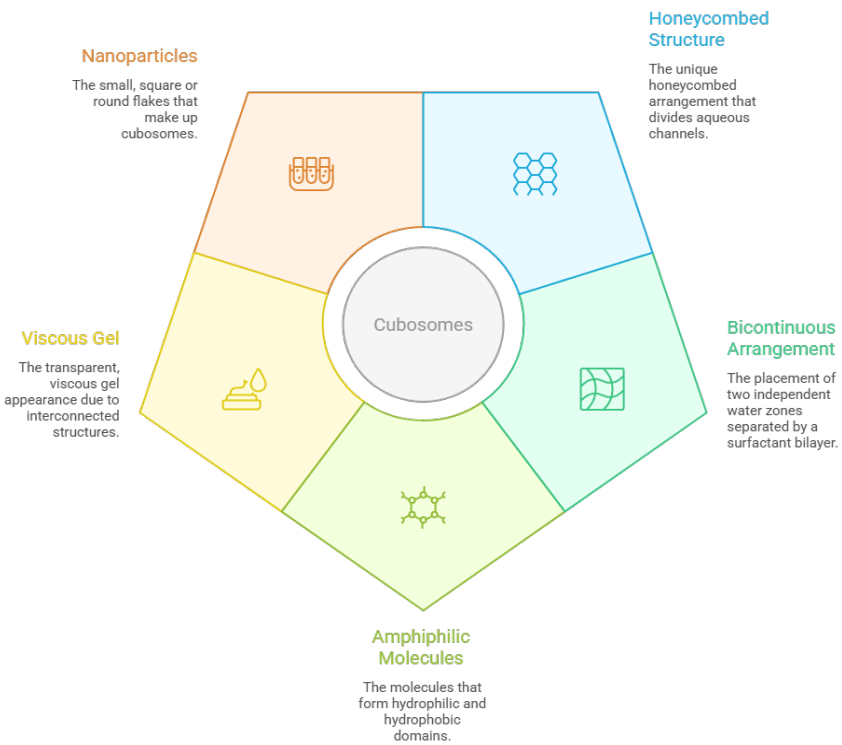


**Fig.1:** Structure of Cubosome

### 2.2 Composition of Cubosomes

#### 2.2.1 AmphiphilicLipids

##### 2.2.1.1 GlycerolMonooleate(GMO)

GMO is a polar, unsaturated monoglyceride with a melting point of 35–37 ◦C, HLB value of 3, and has a translucent and colourless appearance.

Fluid obtained from it becomes monooleate oil,which is a monoglyceride synthesized from oleic acid glycerides and other fatty acids. Monooleate, an amphiphilic lipid, can form lyotropic liquid crystals with many different morphologies[8]. GMOs have hydrophilic and hydrophobic characteristics due to the presence of hydroxyl groups in the head group that can H-bond with water in an aqueous solution and hydrocarbon chains in the tail. Moreover, GMO isbiodegradable, and a biocompatible, and non-toxic, GRAS,and FDA inactive ingredients guide classification widely used as an emulsifier[9]. Recently, Elakkad et al. created a cubosomalnanodispersion loaded with this medication using Pluronic F127 as the stabilizer and GMO as the amphiphilic lipid. to attain a

polydispersity index (PDI) of 0 and the intended particle size of 200 nm. The medication was dissolved in the molten mass to create a uniform dispersion after the combination was heated at 60 degrees Celsius to produce the ideal 10:1 GMO: Pluronic F127 blend with a high colloidal stability and entrapment efficiency[10].

**2.2.1.2 Phytantriol(PHYT)**

Phytantriol or PHYT (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) is a physiological and biocompatible compound derived from natural sources with many cosmetic applications and regarded as an excellent alternative for monoolein in cubosome preparation.Esterases can break down lipid-based substances like glyceryl monooleate, while PHYT's phytanyl backbone may provide more structural stability[11]. Its phytanyl backbone lacks a glycerol moiety, in contrast to monoglycerides. Phytantriol is substantially more resistant to esterase hydrolysis than is GMO, although many fatty acid reactants, including those generated from GMOs, have the drawback of being digested in esterase-catalyzed reactions, which affects the performance of compositions formed from GMOs[12]. Besides, phytantriol also shows similar phase changes as GMO by increasing hydration and temperature. Therefore,phytantriol has been proposed as a non-GMO alternative for the formation of bicontinuous cubic-shaped phases. Phytantriol is water-insoluble and so is biocompatible, biodegradable, and non-toxic. At room temperature in excess water, PHYT forms an inverse cubic Pn3m Pn3m phase which transforms to hexagonal reverse H11 between 40 and $60^{0}$ $^{\circ}$C[13].

**2.2.2 Stabilizers**

Cubosomes exhibit their kinetically inherent instability when distributed in watery media. Particle aggregation occurs when the dispersed particles' hydrophobic faces come into contact with the exterior hydrophilic aqueous media. A surfactant is required to stabilize cubosomes colloidally in order to prevent them from reverting to the bulk cubic phase. The stabilizer prevents particles from attaching by acting as an electrostatic barrier. It maintains a very steady state for the scattered particles.The primary stabilising agents are Pluronics. Poloxamer 407 (F127), a well-known PEO99–PPO67–PEO99 triblock copolymer, was extensively used as a suitable surfactant for the cubosome formation, exposing PPO parts to the water phase at the bilayer surface or in the structure, and PEO chains to the outer aqueous phase[14]. However, the stabilization effect of F127 appears to be different for cubosomes than it is for simple dispersion stabilization, like in emulsions. While the hydrophilic segment (PEO) protrudes in the aqueous media, offering steric shielding, F127 stabilizes the nanocarriers through the adsorption of a hydrophobic segment (PPO) onto the particle surface. The stabilizer affects when cubosomes interact with scattered particles and the way their phase behavior changesdepending on the scattered particles, up to 20% w/w[15].

**2.3 TypesofCubosomes**

Cubosomes can be classified as either liquid or powdered precursors, depending on the method of formulation. One common lipid that can spontaneously form cubosomes is monoolein, which can be diluted with a hydrophobic solvent like ethanol. In this case, nucleation facilitates the production of particles, which then rise as a result of crystallization and precipitation processes. The quid precursor technique offers a quick way to manufacture cubosomes on a larger scale by avoiding the handling of bulk solids and possibly harmful high-energy activities. This technique could be applied to the in situ production of cubosomes[16].

In addition to the liquid cubosome matrix, dehydration surface-active chemicals combined with a suitable polymer can be used to create dry cubosomes. This process, known as spray-drying, can yield cubosomes in powder form based on the conditions of particle encapsulation in the droplets from the emulsion and dispersion. This process involves coating water-soluble, non-cohesive starch with waxy lipid, which typically causes agglomeration[17]. However, cubosomes are better in dry powder form than liquid form to prevent processing bulk water.

## 2. MECHANISM OF FORMATION OF CUBOSOMES

Uniform-sized nanostructured dispersions must be produced from convenient pharmaceutical options. Cubosome formulation features will be primarily determined by the cubosome preparation method. Cubosomal preparations have been widely prepared using two conventional methods, namely, top-down and bottom-up processes (Table 1).

**3.1 Top-Down Approach**

It is the most widely used method in research since it was first described by Ljusberg- Wahren in 1996. This is the most popular method for creating cubosomes, particularly when the polymer lipid is glyceryl monooleate (GMO). Cubosomes with fine dispersion of cubosomes are then produced using this dispersion; high energy levels should be used in the process. A suitable stabilizer is first added to a lipid to create bulk cubic aggregates; energy is then supplied through a homogenizer to make the dispersion[18]. Wörl et al. examined the factors influencing the characteristics of GMO cubosomes based on soybean oil. The temperature during HPH and the F127 content were determined to be extremely important factors based on the findings. Vesicles (distributed nanoparticles of the lamellar liquid crystalline phase) or a structure resembling vesicles are always present when cubosomes are created using the top-down approach. However, the biggest drawback of this strategy is that high energies are not appropriate for the large-scale batch integration of temperature-sensitive bioactive compounds, particularly proteins and peptides[19].

### 3.2 Bottom-Up Approach

The bottom-up approach (i.e., solvent dilution method) is one of the most widely used for cubosome preparation, especially with phytantriol as lipid. Here, a low-energy process is used in which hydrotropes and stabilizers are applied in excess water in order to induce the formation of cubosomes. Hydrotropes are important when deciding to add poorly water-soluble lipids because they solubilize these poorly water-soluble lipids and prevent unwanted liquid crystal formation at high concentrations. This method generates a cooling solvent with mild conditions specifically for temperature-sensitive compounds and provides better long-term stability through a continuous and uniform distribution of the stabilizer. In the rotating drum method, there are two types of precursor formation, which may be liquid or powder, used for the formation of a cubosome. The liquid precursor monolein and ethanol are emulsified with a stabilizer (e.g., poloxamer 407) to form a cubo-gel, then diluted & sonicated to form cubosome nanoparticles. Monoolein adsorbed with polymers (such as starch or dextran) makes up the powder precursor; cubosomes typically develop once this emulsion is hydrated using spray drying techniques. In conclusion, the bottom-up approach helps create a stable cubosome formulation and is energy-efficient[20].

## 3. METHODS OF PREPARATION

### 4.1 High-Pressure Homogenization

Muller et al. also pioneered a high-pressure (piston-gap) homogenization. It is a nanosuspension preparation method of high-energy from the year of 1994. The method consists of circulating a drug suspension through a narrow passage (5–20 μm) under high pressure, 1000–1500 bar, and velocity 500 m/s, typically more than 200 times. Pre-micronization is usually done to prevent clogs, and it reduces the processing time. Emulsifier molecules, for example, egg yolk phospholipids (lecithin), have to be inserted between oil and water, and this will turn the coarse and bottom phase into a fine stable nano-emulsion. Homogenization implies lowering the pressure with the Bellini principle, where vapor bubbles can not only appear but also collapse, leading to very high shear forces, decreasing the particle sizes to the nanometer level. Final sizes are influenced by many factors, such as the pressure, number of cycles, and the type of drug; softer drugs can be used to obtain the smallest sizes (200–300 nm)[21].

A major limitation is that high-pressure homogenization is a very energy-absorbing method, and the drug particles undergo very high power densities ($10^{13}$ W/m3), which can cause the drug particles to become partially or completely amorphous. As the technology scales up from lab to industrial scale, using the same technique with the ability to produce parenteral nanosuspensions continuously. It has been widely used in the preparation of disperse systems, such as parenteral nutrition emulsions, at a lower contamination potential than media milling. The approach has worked in several drugs, significantly enhancing their solubility and oral availability[22].

### 4.2 UltrasonicationMethod

Murgia et al. used this method;quercetin-cubosomes were prepared and stabilized by co-block polymers. The amphiphilic lipid (typically monoolein) is melted and dispersed in a hot aqueous solution of Pluronic®, such as F-108 or Pluronic® F-127, under magnetic stirring. Cubosomes are prepared by using a bath sonicator to disperse the drug in the molten lipid and by ultrasonically dispersing the dispersion of lipid-surfactant solution[23].

### 4.3 Spray Drying Technique

The solvent rapidly evaporates when the atomized lipid-surfactant-solvent mixture comes into contact with the hot air stream during the spray drying process, resulting in the creation of a dry powder of cubosome precursor. The approach is often simple, cost-effective, and typically easy to scale up. Briefly, an ethanol solvent or a binary solvent mixture, like methanol/chloroform, dissolves the amphiphilic lipid if Pluronicis added[24]. A hydrophilic solid carrier in an aqueous phase (such as dextran or sorbitol) is combined with the lipid- ethanol solution, while stirring, to form a low viscosity emulsion. The medication can either be dissolved with the lipid in the organic solvent or mixed with an aqueous solution of the solid carrier. An example of the basic lipid-ethanol-dextran-water quaternary system that can be spray-dried to readily evaporate the organic solvent and water, resulting in a dry lipid-coated powder precursor that can be redispersed in water to create cubosomes[25].

### 4.4 Solvent Evaporation Method

Ou et al. This technique was applied in the preparation of cubosomes containing monosodiumuricosuric Achyranthes bidentata polysaccharide. An alternative technique of powder cubosome production is the solvent evaporation technique and requires the use of a homogeniser or ultrasonicator[26]. It is almost similar to the spray-drying process, the only difference being the employment of a high-energy sonicator. In this method, Lipids are first dissolved in an organic solvent before being dropwise combined with another combination that contains a stabilizer, like Pluronics (F108, F127, F68, P104). The mixture is kept at a high temperature by magnetic stirring. The lipid or aqueous surfactant solution may dissolve the drug. Cubosomes are produced when the mixture is homogenized, and the volatile organic solvent is removed by stirring at higher temperatures. [27]

**Table 1:** The various drugs loaded on to cubosomes for anticancer theranostics and drug delivery

| Sr. No. | PolymersUsed | ActiveIngredient | MethodofPreparation | Application | References |
|---|---|---|---|---|---|
| 1. | Poloxamer 407 (PF127), Monoolein (MO) | Piperine, 20 (S)-protopanaxadiol | Hydration, melting, and vortex mixing | Drug Administration | [28] |
| 2. | Folic acid, Poloxamer 407 (PF127), and monoolein (MO) | 3. Bromopyruvate | High-pressure homogenization in conjunction with the injection technique. | Delivery Targeted Against Tumors | [29] |
| 3. | Poloxamer 407 (PF127), Monoolein (MO) | Hydrochloride of berberine | Emulsification technique | Drug Administration | [30] |
| 4. | NIR-emitting fluorescence probe based on squarain, Pluronic F108 (PF108), and Monoolein | Camptothecin | Ultrasonicprocessing | Theranosticand Bioimaging | [31] |
| 5. | Polyethylene Glycol (PEG), Monoolein (MO), | Carboplatin | Heating, Vortexmixing, Centrifugation, | Drug Administration | [32] |
| 6. | Poloxamer 407 (PF127), Monoolein (MO) | Cisplatin-Metformin | Emulsificationmethod | Delivery Targeted Against Tumors | [33] |
| 7. | Monoolein (MO), RH40, and Polyethylene Glycol 400 (PEG-400) | Curcumin | Sonication and vortex mixing | Anticanceractivity | [34] |
| 8. | Pyridinylmethyl linoleate and monolinolein | Doxorubicin(DOX) | Mixing, Heating, Vortex, Hydration | Anticanceractivity | [35] |
| 9. | Phytantriol with Monoolein (MO) | Doxorubicin(DOX) | Melting, Centrifugation, Hydration | Drug Administration | [36] |
| 10. | N-Oleoyl glycine N-(2-aminoethyl)-oleamide, monoolein (MO) | Doxorubicin(DOX) | Melting | Drug Administration | [37] |
| 11. | Poloxamer 407 (PF127), MES sodium salt, and monoolein | Doxorubicin(DOX) | Dox sol melting and desiccation with molten MO | Drug Administration | [38] |

| | | | | | |
|---|---|---|---|---|---|
| | (MO) | | | | |
| 12. | Monoolein(MO) | Brucea javanica oil with doxorubicin (DOX) | High-Pressurehomogenizer | DualDrug Administration | [39] |
| 13. | Poloxamer 407 (PF127), Monoolein (MO) | ELC-Cu, or Elesclomol Copper Complex | Method of solvent-shifting | Drug Administration | [40] |
| 14. | Monoolein(MO) | Gambogenicacid | Recrystallization of lipids in a homogenous emulsion | Drug Administration in the treatment of cancer | [41] |
| 15. | Phospholipids, Polyethylene Glycol (PEG), and Monoolein (MO) | Meso-Mn (III) chloride-tetraphenylporphine | Hydration, melting, and vortex mixing | Bio-Imaging | [42] |
| 16. | Poloxamer 407 (PF127), Monoolein (MO) | Metformin | Cubic gel disruption | Drug Administration | [43] |
| 17. | Poloxamer 407 (PF127), Monoolein (MO), | Paclitaxel(PTX) | Sonication with a Probe Sonicator | Drug Administration | [44] |
| 18. | Monoolein(MO) | Resveratrol and Pemetrexed | The Hydrotrope method of homogenization | Drug Administration | [45] |
| 19. | RPMI-1640 Poloxamer 407 (PF127), Monoolein (MO), | Resveratrol | Hydration, melting, and vortex mixing | Drug Administration | [46] |
| 20. | Monoolein(MO) | Thymoquinone (TQ) | Homogenization and EmulsificationTechnique | Drug Administration | [47] |

## 4. CHARACTERIZATION OF CUBOSOMES

### 5.1 Particle Size and Zeta Potential

Particle size is determined using dynamic laser light scattering (DLS) with a zeta sizer. This provides a low-effort, non-invasive approach for quantifying the characteristics of the particles in suspension. At 25 ◦C, a light scattering intensity of approximately 300 Hz and three replicates are used after diluting the sample in an appropriate solvent[48]. The average volume-weighted size is used to present the data.The main drawback of all DLS measurements, however, is that larger and heavier particles, which are frequently overestimated, contribute significantly to the overall mean decay rate of a polydisperse solution.

Another crucial characteristic that is defined for liquid-crystalline systems is the zeta potential. Zeta-potential can only be measured by measuring the speed of a charged particle moving under the influence of an applied electric field because it is simply not possible to determine it directly. These statistical factors are therefore essential for pharmaceutical applications in order to identify bonded anionic or cationic particles in solution. Liquid crystalline optical phenomena and polarizing microscopy observations can be used to identify the morphology of liquid crystalline[49]. polymer-coated grids. Conventional transmission electron microscopy (TEM), where materials are dried onto carbon grids before visualization under the

### 5.2 Morphology (TEM/SEM Analysis)

Cryogenic transmission electron microscopy (cryo-TEM) permits direct visualisation of samples in the hydrated state by vitrification of a thin film suspended between

microscope, is discouraged due to complications associated with dehydration[50].

Cryo-TEM serves as a superior complement relative to scatter data since it offers direct visualisation of the structure along with validation of the lattice symmetry.The combination of cryogenic transmission electron microscopy (cryo-TEM) and scattering is the gold standard for structural type characterization of non-lamellar liquid-crystalline dispersions.The cubosomes are faceted cubic particles of cubic shape. Cryogenic field-emission scanning electron microscopy (cryo-FESEM) has recently been described as a valuable imaging complement with respect to the nanostructure of the non-lamellar mesophases[51]. Cryo-FESEM presents dispersions in a frozen state of preservation, ideally close to that found in nature. Rizwan et al. Detailed descriptions of lipid cubic phase nanostructures based on differential geometry, particularly cubosomes, where space is divided into two congruent and non-intersecting water channels by distorting a single continuous lipid bilayer. The dispersions' nanostructure and the non-dispersed phases' microstructure were similar, according to cryo-FESEM[12].

### 5.3 Drug Loading and Entrapment Efficiency

Amount of drug entrapped and drug loading of cubosomes are adoptable using chromatography methods, dialysis, and small-angle X-ray scattering or ultra-filtration methods. Unentrapped drug quantity can further be analysed by UV spectrophotometer, HPLC analysis, and fluorescence correlation spectroscopy. By using a spectrophotometer or radioactivity, the unentrapped drug concentration is determined, which is subtracted from the total drug added in the formulation, and the amount of drug is analyzed[31].

### 5.4 In Vitro Drug Release Studies

Cubosome evaluation begins with an important step from the in vitro drug release studies, which is a key feature in assessing the rate of drug diffusion from the nanostructured carrier and also the mechanism of drug release kinetics. These studies are usually done through dialysis membrane methods or diffusion cells using the simulated physiological conditions for mimicry of the tumor microenvironment[52]. Cubosomes have a controlled and sustained drug release profile because to its unusual bicontinuous cubic structure, which creates a complicated diffusion channel for the medicines within. For anticancer drugs like doxorubicin and paclitaxel, this is particularly helpful for maintaining therapeutic-toxicity ratios with extended dose intervals, reducing systemic toxicity. The release profile can be further adjusted by other factors like lipid content, stabilizer type, particle size, and environmental stimuli like temperature and pH. Additionally, cubosomes have been designed to deliver drugs at specific sites by stimuli-responsive release[53].

### 5.5 Stability Studies

Cubosomes' morphological and organoleptic characteristics over time can be utilized to investigate their physical stability. Particle size distribution, zeta potential, drug content, and cubosome entrapment efficiency at all temperatures can be used to evaluate the potential variation by time at periodic intervals[54]. In liquid crystals, this phase shift is usually accompanied by exothermic or endothermic energy changes. Differential scanning calorimetry (DSC) can be used to detect the phase transition temperature of the binary liquid-crystalline system in order to assess the liquid crystalline stability. Additionally, a rotating viscometer should be used to assess the viscosity of cubosome compositions at different angular velocities. As well, the viscosity of cubosome formulations should be evaluated across various angular velocities employing a rotary viscometer[55].

## 5. DRUG LOADING AND RELEASE IN CUBOSOME

Small-molecule medications, peptides, biologics, or bioactives can be suitably loaded into the cubosomes that will be produced. The medicine can be loaded into the lipid bilayer, attached to the lipid membrane, or localized within the water channels in the cubic phase. These are the three main ways that the Drug can be loaded. Drug moieties can be loaded by either co-lyophilizing with the lipid film and then dispersing, or by adding the medicinal agent to the molten lipid. By executing the dispersion over previously created cubosomes, the incubation process can also be used to load drug moieties into cubosomes.Most proteins, peptides, and small-molecule medications are integrated into the lipid bilayer. Additionally, single or binary lipid compositions,primarily phytantriol and monoolein,were used to create cubosomes. Small-angle X-ray scattering (SAXS) is still the most popular method for quantifying drug loading, despite the availability of many other methods. These investigations therefore indicated the potential of drug delivery carriers, possibly using cubosomes as the drug delivery tool, especially for the delivery of anticancer drugs[56]. Compared to other particles like liposomes, cubosomes have the extra advantage of having a bigger hydrophobic area, which increases the loading capacity of hydrophobic medications while also enabling the loading of hydrophilic ones. Furthermore, curcumin solubilized in phytantriolcubosomes had a higher loading capacity than curcumin liposomes, according to another study.

Furthermore, particle membrane curvature can be changed without regard to particle size, depending on the cubosome lattice structure. This characteristic is particularly important for simulating highly curved structures, which have better membrane loading capacities, a larger membrane mean curvature, and a higher membrane area to volume ratio (Fig. 2)[57].Cubosomes have a cubic structure to encapsulate and release the drugs based on their molecular weight and

polarity, which follows the law of Higuchi- diffusion-controlled kinetics.

$$Q = [D_m C_d (2A - C_d) t]^{1/2}$$

This equation states that the square root of time determines the release (diffusion) of agents from the matrix. A is the main amount of the drug per unit volume of the matrix, t is the time, Dm is the agent's diffusion coefficient in the cubic matrix, Cd is the agent's solubility in the matrix, and Q is the amount of agents released per unit area of the matrix. The amount and rate of medication release can be calculated using this formula[58].

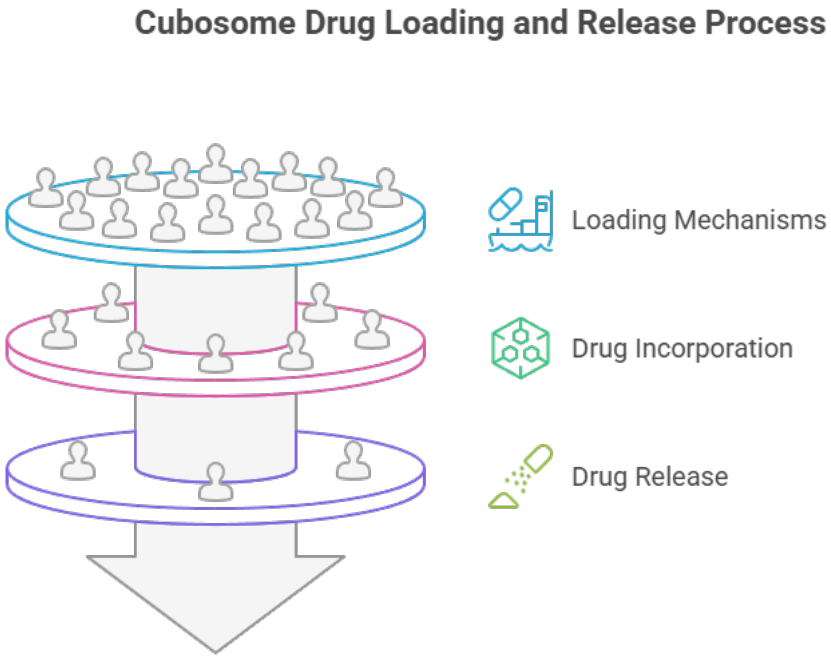


**Fig. 2** Cubosome Drug Loading and Release Process

## 7. TARGETING STRATEGIES IN CUBOSOMES

### 7.1 Passive Targeting (EPR Effect)

In anticancer therapy, cubosomes are traditionally used for passive targeting, which is augmented by the phenomenon known as Enhanced Permeability and Retention (EPR) Effect for solid tumors. However, tumor tissues have abnormally-formed vasculature with wide endothelial gaps and high permeability, which provides a unique advantage for the nanosized carriers such as cubosomes to extravasate and to be effectively deposited into the tumor interstitium[59]. Also, due to the impaired lymphatic drainage system in tumors, these nanoparticles are poorly cleared from the surrounding tissues, resulting in the prolonged presence of the payload at the site of action. Finally, they can passively target through the enhanced permeation and retention (EPR) effect due to their nanometric size, high internal surface area, and lipid bicontinuous cubic structure of the cubosomes. It allows for increased drug concentration within the tumor while reducing drug distribution to other organs and other side effects. In addition, the sustained release property of cubosomes also allows for extended drug exposure within the tumor microenvironment, enhancing the therapeutic effect of the therapeutic agent and decreasing the frequency of administration[60].

### 7.2 Active Targeting (Ligand-Based Targeting)

Active targeting for anticancer therapy, nanocarriers are surface-modified with specific ligands that selectively recognize and bind to overexpressed receptors on the surface of cancer cells. This approach boosts cellular uptake by receptor-mediated endocytosis and therefore achieves a higher drug localization at the tumor site than passive accumulation. Examples of ligands employed are antibodies, peptides, aptamers, and small molecules such as folic acid that bind to folate receptors, which are overexpressed in many cancers. Ligand-functionalized cubosomes are internalized into tumor cells via binding to these receptors, allowing for an effective intracellular delivery of the embedded anticancer agents.

The ligand-receptor specificity reduces off-target effects and thus reduces the toxicity to normal tissues. Moreover, the structure of cubosomes facilitates easy surface functionalization without altering their stability or drug-load capacity[61].

### 7.3 Stimuli-Responsive Targeting

Another sophisticated approach is represented in stimuli-responsive targeting of cubosomes, wherein the nanocarrier system is designed to release the loaded payload upon exposure to certain internal or external triggers in the tumor microenvironment. Such triggers can be acidic pH, high concentration of enzymes, redox gradients, temperature fluctuation, or external stimuli such as light and magnetic fields. A well-known feature of tumor tissues is that the tumor microenvironment is mildly acidic compared to normal tissues. pH-sensitive cubosomescould be designed to change their structure

and release the drugs upon reaching the target site. Likewise, redox-sensitivity is typically based on the difference in the amount of glutathione present in the intracellular environment, which is higher in cancer cells, resulting in the release of the drug. Similarly, localized enzyme-responsive cubosomes can be designed to degrade only in the presence of enzymes that are specific to the tumor. The utilization of these types of smart features in cubosomes increases spatiotemporal control over drug release, leaks less prematurely, and has low systemic toxicity[62].

## 8. ANTICANCERDRUGSLOADEDONTOCUBOSOMES.

The most often used medications that are combined with cubosomes are described below (Table 2).

### 8.1 Paclitaxel

Paclitaxel (PTX) is a first-line chemotherapeutic drug used for treating patients with Non-Small Cell Lung Cancer (NSCLC). PTX enhances the polymerization of microtubules by forming a complex with the β-tubulin that blocks the mitotic spindle, and further arrests the cell cycle at the metaphase-anaphase junction of mitosis, resulting in inhibition of the cell cycle. The microtubules are usually described as unstable and dynamic elements. Conjugation of PTX to β-tubulins stabilizes the microtubules and results in the winding of the dynamic rearrangement of the tubule network that supports interphase and mitotic functions, causing aberrant bundles across the cell cycle[63]. Aleandri et al. Using biotinylated cubosomes, which were stabilized and functionalized by a Biotin (Vitamin H or B7)-based copolymer that enabled the encapsulation of paclitaxel (PTX) as well as the hydrophobic fluorescent dye (MO-Fluo) used for active targeting or cellularinternalization of the nanoparticles. The strategy consisted of incorporating a biotin-conjugated stabilizer (PF108- B) into these novel MO-based cubosomal dispersions, taking advantage of the high affinity of biotin for the sodium-dependent multivitamin transporter (SMVT), which is overexpressed in the membranes of tumor cells. HABA (4'-Hydroxyazobenzene-2-carboxylic acid) is used to quantify this conjugation of PF108 and Biotin in a solution that contains avidin, HABA, and Biotinylated PF108. Moreover, the study also demonstrated a more robust anticancer effect of PTX at a 1 μg/mL concentration level in the biotinylated cubosomes when compared to the free PTX or non-targeted cubosomes. This was further improved by the biotin ligand-mediated enhanced uptake of the carrier in cancer cells through receptor-mediated endocytosis, leading to an increased specific cytotoxicity of PTX on tumor cells and decreased non-specific cytotoxicity of PTX on normal cells and tissues. These cubosomes with biotin on their surface could be used for drug delivery, diagnosis, and for monitoring the therapeutic response[64]. Zhai et al. suggest Mono-olein (MO)-based cubosomes to be potential carriers of PTX for the treatment of ovarian cancer. In conclusion, we suggest that PTX-loaded cubosome- based drug and overall survival modalities can prolong the disease-free progression of a series of cancers[65].

### 8.2 AT101

AT101 is the R-(—)-enantiomer of gossypol, a polyphenol isolated from cottonseed, and a candidate for anticancer drugs and used clinically as a therapeutic agent, mainly in the treatment of Glioblastoma Multiforme (GBM). Induction of cancerous cell death by its apoptotic action leads to its autophagic property-based antitumor effect. Due to the hydrophobicity and low bioavailability of free AT101, it was encapsulated in GMO-based cubosomes using the surfactant Pluronic F-127 with the top-down technique. The in vitro viability cytotoxic studies for AT101, which is embedded in GMO cubosomes and free AT101, and the same concentrations and time of exposure were done on two GBM cell lines (A172 and LN229) and healthy cell types of the central nervous system, astrocytes (SVGA), microglia (HMC3) with determination of cell viability by the colorimetric WST-1 assay. In addition, Nuclear Magnetic Resonance (NMR) diffusometry studies revealed that the drug entrapment efficiency of AT101-loaded cubosomes is 97.7%, and the sustained-release is about 35% over 72 h, which presented strong antitumor activity compared to the free form of AT101. The cytotoxicity of the prepared cubosomes was higher toward the two GBM cell lines (A172 and LN229) compared to healthy central nervous system cells, including macroglia (SVGA)HMC3), thus lowering drug side effects and providing additional confirmation of the targeted drug delivery and resulting cytotoxicity. This study concluded that GMO-AT101 cubosomes would be a potentially great alternative in the treatment of GBM[66].

### 8.3 5-Fluorouracil

5-fluorouracil (5-FU) is a water-soluble antimetabolite compound of the pyrimidine analog subclass. It blocks the synthesis of pyrimidine thymidylate (dTMP), an essential intermediate for DNA replication, by inhibiting the enzyme thymidylate synthase (TS). 5-Fluorouracil (5-FU) for solid tumors,especially many cancers in the colon, stomach, pancreas, liver, rectum, or urinary bladder. 5-FU is usually administered by IV infusion. Nasr et al.conducted a study comparing the contrast of 5-FU-loaded cubosomes with an aqueous solution of free 5-FU. In vitro release studies showed that Cubosomes showed relatively slower release (~4.5 h, after an initial burst), the aqueous solution showed very fast release, with the overall process completed within 1 h.delivering 53.6 ± 3.55% of the drug in the first hour. Also, rat liver bio-distribution studies showed that the liver concentration of 5-FU from the cubosomal formulation was nearly five-fold higher than that of 5-FU solution. While more hepatocellular damage was seen at concentrations of 5-FU higher than 5 mg/kg, the authors speculate that cubosomes enhance the potency of 5-FU delivered at lower doses. The half-maximal inhibitory concentrations (IC50) values were calculated to be

112.70 mg/mL for free 5-FU and 107.78 mg/mL for the cubosomal dispersion, which were derived from the in vitro cytotoxicity studies. The negligible difference between the two values shows that the drug's antitumor activity is not compromised upon loading into the cubosomes[67].

### 8.4 Curcumin

Curcumin (diferuloylmethane), an herbal-origin drug mainly extracted from Curcuma longa L. and a polyphenolic constituent, exerts a wide variety of pharmacological activities, among them, the most popular ones are anticancer, anti-diabetic, anti-inflammatory, antioxidant, hepatoprotective, nephroprotective, myocardial infarction-protective, anti-thrombosis, anti-rheumatic, and hypoglycemic activities. The zeta of curcumin- loaded nano-cubosomes was also measured by the SZ-100 nanoparticle analyzer[68]. This yielded a final zeta potential value of —24 mV,which indicated that there was sufficient charge in the formulation to ensure stability by repelling the cubosomes from one another, preventing agglomeration. In addition, in vitro tests were performed using the dialysis method, which showed rapid drug release (~44.2±2.7% released up to 24h) and sustained drug release up to 81.3±2.6% (over 7 days)[69].

Chang et al. performed refraction-free loading of curcumin on MO, MP, and PT cubosomes. By changing the type of lipid they used to form their liposomes, their studies have shown the ability to modulate entrapment efficiency and curcumin localization in the bilayer. Moreover, the PT-cubosomes presented the greatest entrapment efficacy, as a result of the higher hydrophobicity of the curcumin molecule that penetrated deeper in the lipid bilayer, and this has been confirmed by the comparable low maximum fluorescence emission wavelength. Cubosomal formulations demonstrate up to 2.5 and 3.3-fold greater cytotoxicity of curcumin for the B16F1 and NIH3T3 cell lines, respectively, than that of DSPC-liposomes or free ethanol-solubilized curcumin. The synergistic effect of PT and packed curcumin renders the PT-cubosomes the most cytotoxic of all the formulations. Even at lower concentrations, this formulation can trigger apoptosis. In addition, the highest increase in cytotoxicity of MO-cubosomesnormalized for the NIH3T3cell line was observed in the B16F1 cancer cell line, which indicates their potential application in anticancer treatment modality[70].

### 8.5 Icariin

Icariin (ICA), a principal flavanol glycoside extracted from Herba epimedii (Berberidaceae), has recently been reported to be used as an antitumor agent for Ovarian Cancer Cell Lines (SKOV-3 &Caov 3). The pharmacological role of icariin in breast cancer cells includes inhibition of PI3K/AKT and Raf1/ERK1/2 signaling pathways, cell cycle inhibition, induction of apoptosis, and inhibition of autophagy by overexpression of autophagy-related p53[71]. Secondly, it is also able to regulate mitochondrial transmembrane potential and caspase-3 expression, it induces oxidative stress to promote ROS production in ovarian cancer cells, therefore, displaying its cytotoxic effects. Because of its nature as a hydrophobic molecule, the cubosome-loaded ICA allows for higher efficacy and better antitumor activity when regulatedbased on Box-Behnken statistical design. In vitro release studies showed an initial burst release and a progressive release of ICA upto 96.23 ± 3.231% in a 24-h timeframe for ICA-Cubs (Optimized ICA-loaded cubosomes) as compared to slow and incomplete release of ICA upto 67.34 ± 2.424% in the same timeframe for ICA-Raw (free ICA). Moreover, the notable reduction in release rates of ICA-Raw compared to ICA-Cubs further encourages the use of Icariin-loaded cubosomes as an improved approach for the enhancement of anticancer activity[72].

### 8.6 Cisplatin

Cisplatin (cis-diamminedichloroplatinum) belongs to the family of alkylating agents and is a kind of platinum (II) analog. Through producing an extremely reactive species, which cross-links DNA, creating DNA adducts that inhibit DNA repair, ultimately resulting in DNA damage and apoptosis in the cancer cells[63]. Cisplatin is one of the most widely used chemotherapeutic drugs for solid tumors, but it is most commonly used for metastatic testicular and ovarian cancer. Zhang et al. studied numerous in vitro assays for uncoated and poly-ε-coated cisplatin-loaded cubosomes using the Human Hepatoma (HepG2) cell line.

Further characterization was done by studying the zeta potential, cubosomes in vitro release, as well as entrapment efficiency, and cytotoxicity studies. The Zeta potential was recorded at —24.5 ± 0.3 mV, —22.4 ± 0.4 mV, and — 2.8 ± 0.1 mV for uncoated and coated cisplatin cubosomes, respectively. Coating precedes the complexation of the cubosomal surface, and this corroborates the observed reduction in zeta potential values of coated cubosomes. Moreover, in vitro release studies indicated that the uncoated model manifested an initial burst release of 55 ± 3%, a slow-release post 6 h and no release post 10 h, by contrast, the coated model exhibited a very low initial release of 23 ± 3%, a slow but continual release up to around 25 h, similarly, the cytotoxicity studies showed that the free cisplatin was much more toxic to HepG2 cells than cubosome-loaded HepG2 cells. Because of their substantial initial burst release, the uncoated models exhibited lower cell viability and higher cytotoxicity with respect to the coated models[73]. Finally, the discovery that the cell survival against the coated cubosomes was nearly equal to that of the blank cubosomes suggests that the coating was able to prevent the burst release of a significant amount of the drug.

**Table 2:** Anticancer Drugs Loaded onto Cubosomes

| S. No. | Drug | Cell Line | Key Outcomes | References |
|---|---|---|---|---|
| 1 | Doxorubicin | Breast (MCF-7) | Enhanced cytotoxicity and reduced toxicity | [74] |
| 2 | Paclitaxel | Liver (HepG2) | Sustained release and improved efficacy | [75] |
| 3 | Cisplatin | Colon (HCT-116) | Increased cellular uptake | [76] |
| 4 | 5-Fluorouracil | Liver (HepG2) | Improved bioavailability | [77] |
| 5 | Docetaxel | Lung cancer | Enhanced drug penetration | [78] |
| 6 | Etoposide | Breast cancer | Targeted delivery via ligands | [79] |
| 7 | Methotrexate | Leukemia | Reduced systemic toxicity | [80] |
| 8 | Tamoxifen | Breast cancer | Improved targeting efficiency | [81] |
| 9 | Curcumin | Breast (MCF-7) | Enhanced solubility and apoptosis | [82] |
| 10 | Resveratrol | Prostate cancer | Increased bioavailability | [83] |
| 11 | Gemcitabine | Pancreatic cancer | Controlled drug release | [84] |
| 12 | Vincristine | Leukemia | Improved therapeutic index | [85] |
| 13 | Camptothecin | Colon cancer | Sustained drug release | [86] |
| 14 | Topotecan | Ovarian cancer | Enhanced stability | [87] |
| 15 | Irinotecan | Colorectal cancer | Improved pharmacokinetics | [88] |
| 16 | Sorafenib | Liver cancer | Enhanced anticancer activity | [89] |
| 17 | Imatinib | Chronic myeloid leukemia | Targeted delivery | [90] |
| 18 | Erlotinib | Lung cancer | Improved bioavailability | [91] |
| 19 | Gefitinib | NSCLC | Increased cellular uptake | [92] |
| 20 | Combination drugs (e.g., DOX + PTX) | Multiple cancers | Synergistic therapeutic effect | [93] |

## 9. APPLICATIONS IN ANTICANCER THERAPY

### 9.1 Delivery of Chemotherapeutic Agents

Due to their unique bicontinuous cubic structure and high drug-loading capacity, cubosomes have emerged as effective nanocarrier systems for the delivery of chemotherapeutic agents in anticancer therapy. Such lipid-based nanoparticles are capable of encapsulating both hydrophilic and hydrophobic drugs, and the lipid bilayer helps to protect drugs from degradation while increasing bioavailability. Doxorubicin, paclitaxel, and cisplatin are the most commonly used chemotherapeutic agents, and numerous reports have demonstrated the successful encapsulation of these drugs in cubosomes to enhance their therapeutic profile[94]. Cubosomes are nanoscaled particles that enable better passive targeting accumulation in tumors, and their surfaces are suitable for modification to confer active targeting, resulting in the efficient delivery of drugs to tumor cells[95]. This is accompanied by the additional advantage of controlled and sustained drug release from cubosomes to keep therapeutically effective concentrations of the drug at the tumor site over time. This decreases the frequency of the dosing schedule and reduces the systemic toxicity frequently seen with traditional chemotherapy. In conclusion, utilization of chemotherapeutic agents in

cubosomes markedly improves treatment efficiency while reducing undesirable side effects, and they constitute a useful method for targeted cancer therapy.

### 9.2 Delivery of Genetic Material (siRNA, DNA)

Cubosomes represent a potential carrier system for the delivery of genetic molecules, such as small interfering RNA and DNA, and thus can facilitate the development of sophisticated gene-based therapies. Its unique lipid-based bicontinuous cubic structure serves as a protective compartment that protects the nucleic acid payloads from enzymatic degradation in the biological environment, improving stability and circulation time. Cubosomes can also be surface-functionalized with cationic lipids or targeting ligands, which promote binding and uptake of the negatively charged genetic material. After their uptake, these carriers can assist in endosomal escape and the retake of the genetic payload in the cytoplasm, where siRNA can silence a gene, and DNA can express it. All of this renders cubosomes well-suited to target oncogenes, inhibit tumor function, or restore normal cell function. Moreover, their capacity for regulated, sustained release leads to enhanced transfection efficacy with low off-target effects and toxicity[96].

### 9.3 Combination Therapy

Combination therapy using cubosomes is only a representative example, as these nanocarriers are able to co-deliver therapeutic agents to act on multiple targets with synergistic effects. Due to its unique lipidic cubic structure, cubosomes can simultaneously co-encapsulate different classes of drugs, e.g., chemotherapeutic agents like doxorubicin and genetic materials like small interfering RNAto achieve concurrent chemotherapy and gene silencing[97]. By targeting several pathways involved in tumor development, this approach not only addresses one of the main obstacles to cancer treatment, drug resistance, but it also offers a more individualized approach to managing cancer's progression.Moreover, cubosomes can provide controlled and sustained release of each therapeutic component, thus preserving desired drug ratios at tumor sites over time. The surface can additionally be functionalized for specific delivery so that the combination therapy is delivered specifically to cancer and not normal cells. Combining therapy via a cubosome-based platform achieves the three significant goals, such as improving the treatment effect, minimizing the systemic toxicity, and providing a more holistic and tailored strategy for cancer therapies by incorporating various therapeutic modalities into a single delivery system[98].

### 9.4 Overcoming Multidrug Resistance

Multidrug resistance (MDR) remains one of the major obstacles in cancer therapy, and a better ability of drug accumulation intracellularly will greatly benefit in conquering MDR and lead to enhanced anticancer effect. MDR is frequently mediated by the overexpression of efflux transports such as P-glycoprotein, which actively transport chemotherapeutic drugs out of cancer cells, effectively decreasing their efficacy[99]. Cubosomes can overcome these efflux systems, as they would allow the entry of drugs via endocytosis rather than passive diffusion, which in turn would lead to enhanced intracellular retention of anticancer agents. They can also be designed to co-deliver anticancer agents with agents that modulate resistance or small interfering RNA to silence drug-resistance-related genes. In addition, they ensure sustained exposure of the drug inside tumor cells by means of their controlled release properties to help keep the drug concentration above the therapeutic level over a period of time[100]. Additionally, functionalization of the cubosome surface makes it possible to carry out targeted delivery to resistant cancer cells, reducing off-target side effects.

## 10. LIMITATIONS AND CHALLENGES

Cubosomes have a great potential for use in targeted anticancer therapy, yet they possess some drawbacks and limitations, e.g., poor stability, poor scale-up ability, or toxicity. However, stability is of particular concern due to their tendency to aggregate, transition phases, or leak drugs prematurely as they are subject to environmental factors that change temperature, pH, and ionic strength, which may lead to infiltration of the phase and loss of structure and therapeutic activity[101]. As a result, process characteristics cannot be correlated with microstructure and property, and because of the complexity of large-scale manufacture, it would require stringent control of formulation variables such as lipid composition, homogenization pressure, and processing conditions to ensure uniformity and reproducibility. This renders the industrial scale-up expensive and technically challenging. For example, different lipids, surfactants, or surface-modifying agents used in the preparation of liposomes may lead to cytotoxicity or immunogenicity with high potential to cause long-term biocompatibility problems, if their concentrations are not carefully adjusted. At the nanoscale, the behavior of these components is entirely different and should be investigated in vitro and in vivo, even if many of them are considered safe[102]. Consequently, these challenges need to be overcome to make the most of the clinical and commercial success of cubosome-based drug delivery systems in cancer treatment.

## 11. FUTURE PERSPECTIVES

Future perspectives of cubosomes as a targeted drug delivery system are very appealing for anticancer applications and are quite likely to become a reality as nanotechnology and biomedical research continue to advance. Recently developed strategies are emphasising the construction of multifunctional cubosomes satisfying the desired high specificity along with specific ligand-based surface modification for high target efficiency and stimuli-responsive and dynamic structure to the tumor microenvironment for high release efficiency[103]. Prompted delivery of nucleic acids, including but not limited to small interfering RNA, coupled with gene therapy, is anticipated to broaden their therapeutic option beyond classical chemotherapy. Lipid engineering and

formulation innovations are also designed to enhance stability, scalability, and reproducibility for clinical application[104]. Combining imaging agents in a cubosomeis also attracting interest toward theranostic applications, where diagnosis and therapy are integrated into one platform. In addition, personalized medicine strategies could use cubosome systems to deliver drugs according to the biology of each patient[105]. These challenges notwithstanding, ongoing research and breakthroughs in technology may continue placing cubosomes as a potential platform for next-generation anticancer therapy.

## CONCLUSION

As a unique nanocarrier system with a bicontinuous cubic structure, cubosomes have high drug-loading capacity and can be used for the delivery of a large variety of anti-cancer drugs. This improves therapeutic efficacy and minimizes systemic toxicity, providing controlled and sustained drug release and increasing tumor targeting by passive, active, and stimuli-responsive mechanisms. Cubosomes have been successfully shown as a delivery vehicle for a large number of anticancer drugs to enhance bioavailability, cellular uptake, and anticancer activity. However, despite all of these advantages, there remain some challenges that need to be addressed for successful clinical translation to occur, including stability issues, large-scale production, and potential toxicity. This limitation and others are likely to be addressed with continued research and the advances in technology that will broaden the scope of application of cubosomes. In conclusion, cubosomes are a promising passive and active drug delivery platform that can help develop more effective cancer treatments in the future.

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