iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 13 **Issue:** XI **Month of publication:** November 2025

DOI: https://doi.org/10.22214/ijraset.2025.75772

www.ijraset.com

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue XI Nov 2025- Available at www.ijraset.com

Cubosomes: A New Approach in Drug Delivery

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Abstract: ***Cubosomes are bi-continuous cubic liquid crystals which can encapsulate hydrophilic, lipophilic and amphiphilic drugs. Cubosomes have emerged as unique drug targeting systems mainly due to their excellence in delivery of poorly aqueous soluble drugs. This review emphasizes on major applications and future prospectives of cubosomes as a novel drug delivery systems. The article leaves a note on methods of preparation of cubosomes with an added information about the mode of drug release from cubosomes which is diffusion. The major theories proposing the cubic nature of these cubosomes has also been discussed. Cubosomes, thus are a choice of delivery systems of drugs to lengthen the duration of action and decreasing toxicity by targeting.***

Keywords: Cubosomes, Bi-continuous liqud crystals, Controlled drug delivery, Cancertherapy, Transfection.

I. INTRODUCTION

Drug delivery systems are designed to release a medication directly at its intended site of action. Controlled drug delivery systems provide a steady release of the drug over a specified period, helping prevent drug build-up in the body and reducing potential toxic effects. These are now being regarded as Novel Drug Delivery Systems (NDDS). Due to several cons of traditional delivery systems, in the modern- day approach NDDS are popularly used. Reduced dosage frequency, improved site-specificity, less harmful side effects, resistance to degradation, particularly in the acidic stomach environment, and greater bioavailability are some of their observable advantages. Results indicate that NDDS is a viable strategy to address serious illnesses. [1]

Drug administration increases a medication's effectiveness. However, the rate of active release, vehicle stability, and ease of preparation must all be managed in order to create ideal drug release profiles. An ideal delivery vehicle must successfully blend each of these qualities. [2] They create supra-assemblies, which are often used as effective delivery modules. Active components like liquid-crystalline aggregates or cross-linked gel networks are loaded, stabilized, and subsequently dispersed. Drug release rates affect the therapeutic effects of all drug delivery methods, including liposomes. Among these delivery systems cubosomes have emerged as a distinctive class of lipid based nanocarriers characterized by their unique bio-continuous cubic liquid crystalline structure. These self-assembled structures form spontaneously when specific amphiphilic molecules are dispersed in a aqueous environment under appropriate conditions.[3]

II. DIFFERENCE BETWEEN CUBOSOMES AND LIPOSOMES

TABLE 1

CHARECTERISTICS	CUBOSOMES	LIPOSOMES
Structure	A biologically compatible continuous cubic liquid crystalline phase featuring a honeycomb-like structural pattern.	Vascular bilayer structure
Stability	Highly stable, non-toxic and biodegradable. More resistant to breakage than liposomes.	Less stable, more prone to degradation and drug leakage specially understress.

Drug Loading	Capable of encapsulating hydrophilic, hydrophobic and amphiphilic drugs simultaneously Has a higher loading capacity for hydrophobic drugs.	Primarily designed for encapsulating either hydrophilic (liposomes) or hydrophobic (solid lipid nanoparticles) drugs.
Release profile	Offers both targetted and sustained drug release, controlled by the tortuous diffusion pathway through the cubic network. [4]	Varies widely; some systems offers sustained release but others have rapid release.
Administration	Applicable via multiple routes- oral, parentral, transdermal and mucosal routes.	Varies depending on the system; parentral and oral are common. But other routes may face more limitations.
Viscosity	Low viscous compared to the bulk cubic phase, making them easier to handle and formulate.	Varies depending on the formulation; liposomes have lower viscosity compared to cubosomes.

III. TYPES OF CUBOSOMES

Based on their morphological structure, they can be either primitive or diamond. [Fig. 1] Upon hydration with water, these lipids spontaneously form cubic liquid crystalline phases arranged in primitive or double diamond assemblies with the phase transition between 43°C and 80°C.

1) Primitive: Lipids self-assemble into a three-dimensional, bio-continuous cubic liquid crystalline structure to form a sort of cubosome.

2) Diamond:A type of nanostructure made from lipid molecules that self-assemble into a three-dimensional, bio-continuous cubic phase with a "diamond" or Pn3m crystallographic symmetry.

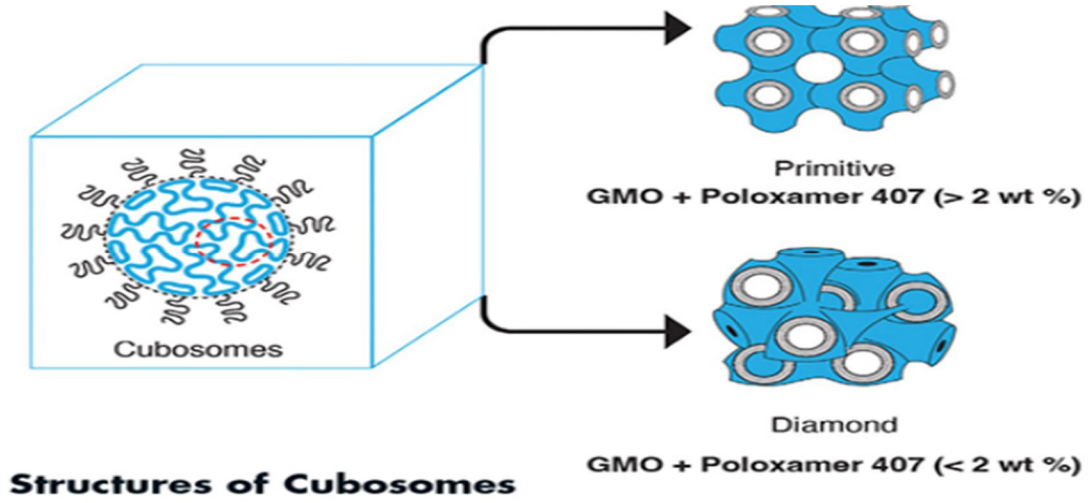

Fig1: Types of cubosomes

IV. STRUCTURE AND COMPONENTS OF CUBOSOMES

Cubosomes are bio-continuous cubic-phase liquid crystals and self-assembling liquid-crystalline particles with solid-like rheology. The cubic phases have extremely high viscosity because to their intriguing bio-continuous topologies.

At the micron scale, coarse cubosomes exhibit the same Pn3m/QIID (Diamond or D-surface) structure as their corresponding bulk cubic phase. However, after homogenization, the Im3m/QIIP (Primitive, Schwarz, or P-surface) structure becomes predominant, likely because of added polymer or other influencing factors. [5]

The structure aids in preserving the stability and effectiveness of active components like proteins and vitamins. Cubosomes have an endless lifespan and are thermodynamically stable. Cubosome colloidal dispersions can be stabilized by adding polymers.

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue XI Nov 2025- Available at www.ijraset.com

A. Amphiphilic Lipids

GMO (Rylo MG 19 or glycerol monooleate/monoolein) and phytantriol (PHYT) are currently the most widely used amphiphilic lipids for producing cubosomes. In the presence of excess water, these lipids typically form a Pn3m/QIID (Diamond or D-surface) structure, with GMO doing so from room temperature up to about 43 °C, and PHYT above roughly 80 °C. They are biocompatible, exhibit well-characterized bulk phase behaviour, and have recently been approved for in vivo applications. [6]

B. Stabilizers

When cubosomes are dispersed in water, their hydrophobic regions become exposed to the surrounding aqueous environment, leading the particles to clump together and lose stability. To maintain their colloidal stability and prevent them from reverting back to the bulk cubic phase, a surfactant is required. This stabilizing agent forms a protective barrier around the particles, reducing their tendency to interact closely. Pluronics are the most commonly used stabilizers for this purpose.

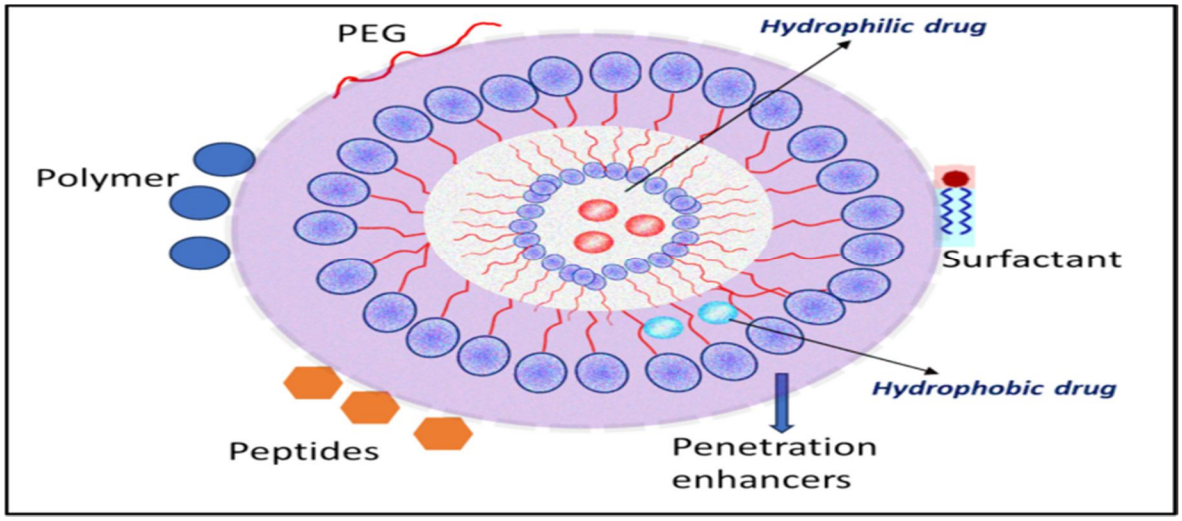

Fig 2: Structure of cubosomes

V. THEORIES ON CUBIC NATURE OF CUBOSOMES

There are four theories proposed on cubic phase structure of cubosomes:

A. Fontell and Drew theory

Cubic phases can occur in ternary mixtures containing an amphiphile, oil, and water, including systems made with different monoglycerides. Monoglycerides are polar lipids with limited solubility in water and display aqueous-phase behaviour similar to that of non-ionic surfactants. According to Lutton's findings, monoglycerides with hydrocarbon chains ranging from C-12 to C-22, especially monoolein,tend to occupy a larger cubic phase region. Monoolein, a C-18 monoglyceride, is an unsaturated fatty acid derivative. [7]

B. Gustafson et al. Theory

Cubosomes are essentially single-crystal structures containing dispersed lamellar liquid-crystalline particles, within which unilamellar vesicles can be observed. Increasing the proportion of polymer relative to monoolein promotes the development of larger vesicular structures. [7]

C. Schwart, Jacoband Anderson theory

In non-ionic surfactant systems, cubic forms are seen between lamellar and hexagonal liquid-crystalline phases. The monoolein-water system is unique because of its cubic phase region, which has a wide range of content and temperature.

Conversely, concepts for surfactant packing are becoming closer. Monoolein typically exhibitscubic phases that are reversed or inverted, signifying polar medium phases, because of to its lipophobic head and hydrophobic tail. Therefore, minimum surfaces and differential geometry can be applied todepict cubic phase structures.

D. System forming theory

Cubosomes can be generated in binary and ternary systems when there exists a notable miscibility difference between the cubic phase and the solvent. Lamellar bilayer caps can provide colloidal stability by isolating hydrocarbon chains from water and protecting the cubic bilayer openings resulting from fragmentation.

VI. CHARACTERIZATION OF CUBOSOMES

A. Particle Size Determination

The particle size of any nanocarriers was proven toeffect its function in vaccine and chemotherapy.The particle size range of cubosomes typically should vary in between 10-500n

The distribution of sizes of cubosomes was measured using a Zetasizer.

Example:The average particle size of capsaicin loaded cubosomes was 251nm.[8]

B. Encapsulation Efficiency

Encapsulation efficiency is the amount of material successfully encapsulated upon the total amount of material added initially,expressed in percentage. Encapsulation efficiency (EE) was evaluated using a membrane dialysis method.[9]

The EE was calculated by the following formula:

$$EE\%=(C_{\text{total con}}-C_{\text{free con}})/C_{\text{total con}}\times 100\%$$

From the above formula the Encapsulation Efficiency(EE) of capsaicin loaded cubosomes was found to be 97.14%.[5]

Such high EE could be attributed to the unique cubic nature and the property of capsaicin.

C. Invitro Drug Release

It is a laboratory test that measures the rate and extent at which a drug is released from its pharmaceutical formulation.

Example: The release of capsaicin from cubosomes in aqueous media at 37°C was calculated with use of dialysis. The cubosomal sample (5 ml) was transferred into a dialysis bag, kept into a flask and filled with 250 ml of water. Flask was later placed in a rotary incubator shaker at 50 rpm and 37°C. An aliquot of 5 ml was taken periodically and replaced with the same volume of water.The capsaicin release profile from cubosomes was about 41%.[5]

Therefore cubosomes provide a sustained release system for capsaicin.

D. Zeta Potential

It is the measure of the electric potential at the slipping plane of a colloidal particle,which indicated the magnitude of the electrostatic forces between particles.

In general zeta potential of cubosomes is typically negative ranging from -19mV to -49Mv.

TRANSMISSION ELECTRON MICROSCOPY:

TEM images of capsaicin-loaded cubosomes, demonstrated that the cubosomes are of irregular hexagonal shape were well dispersed as individual particles.[5]

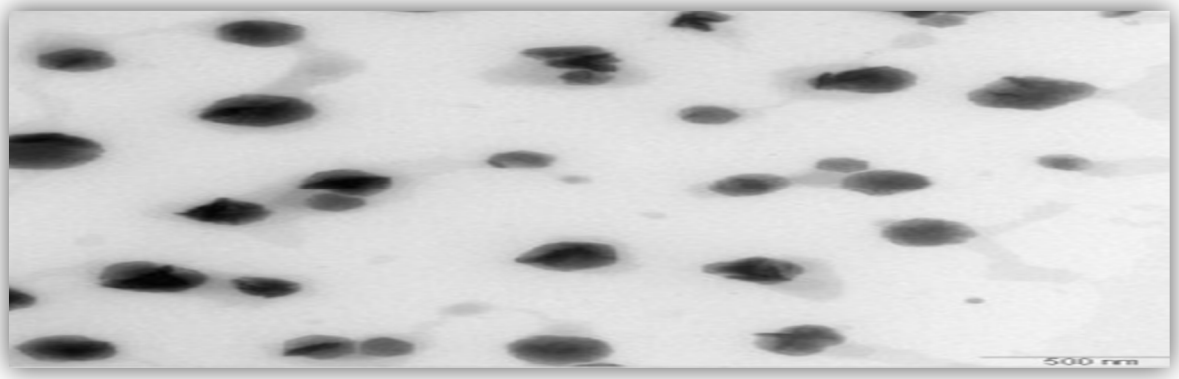

Fig 3: TEM of cubosomes

VII. ADVANTAGES OF CUBOSOMES

A. Drug Delivery and loading

1) High drug loading: The wide internal surface area of cubosomes facilitates high capacity to load drugs.
2) Versatile encapsulation: They can encapsulate lipophobic, hydrophobic and amphiphilic drugs.
3) Controlled release: The cubic nature provides controlled release of drugs for prolonged period of time.
4) Protection: They offer protection to drugs from enzymatic degradation.

B. Physico-chemical Properties

1) Stability: Cubosomes are thermodynamically stable making the drug delivery system physically stable and long lasting.
2) High surface area: The high internal surface area of cubosomes offers benefit for better interaction with biological membranes.

C. Safety and compatibility

1) Bio-compatibility: Cubosomes are prepared from bio-compatible lipids. Thus, can be used for medical purpose.
2) Low toxicity: Cubosomes are relatively less stable.

D. Other advantages

1) Ease of preparation
2) Excellent solubilization
3) Better functionalization for tissue targeting

VIII. DISADVANTAGES OF CUBOSOMES

1) Production challenges: The high viscosity of cubosomes can make large scale manufacturing difficult.
2) Stability issues: Cubosomes can lead to drug leakage during manufacture, storage and *in vivo* drug delivery.
3) Low advantage of hydrophilic drugs: Due to high aqueous content entrapment of hydrophilic drugs can pose problems.
4) Limited control over drug delivery: Studies suggested that cubosomes may not offer regulated drug delivery for polymer-based drugs.
5) Susceptibility to environmental conditions: Any changes in environmental conditions can lead to phase transition of cubosomes.
6) There is always a chance of growth of particle, in case if they were kept untouched for prolonged period of time.
7) The channels of cubosomes may be difficult for bigger medications to penetrate, and there is a chance that pharmaceuticals will damage the bio-continuous liquid crystalline phases lattice structure.

IX. MECHANISM OF DRUG RELEASE

Drug diffusion is the prime mode of drug release. The driving force for diffusion is the concentration diffusion across cubosomes. Consequently, the Higuchi or Fick's diffusion equation and the rate of release of drug from cubosomes are typically coincidental. The various factors which effect drug release are solubility of drug, partition coefficient, diffusion coefficient, geometry of cubosomes, pore size, temperature and release medium's ionic concentration.

Diffusion was confirmed as the mode of drug release based on investigations performed on release of some hydrophilic drugs from cubic and reverse hexagonal liquid crystalline.

Example: release profile of 14C-Glucose from cubosomes. [10]

However, because of its affinity for the lipophilic part of cubic phase, the hydrophobic medication find it difficult to exit from the cubosomes *in vitro*. Therefore, the release rates of lipophilic drug loaded cubosomes in digestive media [0.1M HCL] and aqueous media [pH 6.5] were examined, and later found that there is increase of drug release rate in digestive media.

Example: In investigations, there is an increased rate of relase of drug from cubosomesof Silymarin than capsule formulation of Legalon. [11]

X. PREPARATION METHODS

Cubosome preparation can be achieved by two approaches: top-down [TD] and bottom-up[BU] techniques.

A. Top-down Approach

This method consists of two steps, is the widely used method for preparation cubosomes. Using high-energy techniques like high-pressure homogenization or sonication, the primery step involves preparing a viscous bulk cubic phase by adding lipid phase to a stabilizer to avoid formation of aglomerates.

The second step is to scatter the previous processes resulting in a hydrophilic solution ultimately producing cubosomes. The TD method was shown in Fig. 4. The TD method was 1st reported by Ljusberg-Wahren. It is widely used because of its rapid method which forms cubosomes with a size below 200 nm and low polydispersity. However it also have cons like small scale feasibility only.

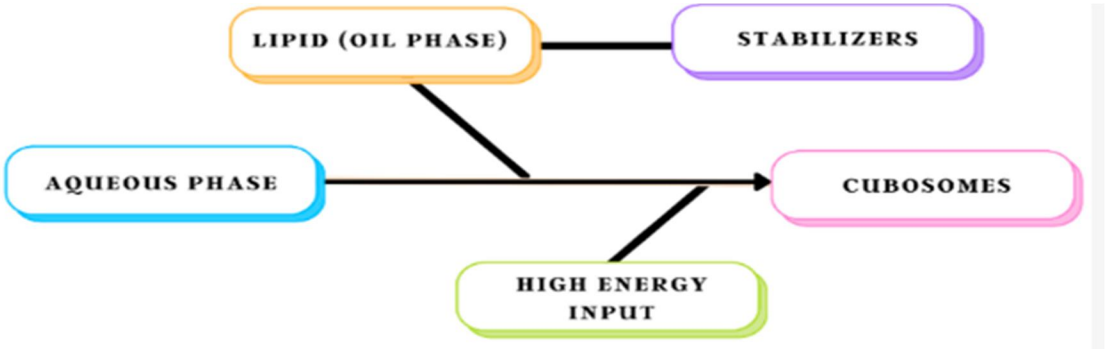

Fig 4: Top-down method

B. Bottom-up approach

A single-phase solution is diluted into a two-phase system made up of cubosomes and an aqueous phase in the BU method, have another name as the solvent dilution, liquid precursor, or hydrotope method. When too much water is added, lipid droplet nucleation takes place, the mixed phase separates on its own, and cubosomes precipitate. The primary benefit is that low energy is needed for the preparation of the dispersions, which enables large-scale preparation and is especially favoured when delicate substances are incorporated as shown in Fig. 5.The primary issues with the BU approach, however, are the challenges of regulating particle size and the presence of solvent in the final sample, which is incompatible with solvent-sensitive substances in the cubosomes, such proteins, and may not be suitable for use in medical applications. This is a recent method introduced by Spicer and coworkers. [13]

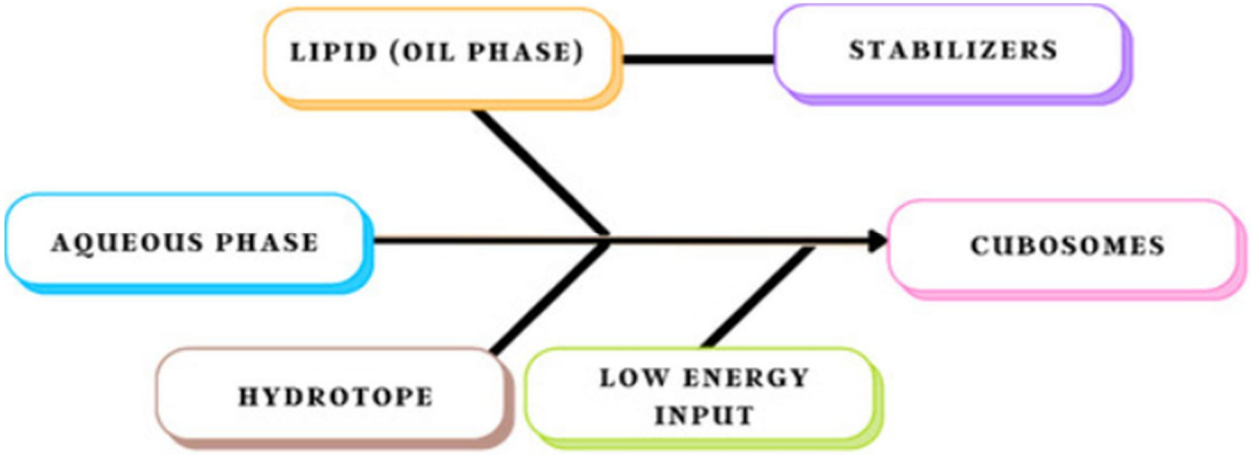

Fig 5: Bottom-up method

C. Heat treatment

Muir et al. discovered that cubosome formation can occur when a bufferd saline solution of phosphate (PBS) is added to a biphasic lipid system that contains a lipid possessing charge, dodecyl dimethylammonium bromide (DDAB). The bilayer system is altered and the bio-continuous cubic phase is restored when phosphate buffer saline is added into the DDAB, creating a charge-shield on its surface. Since heat treatment only makes the transition from non-cubic vesicles to well-ordered cubic particles easier, it is not, strictly speaking, an integrated procedure for the creation of cubosomes. [13] According to the research, this method reduces the lower size of particles associated with vesicles, generating bigger cubic phases with good colloidal stability and limited particle dispersion.

It was obvious that the changeover takes place all over the heat-treatment process. The transition may result from a reduction in solubility and stability brought on by high temperatures.

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue XI Nov 2025- Available at www.ijraset.com

D. Spray Drying

A carefully designed nozzle sprays both phases, producing suspension of droplets that hit a hot, dry airflow. The excess water rapidly evaporates, leaving a dry residue of powder particles encased in a polymer that was previously dissolved. Spray-drying is increasingly widely used in consumer products like meals and detergents since it is simple to scale up. Additionally, preloading actives into cubosomes prior to drying is made easy by the technique. Lastly, the surface charecteristics of hydrated cubosomes are determined by coating polymer on powder and can be altered by selecting the ideal encapsulating polymer as depicted in Fig. 6

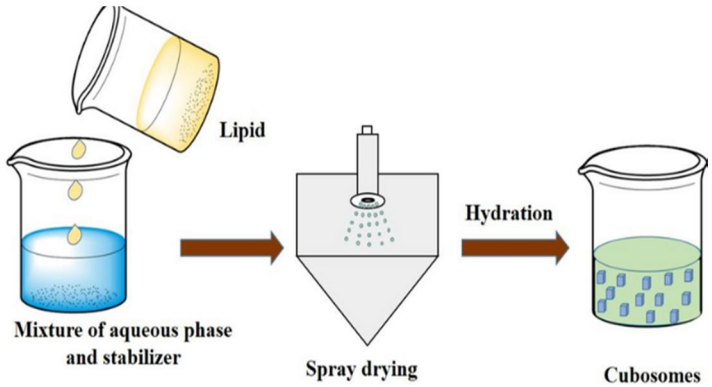

Fig 6: Spray drying

XI. APPLICATIONS

Cubosomes are bio-continuous cubic phased crystals of liquid having a numerous-properties that offer a possible chance to use them as carriers for wide range of pharamaceutical agents. For the cubosomes to functioneffectively as drug transport vehicles, sufficient amounts of small-molecule medications must be loaded.

Numerous studies have employed cubosomes to encapsulate antitumor drugs, with efficiency of encapsulation from 71 to 103%. Consequently, these studies demonstrate cubosomes potential as a drug delivery system, mainly for anticancer drugs. [14]

The pharma manufacturers have been continuously working to apply cubosomes as stabilizers and pollutant adsorbing agents in cosmetics. Recently in a study it was known that formulation of cubosomes in Alpha-Lipoic Acid in cubosomes proved excellent positives on reduction of fine lines on face with complete resolution and enhanced skin texture at upper lip area in volunteers.

The bio-continuous structures formed in sin layers of human beings are similar to those observed in cubosomes, according to recent research, which gives a future in improving our knowledge of and ability to treat skin transport. Commercial treatments for periodontal disease have been produced using combinations of cubosome-based triglyceride–monoolein and the antibiotic metronidazole.

Cubosomes are thought as a great option for oral medication delivery. They improve the oral efficacy of poorly watersoluble drugs. Cubosome formulations containing the protein ovalbumin were created with a high entrapment efficiency and slow-release behaviour *in vitro*, demonstrating cubosomes promise as a cutting-edge vaccine delivery method. Furthermore, the encapsulated medication is shielded from gastrointestinal system breakdown by the distinctive crystalline structure of cubosomes.

Cubosomes facilitate the drug's easy penetration of the skin's and mucosal epidermis, improving the drug's bioavailability. Studies showed that the apparent permeability and bioavailability of dexamethasone and flurbiprofen, which are utilized as carriers for eye therapy, were significantly enhanced by cubosomes. [15], [16]

Cubosomes have become a great drug delivery technology because of their unique solubilization, very effective encapsulation, prolonged release behavior, and in vivo stability. In comparison to the traditional somatostatin solution, it was discovered that the half-life of somatostatin cubosomes injected intravenously in rats was enhanced.

Cubosomes give a promising transdermal drug delivery method due of their special structure. Microneedles and cubosomes are combined to administer vaccines through the skin. The use of microneedles improved the aqueous peptide mixture's penetration into the skin's layers, and cubosomes demonstrated a longer peptide retention period. The special qualities of liquid crystals provide the foundation for cubosome topical administration. The liquid crystal systems' bio-adhesive properties make it easier for medications to reach mucosal surfaces like the vagina, buccal, and ocular. [5], [17]

A. *Cancer therapy*

Cubosomes are used to deliver anticancer medications in several investigations, with encapsulation efficiencies ranging from 71 to 103%. [18]

Doxorubicin, cisplatin, paclitaxel, curcumin, and quercetin are a few examples of molecules that have been successfully encapsulated in cubosomes, either alone or in some kind of simultaneous encapsulation. [19]

Zhai et al. studied the efficacy of monoolein cubosomes loaded with paclitaxel in the treatment of ovarian cancer. [20]

An innovative work studied the use of cubosomes as simultaneous carriers of doxorubicin, a chemotherapeutic, and Lutetium-177, a radionuclide, thus allowing for a combined chemo- and radiotherapy.

TABLE 2
LIST OF ANTICANCER DRUGS DELIVERED USING CUBOSOMES

ACTIVE INGREDIENT	APPLICATION
Campothecan	Theranostic and bioimaging
Doxirubicin[DOX]	Tumour targeted delivery
Paclitaxel [PTX]	Drug delivery
Dacarbazine	1stline chemotherapy medication against melanoma
5-Fluoro uracil[5-FU]	Treatment of GIT cancers and hepatocellular tumour
Folic acid modifies etoposide cubosomes	Breast cancer
Cisplatin and paclitaxel loaded cubosomes	Liver cancer
Cisplatin and metformin nano-cubosomes	Colo-rectal cancer

B. *Transfection*

Nucleic acids are used in gene therapy to modify the genetic makeup of certain cells. For instance, it can be used to cure monogenic illnesses by replacing a mutant or absent gene with a normal allele. [21]

Sarkar et al. provided an example of a successful cubosome transfection. Different combinations of monoolein doped with cationic lipids were employed in the synthesis of cubosomes loaded with antisense green fluorescent protein (GFP). Cubosomes were applied to Chinese hamster ovary cells that expressed GFP. The findings enabled us to draw the conclusion that the knockdown efficiency is dependent on the cationic lipid used and that some of the combinations show a gene knockdown efficiency comparable to lipofectamine (a commercially available liposomal-based formulation) for up to 48 hours, with a higher efficiency after 72 hours.

C. *Other applications*

TABLE 3
DRUGS DELIVERED USING CUBOSOMES

DRUG NAME	APPLICATION
Dexamethasone	Treatment of anterior eye inflammation
Pilocarpine	Treatment of closed-angle glaucoma
Colchicine	Gout management
Clotrimazole	Treatment of fungal infections
Norfloxacin	Otitis
Ketoconazole	Hydrogel for fungal infections
Diclofenac sodium	Percutaneous inflammation

XII. FUTURE PROSPECTIVES

Topical drug delivery systems (TDDS) are beingmostly used for treating and managing various skin and ocular diseases. More than 90% of the ophthalmic products available in the global market today fall within the topical drug delivery category. The WHO reports that approximately 76 million people are affected by glaucoma, 1.5 million by fungal keratitis, and nearly 40% of people suffer from allergic conjunctivitis Conventional TDDS includes micronized therapeutic embedded creams, gels, ointments, pastes, suspensions, emulsions, powders, and lotions.

The management of skin and ocular disease conditions using conventional topical drug delivery is associated with certain challenges, including retention, irritation, allergic reactions, drug-induced hypersensitivity, high drug doses, frequent dosing, reduced penetration, diminished therapeutic efficacy, and increased metabolism with regionally distributed CYP450 enzymes when applied topically. Cubosomes have shown prominent results in achieving wide applications for topical drug delivery. Cubosomes are liquid crystalline structures that are well retained in the skin and eye regions, providing a suitable environment for loading hydrophobic, hydrophilic, or both types of drugs, exhibiting bio adhesion, and sustaining drug release to achieve a maximum therapeutic effect in topical treatments.

Multidrug resistance (MDR) poses a significant challenge in cancer treatment by reducing the efficacy of therapies.There is a high chance for rapid blooming of cubosomes as a drug delivery for targeting cancers.

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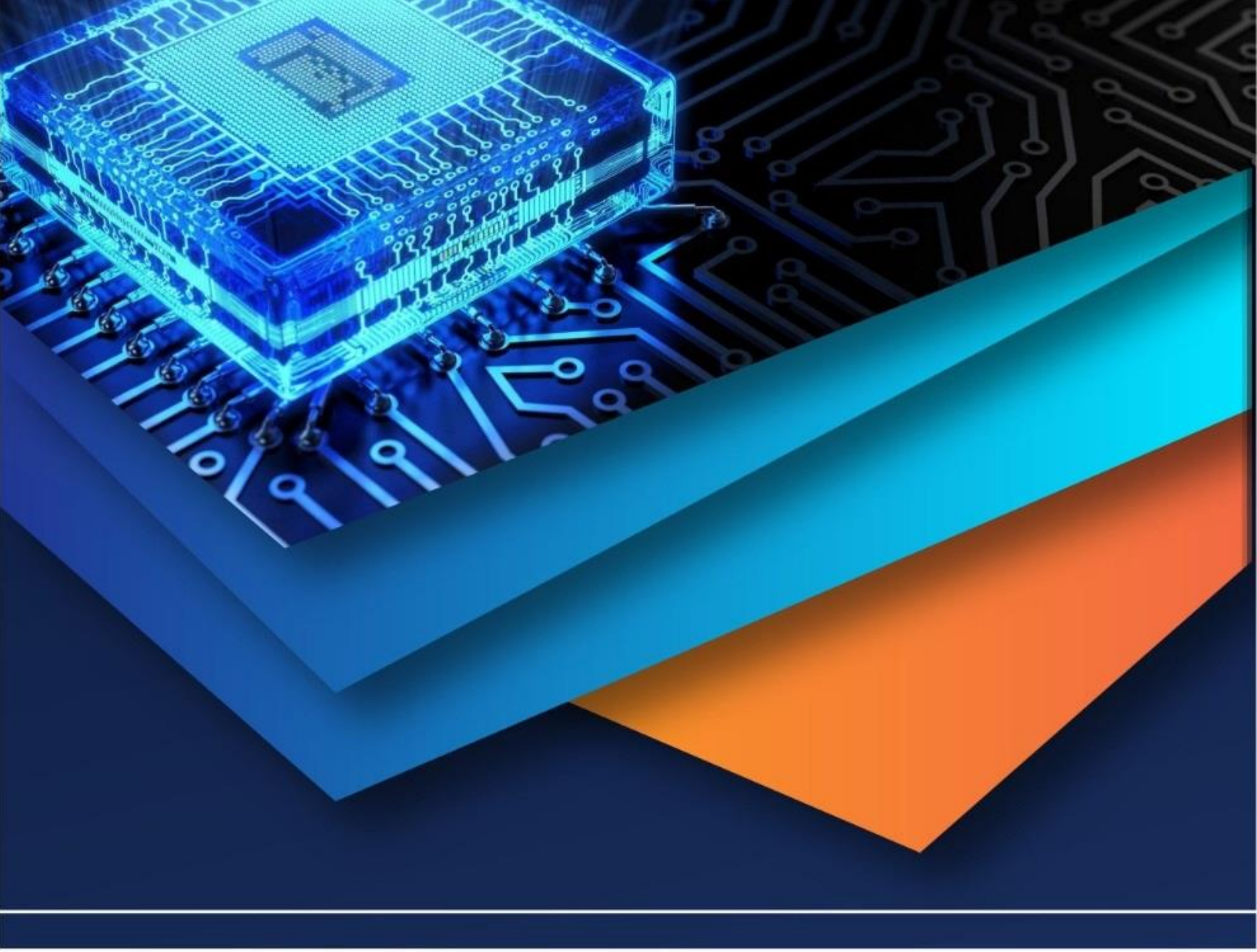

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IMPACT FACTOR: 7.429

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