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Cubosome: A Potential Nanocarrier in Cancer Therapy

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Abstract
Cubosomes, a specific type of lyotropic nonlamellar liquid crystalline nanoparticle (LCN), has emerged as a highly promising drug delivery system, particularly in the field of cancer treatment. These versatile nanoparticles can be administered through various routes, including oral, topical, and intravenous delivery, making them suitable for a wide range of theranostic applications. Extensive research efforts have been dedicated to enhancing the efficiency of cubosomes, optimizing their manufacturing techniques, characterizing their unique properties, improving their target selectivity, and precisely controlling the release of anticancer drugs encapsulated within them. Despite these significant advancements, the clinical translation of cubosomes has been relatively slow, and further robust evidence is required to establish their efficacy and safety in real-world clinical settings. Continued research and comprehensive validation studies are essential for realizing the full potential of cubosomes as a breakthrough drug delivery system for improved cancer treatment outcomes. This comprehensive review provides an overview of the advancements in cubosome development and their multifunctional applications in cancer treatment, focusing on the latest research findings and reports.

Keywords: LCN, theranostics, cancer.

1. Introduction

Cancer encompasses a range of diseases distinguished by irregular gene function and changes in gene expression patterns. It occurs when the body's cells undergo uncontrolled growth and invade nearby tissues. In the year 2020, it was projected that there would be approximately 19 million newly diagnosed cancer cases and approximately 10 million deaths related to cancer globally [1]. Aside from health problems, cancer is also seen as an economic problem, even in industrialized nations. The total cost of Medicare in the United States is projected to rise from 3.6 trillion in 2018 to 6 trillion in 2027 [2]. Cancer can be treated in many different ways, including surgically removing the tumour or using medicines including chemotherapy, immunotherapy, radiation, hormone therapy, targeted therapies, and stem cell transplant. Each technique available for cancer patient therapy has specific and universal benefits and downsides [3]. These benefits and drawbacks are always assessed individually, which is why treatment plans usually combine several therapeutic modalities to provide cancer patients with a

wider range of effective therapies [4]. Humans have a high incidence of solid-tumor-related cancer, necessitating invasive cancer treatments including chemotherapy and surgery to remove tumours if possible, followed by chemotherapy and radiation to eradicate any remaining tumour cells [5]. Chemotherapy has been a key component of solid cancer therapies for many years, and its use has increased significantly in recent years as a result of the benefits of adjuvant chemotherapy. After having chemotherapy for solid tumours, patients with excellent performance status have a higher probability of surviving and a better quality of life. Patients with poor performance status, on the other hand, had a higher likelihood of experiencing symptomatic improvement after chemotherapy [6]. Since the introduction of the first chemotherapy agent, methotrexate, in 1956, chemotherapy has been used successfully to treat numerous cancer patients. Despite being a crucial component of cancer treatment, chemotherapy confronts several difficulties. First of all, it affects each person differently and might not even work as well for some. Second, rather than efficiently treating the patient, the severe side effects may worsen their quality of life. Last but not least, due to their scarcity and high price, many drugs are out of the reach of the majority of people[7]. There are several ways to increase the effectiveness of chemotherapy and reduce its negative effects, including the development of novel medicines. Nevertheless, the most recent study estimates that it would cost USD 2.6 billion to create a new drug that is authorized for commercialization [8] . Innovative drug delivery techniques can reliably administer chemotherapeutic drugs with minimal side effects, reducing drawbacks such as side effects and costs by ensuring precision, focused administration, and low-dose loading. and needs for drug delivery.

One of the major issues and requirements for medication delivery is selective targeting of the illness location inside the body. and needs for drug delivery [9]. Chemical agents are often equally disseminated throughout the body, allowing the medications to reach every organ system component. Chemotherapeutic medicines, in contrast, are intended to just target the tumour site, or the bulk of the chemicals are thought to reach the target location. This is because of the unneeded adverse effects that chemotherapeutic medications may have on those healthy bodily parts. In light of the fact that a targeted drug delivery system contains two components, it must fulfill two functional requirements. and needs for drug delivery [10].

The main role of carrier is to locate the target, which allows the medications it contains to work more effectively[11]. These drug delivery methods are frequently used with good results, and they use special particles classified as nanoparticles. The majority of the time, materials having a particle size between 1-100 nm are referred to as nanoparticles [12]. Nanoparticles have become increasingly essential in various scientific fields, including drug delivery, diagnostics, bioengineering, and sensors, due to their diverse nanophases and nanostructures [13]. They serve as a vital link between the physical and biological sciences, particularly in the development of pharmaceuticals and biomedicine.

The success of therapy relies on the bioavailability and steady-state concentration of nanoparticles in the bloodstream, with the surface properties playing a crucial role. Modifying nanoparticles to increase their hydrophilicity allows them to remain in the bloodstream for longer durations, leading to higher drug concentrations at the tumor site and an extended overall half-life [14]. Polyethylene glycol (PEG) is commonly used to enhance hydrophilicity. Surface modifications offer advantages such as evading recognition by opsonic proteins in the bloodstream, preventing opsonization and phagocytosis

[15]. This reduces nanoparticle toxicity and enhances therapeutic potential, even at lower doses. For example, doxorubicin-loaded PEGylated liposomes exhibit considerably lower cardiotoxicity compared to free doxorubicin [16].When selecting nanoparticle drug delivery materials, factors such as pathophysiology, tumor features, and size are considered. The precise form, size, and surface characteristics of nanoparticles are crucial for therapeutic efficacy [17]. Nanoparticles smaller than 10 nm are generally not recommended in cancer treatment due to increased renal filtration and potential toxicity. Particle sizes between 10 nm and 100 nm are preferable as they remain in the circulatory system for longer periods and release therapeutic agents at the target site without significant systemic effects [18].

Various types of nanoparticles, including organic, inorganic, and hybrid nanoparticles, have been developed and employed. Organic nanoparticles like lipoproteins, polymeric nanoparticles, and polymeric micelles are commonly used [19]. Liposome-based nanoparticles are effective in treating breast and prostate cancer due to their ability to selectively interact with cancer cells without harming healthy cells. Polymer-based nanoparticles, such as polylactic-co-glycolic acid, offer improved biodegradability and compatibility as drug carriers. Polymeric micelles enhance the absorption and efficient delivery of insoluble anticancer agents to the target site [20]. Overall, nanoparticles have revolutionized drug delivery by providing precise control over physical properties and enabling targeted and efficient treatment approaches in cancer and other diseases..Inorganic nanoparticles, such as gold, carbon nanotubes, magnetic nanoparticles, and silica nanoparticles, have been the subject of several studies. They offer a number of advantages over organic materials, such as greater drug accumulation in tumours, which is supplied by gold, and the encapsulation of the greatest amount of anticancer drugs in silica [21]. Finally, a number of hybrid nanoparticles have shown significant anticancer action, including liposome-silica hybrids, chitosan-carbon hybrid nanotubes, cell membrane-coated nanoparticles, and lipid-polymer hybrid nanoparticles [22].

Cubosomes, a bicontinuous cubic phase liquid crystals having a number of intrinsic properties that make them a potentially versatile vehicle for the delivery of different therapeutic actives [23]. These nanoparticles, like traditional controlled drug delivery systems, utilise surfactant and polymer systems to create supra-assemblies, which are frequently utilized as active transport vesicles. The bicontinuous lipid and water zones of these surfactants' assembled bilayers are distorted into 3-dimensional, minimum surface-forming, periodic, densely packed structures, which further resemble a "honeycomb" structure. The cubosomes are often created by laborious, time-consuming procedures that need a lot of energy input. They are first made by breaking up the cubic lipid-water phase into a three-phase area that includes a liposomal dispersion. Although these particles vary structurally from liposomes and may hold amphiphilic, lipid-soluble, and water-soluble actives, they have been given the term cubosomes [24]. Three cubosome structures—Im3m/QII P (Primitive, Schwarz, or P-surface), Pn3m/QII D (Diamond or D-surface), and Ia3d/QII G (Gyroid or G-surface)—have been recognized as canonical based on changes in nodal surfaces [25]. Additionally, the cubosomes produced in aqueous surfactant systems at relatively higher amphiphile concentrations often exhibit a sufficient level of molecular orientation to be described by structural symmetry [24]. The production of cubosomes is based on the idea that when loaded with proteins, stabilizers, and the desired chemical or medicament, the lipid mixture self-assembles to characterize as a lipid bicontinuous cubic phase. A variety of mixes may be employed to create a variety

of dispersed mesophases, such as micellar cubic, hexagonal, and sponge phases, by making additional adjustments to the combinatorial systems and using different other lipids [26]. The most commonly sought lipids used in the formation of cubosomes are Phytantriol and Monoolein (Rylo MG 19 or Glycerol Mono-oleate (GMO)), which under excess water conditions exhibit a Pn3m/QII D (Diamond or D-surface) morphology in temperatures ranging from room temperature to 43 ◦C and to above 80 ◦C respectively. Additionally, these lipids are biocompatible, well-characterized in the bulk phase, and have recently been approved for in vivo study [27].

Three macroscopic states of the cubic phase—the precursor, bulk gel, and particle dispersion phases—are often seen during the manufacture of cubosomes [24]. Typically, the precursor state is a solid or liquid substance that, in response to an external stimulus, such as coming into contact with another liquid, transforms into the cubic phase. Although it resembles a solid, the bulk gel cubic phase is rigid, optically isotropic, and in equilibrium with water. It may also be further distributed into cubosomes. Additionally, the top-down and bottom-up techniques to cubosome preparation may be generally categorised based on the type of energy sources used in breaking the bulk phases. Top-down procedures encompass the use of sonication and high-pressure homogenization in contrast to bottom-up approaches, which strive to decrease energy inputs by using hydrotropes. Both strategies use the same stabilising agent, Poloxamer-407 (P407 or Pluronic F127 or PF127), which is a triblock polymer made of polyethylene oxide, polypropylene oxide, and polyethylene oxide (PEO-PPO-PEO) copolymer. It helps stabiliselyotropicnonlamellar liquid-crystalline nanoparticles (LCNs) by maintaining the nanoparticles' liquid-crystalline internal structure and by creating a stable network [28]. Additionally, the hydrophilic properties of the polymer are attributed to its PEO component, whilst its hydrophobic properties are explained by its PPO component. The hydrophobic PPO block's ability to adsorb or be functionalized on the particle surface is explained by P407's stabilising actions. In addition, the hydrophilic PEO segment extends into the surrounding aqueous environment, providing steric shielding [29]. However, the amount of P407 dispersions employed in relation to the specific liquid crystal lipid can have an influence on the structural types of the dispersions [30]. Recent developments have shown the effectiveness of additional stabilisers as superior substitutes for the P407 copolymer, including Cremophor, Myrj 59, PEGylated-phytanyl phase in an excess of water [14]. For the synthesis of complex dispersions that comprise vesicles as well as cubosomes with time-dependent ratios of each particle type, high-pressure homogenization and sonication are encouraged [23].

Similar to their starting bulk cubic phase, the coarse cubosomes on the micron scale have a Pn3m / QII D (Diamond or D-surface) form. In contrast, Im3m/QII P (Primitive, Schwarz, or P-surface) morphology predominates during homogenization, which may be related to the additional polymer or other reasons [32]. Simple hydration of monoolein results in the formation of cubosomes, and the polymers provide these nanoparticles colloidal stabilisation [33]. When the necessary solvent system is properly hydrated with the dry powder precursors, cubosomes are created [34]. A lipid/stabilizer combination microfluidized, then subjected to a high-temperature thermal treatment followed by chilling, produces liquid crystalline dispersions with a constrained size distribution [35]. The combination of lipids that form liquid crystals in ethanol or other organic solvents is dispersed using an excessive amount of water (or the preferred solvent system), which further promotes the spontaneous formation of cubosomes [36].

2. Loading drug in cubosome

The synthesized cubosomes need to be loaded with sufficient levels of small-molecule medicines, peptides, biologics, or bioactives in order to function as a possible drug delivery system. The three primary methods of loading the cargo include localising the medicine in the water channels of the cubic phase, attaching to the lipid membrane, and loading inside the lipid bilayer [10]. The medicinal agent might be added to the molten lipid [37] or the latter colyophilized with the lipid film [38] before dispersion to load the drug moieties. Alternately, the incubation process might be used to load drug moieties onto cubosomes that have already been created after they have been dispersed. The majority of small-molecule medications, peptides, and proteins are loaded into the lipid bilayer despite the fact that there are several ways to do so. These cubosomes are also made using single or binary lipid compositions, primarily phytantriol and monoolein. Cubosomes have been used in a number of trials to deliver anti-cancer medications, with encapsulation efficiencies varying from 71 to 103% [39]. Although other techniques may be used to measure drug loading, small-angle X-ray scattering (SAXS) is still the most used [40]. The promise of employing cubosomes as a drug delivery method, particularly for the administration of anti-cancer drugs, is therefore highlighted by these investigations.

3. Cancer therapy

Globally, cancer is one of the most prevalent diseases which poses an important clinical challenge, owing to its high rates of incidence. GLOBOCAN 2020 estimates reveal the diagnosis of 19.3 million cancer cases, with ~10 million deaths in 2020 [41,42]. This marks cancer as a critical barrier to increasing life expectancy and is also deemed the leading cause of death worldwide [41]. With the advent of biotherapeutic interventions, biomacromolecular drugs have recently garnered substantial attention, primarily in the field of drug discovery and development, due to advance in-vivo functions. Over the recent years, a plethora of drug delivery strategies have been devised for the administration of these biomacromolecular drugs, to overcome the difficulties in their dispensing, like drug instability and restriction by physiological membrane barriers. The uneven vascular structure, thick stroma, and many supportive cells, such as cancer-associated fibroblasts and tumor-associated macrophages (TAM) [43] are among the most noticeable features of the tumour microenvironment. Because the responsiveness of these nanobiotechnological modalities solely depends on the release of the active constituents under specific stimuli like enzymes, temperature, pH, redox potential, or other external stimuli based on their distinct physicochemical parameters, these characteristics have been effectively used in the diagnosis and treatment of cancer. Additionally, the development of highly effective active drug carrier systems is sped up by the synergistic fusion of several nanoparticles with target ligands. The sensitivity of in vivo real-time diagnosis for next-generation precision medicine can also be significantly improved by combining nanotechnology with a contrast agent [44]. The effective and patient-compliant delivery of biomacromolecular drugs using novel drug delivery systems guarantees a significant increase in bioavailability, drug half-life prolongation, and improved patient compliance, thereby enhancing their efficacy and potential for clinical applications [42].

4. Application of Cubosome in cancer

Most of the drugs used for cancer therapy are described below in correlation with cubosome:

A. Cisplatin

A subset of platinum (II) analogs, cisplatin (cis-diamminedichloroplatinum) is an alkylating agent. It works by generating an incredibly reactive moiety that promotes DNA cross-linking, resulting in DNA adducts, which then prevent DNA repair, causing DNA damage and death in cancer cells [45]. Cisplatin is one of the most often used chemotherapeutic drugs for solid tumours, however, it works best for metastatic ovarian and testicular cancer. The Human Hepatoma (HepG2) cell line was used in a number of investigations on uncoated and poly-coated cisplatin-loaded cubosomes by Zhang et al. For additional characterisation, cubosomes' entrapment, release, and zeta potential were examined in vitro. Cytotoxicity tests were also carried out. The complexation process of the cubosomal surface occurs after it is coated, and this is confirmed by observing a decrease in the zeta potential values of the coated cubosomes. In addition, in vitro release experiments showed that the uncoated model had an initial burst release of 55 ± 3%, followed by a slow release after 6 hours, and no further release after 10 hours. However, the coated model exhibited only 23 ± 3% initial release, with a slow but continuous release over a period of approximately 25 hours.

Moreover, cytotoxicity studies indicated that the free cisplatin, when compared to the cumbersome-loaded ones, exhibited significantly higher toxicity toward HepG2 cells. Due to their significant initial burst release, the uncoated models exhibited lower cell viability and higher cytotoxicity compared to the coated models [46]. These findings suggest that the coating effectively prevents the rapid release of large quantities of the drug. This is supported by the observation that the cell viability of the coated cubosomes is nearly equivalent to that of the blank cubosomes, indicating the coating's ability to mitigate the adverse effects associated with the high initial drug release.

B. Paclitaxel

Paclitaxel (PTX) is commonly used as a frontline chemotherapy drug for Non-Small Cell Lung Cancer (NSCLC). PTX binds to β-tubulin, leading to the obstruction of the mitotic spindle and arresting the cell cycle at the metaphase-anaphase junction of mitosis. This inhibition of the cell cycle is achieved by enhancing polymerization and stabilizing the microtubules, which are responsible for maintaining normal cellular functions throughout the cell cycle [45]. Aleandri et al. focused on biotinylatedcubosomes, which were stabilized and functionalized using a Biotin-based copolymer. These cubosomes had the ability to transport PTX and a hydrophobic fluorescent dye (MO-Fluo) for active targeting and cellular internalization. The cubosomes were characterized using chemical and physical techniques such as small-angle X-ray scattering (SAXS) and dynamic laser light scattering (DLS), and their efficacy in delivering PTX to HeLa cells was confirmed.

The novel cubosomal dispersions, designed with a biotin-conjugated stabilizer (PF108-B), exhibited a high affinity for the sodium-dependent multivitamin transporter (SMVT) that is overexpressed in tumor cell membranes. The conjugation of PF108 and Biotin was quantified using HABA (4'-Hydroxyazobenzene-2-carboxylic acid) in the presence of avidin. The research demonstrated a significantly increased anticancer effect of PTX at a concentration of 1 μg/mL in the biotinylatedcubosomes compared to free PTX or non-targeted cubosomes. The biotin ligand promoted cancer cell uptake through receptor-mediated endocytosis, enhancing PTX efficacy against tumor cells while reducing toxicity to healthy cells and tissues. These biotinylatedcubosomes hold promise for drug delivery, diagnosis, and therapeutic monitoring. Other studies by Murphy et al. and Chang et al. reported response rates of 21% and 24%, respectively, for a 24-hour infusion regimen of PTX in NSCLC treatment [47]. Zhai et al. investigated the potential of Mono-olein (MO)-based cubosomes as carriers

for PTX in ovarian cancer treatment. These lipid nanoparticles enabled higher PTX loading (up to 10 wt% of MO) and were bio-conjugated with Epidermal Growth Factor Receptor (EGFR) antibody fragments for active targeting of cancer cells. The nanoparticles exhibited higher cytotoxic activity against the Human Ovarian Cancer Cell Line (HEY) compared to free PTX. In mouse models of HEY-derived ovarian cancer, intraperitoneal injection of PTX-loaded cubosomes significantly reduced tumor burden and improved in vivo survival compared to free PTX administration [48]. Overall, PTX-loaded cubosome-based drug delivery systems have the potential to prolong disease-free progression and improve overall survival in late-stage cancers.

C. 5-Fluorouracil

5-fluorouracil (5-FU) is a water-soluble compound classified as an antimetabolite and belonging to the sub-class of pyrimidine analogs. It functions by inhibiting DNA replication through the suppression of the thymidylate synthase (TS) enzyme, which ultimately hinders the synthesis of pyrimidine thymidylate (dTMP). Typically administered via intravenous infusion, 5-FU is commonly used in the treatment of solid malignancies, particularly those affecting the colon, stomach, pancreas, liver, rectum, or urinary bladder.

In a study conducted by Nasr et al., 5-FU-loaded cubosomes were compared to an aqueous solution of free 5-FU. In vitro release studies demonstrated that the aqueous solution exhibited rapid release, lasting only about 1 hour, whereas the cubosomes displayed a relatively slower release, lasting approximately 4.5 hours. The cubosomes exhibited an initial burst release, with 53.6 ± 3.55% of the drug being released within the first hour. Biodistribution studies conducted in rat liver showed that the concentration of 5-FU in the liver was nearly five times higher with the cubosomal formulation compared to the 5-FU solution. Although higher concentrations of 5-FU resulted in greater hepatocellular damage, the use of cubosomes is believed to enhance the effectiveness of lower doses of 5-FU. The half-maximal inhibitory concentrations (IC50) obtained from in vitro cytotoxicity studies were calculated to be 112.70 mg/mL for free 5-FU and 107.78 mg/mL for the cubosomal dispersion. The insignificant difference between these values indicates that the antitumor activity of the drug has not been negatively affected by its loading onto the cubosomes [49].

D. Doxorubicin

Doxorubicin is a potent chemotherapy drug used primarily to treat solid tumors in various parts of the body, including the breast, ovaries, thyroid gland, urinary bladder, as well as neuroblastomas, sarcomas, and lung cancers. Its mechanism of action involves inhibiting the synthesis of DNA and RNA by intercalating between the DNA strands. Activation of the topoisomerase-2 enzyme, along with the formation of quinone-type free radicals, leads to the cleavage of the DNA strands.

Glycerol Mono-oleate (GMO)-based cubosomes serve as effective carriers for both Doxorubicin and the radionuclide Lutetium-177 (177Lu), which is a low-energy β-emitter. These cubosomes are also doped with a chelating agent known as DOTAGA-oleylamine conjugate (DOTAGA-OA), which forms stable complexes with Lutetium-177. The hydrophilic nature of the DOTAGA-metal complex allows it to be released from the channels of the cubosomes. However, the presence of the oleylamine hydrophobic chain, utilized in the synthesis of DOTAGA-OA, impedes the release, as it acts as a lipophilic barrier.

Cytryniak et al. conducted studies that demonstrated a decrease in the metabolic activity of HeLa cells, confirming the antitumor efficacy of these cubosomes. Their findings revealed that while the increase in cytotoxicity from the conjugated DOTAGA-OA-177Lu in a single cubosome was minimal, statistically significant enhancements in cytotoxicity were observed after short incubation times (e.g., 24 hours). Despite this, the multifunctional cubosomes allow for a reduced dosage of chemotherapy drugs, thereby minimizing the risk of adverse effects, especially in the case of doxorubicin, which is known for its strong cardiotoxic effects [50].

E. Ekariin

Icariin (ICA) is a naturally occurring antitumor compound derived primarily from Herbaepimedii. It is a flavanol glycoside and has recently been utilized for the treatment of ovarian cancer cell lines (SKOV-3 and Caov 3). Icariin exerts its pharmacological effects through various mechanisms, including the inhibition of PI3K/AKT and Raf1/ERK1/2 signaling pathways, cell cycle arrest, induction of apoptosis, and inhibition of autophagy by increasing the expression of autophagy-related p53 in breast cancer cells [51].

Moreover, Icariin modulates the mitochondrial transmembrane potential and the expression of caspase-3, leading to the generation of reactive oxygen species (ROS) in ovarian cancer cells, thereby exerting a cytotoxic effect. To enhance its efficacy, ICA was loaded into cubosomes due to its hydrophobic nature. The optimization of ICA-loaded cubosomes was achieved using a Box-Behnken statistical design. In vitro release studies comparing ICA-Raw (free ICA) and ICA-Cubs (optimized ICA-loaded cubosomes) demonstrated that ICA-Cubs exhibited an initial burst release followed by a gradual release, reaching 96.23 ± 3.231% release within 24 hours. In contrast, ICA-Raw showed a slow and incomplete release, with only 67.34 ± 2.424% release within the same time frame. The significantly reduced release rates of ICA-Raw compared to ICA-Cubs highlight the potential of cubosomes loaded with Icariin as a more effective intervention for achieving enhanced antitumor activity. Furthermore, the enhanced ICA-Cubs demonstrated moderate non-cytotoxicity on normal EA.hy926 endothelial cells, indicating a more targeted action and suggesting the potential for a selective antitumor effect [52].

F. Etoposide

Etoposide (ETP) is a semi-synthetic compound derived from podophyllotoxin, a plant glycoside. It is commonly used as a chemotherapeutic agent for treating lymphoma, leukemia, neuroblastoma, lung cancer, testicular cancer, and ovarian cancer. ETP exerts its anticancer properties by inhibiting the topoisomerase-2 enzyme, which is responsible for cleaving DNA strands and causing cell cycle arrest in the G2 phase.

In a study conducted by Tian et al., folate-modified cubosomes containing Etoposide (ETP-Cubs-FA) were synthesized, along with normal Etoposide-loaded cubosomes (ETP-Cubs). These cubosomes were synthesized using bulk gel fragmentation under 1500 bar homogenization conditions, resulting in a narrow size distribution with an average particle size of approximately 180 nm. Glycerol Monooleate (GMO)-based cubosomes were loaded with Etoposide, and the cubosomal surface was embedded with the P407-FA stabilizer. The incorporation of P407-FA facilitated active targeting of the tumor through the folate-mediated pathway, taking advantage of the overexpression of folate receptors on malignant cancer cells, thereby promoting a targeted antitumor effect [53].

Furthermore, the cytotoxic effects of ETP-Cubs and ETP-Cubs-FA were tested in vitro on the human breast adenocarcinoma cell line (MCF-7). The antiproliferative action of free ETP was evaluated using the MTT assay. The in vitro release of ETP from the cubosomes demonstrated sustained release, with approximately 82.5% release observed after 36 hours, in contrast to the administration of the free drug. The study demonstrated that ETP-Cubs-FA exhibited a greater cytotoxic effect on MCF-7 cells compared to free ETP and non-transformed ETP, primarily due to the active folate targeting. This was further validated by in vivo tumor imaging using Rhodamine B.

Overall, the folate-modified cubosomes loaded with Etoposide showed promise for enhancing the cytotoxic effects and targeted antitumor activity, offering a potential strategy for effective cancer treatment [53].

G. Curcumin

Curcumin, a natural compound derived from Curcuma longa L., exhibits a wide range of pharmacological activities including anticancer, antidiabetic, anti-inflammatory, antioxidant, hepatoprotective, nephroprotective, myocardial infarction protective, thrombosis suppressing, antirheumatic, and hypoglycemic effects. The zeta potential of curcumin-loaded nano-cubosomes was quantified using the SZ-100 nanoparticle analyzer [54]. The resulting zeta potential value of -24 mV indicated that the formulation was stable, as there was sufficient charge to prevent cubosome aggregation through electric repulsion. In vitro tests were conducted using the dialysis method, which revealed a rapid release of approximately 44.2 ± 2.7% of the curcumin within 24 hours, followed by sustained release of up to 81.3 ± 2.6% over the next 7 days [55]. Chang et al. conducted a study demonstrating the successful loading of curcumin onto cubosomes composed of monoolein (MO), monopalmitolein (MP), and phytantriol (PT). The study indicated variations in entrapment efficiency and curcumin localization within the bilayer based on the lipid composition. PT-cubosomes exhibited the highest entrapment efficiency, attributed to deeper curcumin molecule penetration into the hydrophobic region of the lipid bilayer, as supported by the relatively lower maximum fluorescence emission wavelength.

Cytotoxicity studies on B16F1 and NIH3T3 cell lines demonstrated significantly higher cytotoxicity of curcumin in cubosomal formulations compared to DSPC-liposomes or when freely solubilized in ethanol. The PT-cubosomes exhibited the most pronounced cytotoxic effect among all formulations, inducing apoptosis even at low concentrations, likely due to the synergistic interaction between PT and the loaded curcumin. Additionally, MO-cubosomes showed the greatest increase in cytotoxicity specifically in the B16F1 cancer cell line, suggesting their potential utility in anticancer treatment modalities [56].

H. A101

AT101, a potential anticancer drug, is the R-(−)-enantiomer of gossypol, a polyphenol derived from cottonseeds. It is primarily used in the treatment of Glioblastoma Multiforme (GBM). AT101 exerts its antitumor effect by inducing apoptosis and autophagic cell death in cancer cells. However, due to its hydrophobic nature and limited bioavailability in its free form, it was encapsulated in Glycerylmonooleate (GMO)-based cubosomes using the surfactant Pluronic F-127, employing the top-down technique.

In vitro cytotoxicity studies were conducted to compare the viability of GBM cell lines (A172 and LN229) and healthy central nervous system cells, including astrocytes (SVGA) and microglia (HMC3), using AT101-embedded GMO cubosomes and free AT101. The cell viability was determined using the colorimetric WST-1 assay. The drug entrapment efficiency of the AT101-loaded cubosomes was found to be 97.7% through Nuclear Magnetic Resonance (NMR) diffusometry studies. The observed sustained release of the drug was approximately 35% over 72 hours, demonstrating potent antitumor activity compared to the free form of AT101.

The prepared cubosomes exhibited greater cytotoxic activity against the GBM cell lines (A172 and LN229) compared to healthy central nervous system cells, including astrocytes (SVGA) and microglia (HMC3). This selective cytotoxicity reduces the potential side effects of the drug, highlighting the targeted drug delivery and its subsequent cytotoxic effect. Overall, this study concluded that GMO-AT101 cubosomes present a promising alternative for the treatment of GBM [57].

I. Docetaxel

Docetaxel (DTX) is a highly effective first-line anticancer drug used primarily for breast and ovarian tumors. As a member of the taxane group of chemotherapeutic agents, it exerts its pharmacological effect by inducing apoptosis through the phosphorylation of B-cell lymphoma 2 (Bcl-2) or by promoting the polymerization of tubulin monomers, leading to cell death [58].

Glycerol monooleate (GMO)-based cubosomes were utilized as a drug delivery vehicle for Docetaxel, along with stabilizers Pluronic-F127 and Pluronic-F68. A controlled drug delivery system was developed by incorporating DTX-Cubosomes (DTX-Cubs) into a thermo-responsive gelling depot system, which remains in liquid form at room temperature and transforms into a gel at higher temperatures. In vitro DTX release studies were conducted using different concentrations of Pluronic F127 and Pluronic F68. The formulations containing varying concentrations of PF127 (18-20% w/v) remained in liquid form at room temperature, while the formulation with a higher concentration of PF127 (20% w/v) formed a stable gel at lower temperatures, as observed through the "test-tube tilting" method. Additionally, the preparations containing 18-19% w/v of PF68 exhibited lower gelation temperatures compared to those containing 19-20% w/v of PF68 [59]. However, contrasting studies suggest that the sol-gel transition temperature of PF127-based thermo-responsive gels decreases with the addition of PF68. This decrease is attributed to the higher hydrophilicity of PF68, which may disrupt the hydration around the hydrophobic portion of PF127 molecules [60].

The zeta potential, polydispersity index, and mean particle size of the blank cubosomes (CD1) were measured as -54.1 ± 1.57 mV, 0.162 ± 0.014, and 184.9 ± 2.47 nm, respectively. In comparison, the DTX-loaded cubosomes (CD2) had zeta potential, polydispersity index, and mean particle size values of -47.4 ± 1.80 mV, 0.173 ± 0.019, and 220.9 ± 3.02 nm, respectively, with a DTX entrapment efficiency of 94.74 ± 3.41% w/w. In vitro drug release studies using Franz diffusion cells indicated a short lag time of 3 minutes and 5 seconds, with approximately 97% of the drug being released within 12 hours. When incorporated into the thermo-responsive depot system, the preparation exhibited an initial burst release of 21.48 ± 1.59% and released about 39.83 ± 3.27% of the drug over 12 hours, demonstrating its potential as a controlled drug delivery system. Cubosomes not only enable high loading of lipophilic drugs but also serve as reservoirs for sustained drug release. However, more advanced studies, including detailed microscopic characterization of cubosome formations and drug loading, would further enhance our understanding of this subject [59]. In conclusion, the incorporation

of DTX-Cubosomes into a thermo-responsive depot system enables controlled drug release, leading to improved antitumor efficacy.

J. Methotrexate

Methotrexate (MTX), a widely used cancer drug, belongs to the class of folate antagonists, which are a subclass of antimetabolites. MTX is commonly employed in the treatment of various cancers such as leukemia, lung, breast, head and neck, skin, and uterine cancers. It is also used for managing psoriasis, rheumatoid arthritis, and other autoimmune disorders. The pharmacological mechanism of MTX involves competitive inhibition of the enzyme dihydrofolatereductase (DHFRase), which hinders the conversion of dihydrofolic acid (DHFA) to tetrahydrofolic acid (THFA). THFA is crucial for the synthesis of thymidine, an essential component in DNA synthesis.

In a recent study by Janakiraman et al., methotrexate-loaded cubosomes (MTCs) were investigated. Different cubosomal formulations (MTCs 1 to MTCs 8) were developed, each with varying ratios of Poloxamer 188, cetylpalmitate, and water. The zeta potential values of the cubosomes ranged from -33.0 ± 0.21 mV to -7.84 ± 0.03 mV, indicating the stability and good dispersion characteristics of the formulations. Comparative analysis of the drug release profiles between the cubosomes and free MTX demonstrated that the cubosomes exhibited an initial burst release of 80.4 ± 0.9% of the drug, lasting approximately 1.5 hours, followed by sustained release for up to 8 hours. In contrast, free MTX showed a release of only 7.2 ± 1.1% of the drug within the same time period [60].

5. Theranostic application of cubosome.

The term "theranostic" refers to the combination of therapy and diagnostics in the treatment of any condition. It originated from the Greek words "thera" meaning therapeutic, and "nostic" meaning diagnostic [61]. Theranostic approaches have been developed to improve patient care by integrating diagnostics with therapy, particularly in the context of cancer. The identification of biomarkers expressed in cancer cells but not in normal cells is crucial for the development of nanomaterial-based theranostics in cancer treatment. Furthermore, the nanomaterial formulations used must be safe, inert, and compatible with the body's systems [62]. Several studies have explored the theranostic potential of cubosomes in cancer treatment. For instance, Zhang et al. created cubosomes using RYLO and a stabilizer called Poloxamer 407. They loaded cisplatin and paclitaxel into the cubosomes and coated them with poly-ε-lysine to achieve sustained drug delivery and enhanced effectiveness. They demonstrated the sustained drug delivery capability of cubosomes using HepG2 cells. Therapeutic potential was evaluated using impedance measurement and fluorescent imaging techniques [63].

In another study, surface modification of cubosomes with folate was investigated to confer theranostic activity on cancer-specific cubosome formulations. Folate receptors are known to be widely present in various tumor sites, making folate-conjugated cubosomes a promising approach for selective targeting of cancer treatment. Tian et al. developed folate-modified cubosomes loaded with etoposide stabilized with GMO and P407. The therapeutic efficacy of these cubosomes was evaluated in vitro on MCF-7 cells and in animal models. They observed a significant increase in drug accumulation when folate cubosomes were used, as demonstrated by the comparison of targeted cubosomes loaded with Rhodamine B against non-targeted cubosomes and free Rhodamine B. In vivo studies on mice with MCF-7 xenografts showed that folate-modified cubosomes were more successful in targeting tumor

cells. These findings indicate the theranostic potential of cubosomes when combined with imaging and therapeutic capabilities [64].

In another study conducted by Park et al., the interaction between the receptor (folate) and ligand (doxorubicin) was demonstrated using folate cubosomes loaded with doxorubicin. The researchers found that the functionalized cubosomes, which contained folate, exhibited enhanced efficiency in delivering doxorubicin. This resulted in increased anticancer efficacy through apoptosis in an in vitro Hela cell culture [65]. Similarly, Godlewska et al. observed a similar pattern of anticancer effectiveness when comparing folate-decorated monooleincubosomes to folate-free cubosomes [66]. These studies collectively highlight the rapid and enhanced utilization of cubosomes in drug delivery and diagnostic applications in cancer, particularly through the intervention of theranostics.

6. Conclusion

Cubosomes are unique nanoscale structures that consist of a bicontinuous cubic-phase colloidal dispersion in water. They act as stabilizers for surfactants and are capable of transporting various chemical substances in both living and non-living systems. With diameters ranging from 10 to 300 nm, cubosomes possess several advantageous characteristics, including bio-adhesive properties, improved dermal penetration, ease of formulation, high drug loading capacity, excellent stability at any dilution level, resistance to breaking, and protection of sensitive pharmaceuticals within the cubic phase. Furthermore, they are cost-effective, biocompatible, and safe to use. However, challenges such as increased viscosity during large-scale manufacturing and concerns regarding hydrophilic drug retention still need to be addressed.

Cubosomes have demonstrated efficacy as drug delivery systems in various dosage forms, including oral, topical, ocular, and parenteral administration. One of their key advantages is their ability to accommodate poorly water-soluble drugs and target specific sites in the body. In the field of cancer research, advanced studies are exploring the incorporation of anticancer drugs into cubosome formulations as carriers, with the aim of significantly improving cancer treatment outcomes. The use of cubosome-based anticancer drug delivery systems has shown minimal adverse effects on patients, aligning with previous research in this area. This reduction in adverse effects can alleviate the discomfort experienced by cancer patients during their treatments. Theranostics, which combine therapy and diagnostics, have further enhanced the therapeutic value of anticancer drugs by enabling simultaneous diagnostic applications and targeted delivery to tumor sites.

In the future, cubosome-mediated targeted nanoparticle cancer drug carriers have the potential to revolutionize cancer therapy and improve the quality of life for cancer patients. However, advancements in cubosome technology are necessary before they can be successfully implemented in clinical practice.

7. Conflict of Interest

The authors report no conflict of interest.

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9. Authors' Biography

Jitakshara Das (Corresponding Author): Wrote the article, designed the references as per the journal's requirements. Assistant Professor MadhuchandraLahan comprehended the idea, provided the guidance and resources and reviewed the article as per the journal's requirement.

10. References

1. Compton C. *Correction to: Cancer: The Enemy from Within*.; 2021. doi:10.1007/978-3-030-40651-6_13
2. Vokinger KN., Hwang TJ., Grischott T . Prices and clinical benefit of cancer drugs in the USA and Europe: a cost–benefit analysis. *Lancet Oncol*. 2020, 21(5), 664-670. doi:10.1016/S1470-2045(20)30139-X
3. Naoum GE., Morkos M., Kim B., Arafat W. Novel targeted therapies and immunotherapy for advanced thyroid cancers. *Mol Cancer*. 2018, 17(1), 1-15. doi:10.1186/s12943-018-0786-0
4. Dang YF., Jiang XN., Gong FL., Guo XL. New insights into molecular mechanisms of rosiglitazone in monotherapy or combination therapy against cancers. *Chem Biol Interact*. 2018,296, 162-170. doi:10.1016/j.cbi.2018.09.019
5. Ferlay J., Colombet M., Soerjomataram I. Cancer statistics for the year 2020: An overview. *Int J Cancer*. 2021, 149(4), 778-789. doi:10.1002/ijc.33588
6. Skoetz N., Will A., Monsef I., Brillant C., Engert A., von Tresckow B. Comparison of first-line chemotherapy including escalated BEACOPP versus chemotherapy including ABVD for people with early unfavourable or advanced stage Hodgkin lymphoma. *Cochrane Database Syst Rev*. 2017, 2017(5). doi:10.1002/14651858.CD007941.pub3
7. Krikorian S., Pories S., Tataronis G. Adherence to oral chemotherapy: Challenges and opportunities. *J Oncol Pharm Pract*. 2019, 25(7), 1590-1598. doi:10.1177/1078155218800384
8. DiMasi JA., Grabowski HG., Hansen RW. Innovation in the pharmaceutical industry: New estimates of R&D costs. *J Health Econ*. 2016, 47, 20-33. doi:10.1016/j.jhealeco.2016.01.012
9. Timucin AC., Basaga H., Kutuk O. Selective targeting of antiapoptotic BCL-2 proteins in cancer. 2018, 1-30. doi:10.1002/med.21516
10. Paper R, Manish G, Vimukta S. Targeted drug delivery system : A Review. 2011;1(2).
11. Khan I., Khan M., Umar MN., Oh D hwan. Nanobiotechnology and its applications in drug delivery system : a review. Published online 2015, 1-5. doi:10.1049/iet-nbt.2014.0062
12. Ban, D.K.; Paul, S. Protein corona over silver nanoparticles triggers conformational change of proteins and drop in bactericidal potential of nanoparticles: Polyethylene glycol capping as preventive strategy. Colloids Surf. B Biointerfaces 2016, 146, 577–584.
13. Owens, D.E., III; Peppas, N.A. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int. J. Pharm. 2006, 307, 93–102
14. O'Brien, M.E.; Wigler, N.; Inbar, M.; Rosso, R.; Grischke, E.; Santoro, A.; Catane, R.; Kieback, D.; Tomczak, P.; Ackland, S.J.A.o.o. Reduced cardiotoxicity and comparable efficacy in a phase IIItrial of pegylated liposomal doxorubicin HCl (CAELYX™/Doxil®) versus conventional doxorubicin forfirst-line treatment of metastatic breast cancer. Ann. Oncol. 2004, 15, 440–449.
15. De Villiers, M.M.; Aramwit, P.; Kwon, G.S. Nanotechnology in Drug Delivery. Springer Science & Business Media: Berlin/Heidelberg, Germany, 2008

16. Hong, S.; Choi, D.W.; Kim, H.N.; Park, C.G.; Lee, W.; Park, H.H. Protein-based nanoparticles as drug delivery systems. Pharmaceutics 2020, 12, 604.
17. Qu, M.-H.; Zeng, R.-F.; Fang, S.; Dai, Q.-S.; Li, H.-P.; Long, J.-T. Liposome-based co-delivery of siRNA and docetaxel for the synergistic treatment of lung cancer. Int. J. Pharm. 2014, 474, 112–122.
18. Li, W.; Cao, Z.; Liu, R.; Liu, L.; Li, H.; Li, X.; Chen, Y.; Lu, C.; Liu, Y. AuNPs as an important inorganic nanoparticle applied in drug carrier systems. Artif. Cells Nanomed. Biotechnol. 2019, 47, 4222–4233.
19. Sailor, M.J.; Park, J.H. Hybrid nanoparticles for detection and treatment of cancer. Adv. Mater. 2012, 24, 3779–3802
20. G. Van Meer, A.I.P.M. de Kroon, Lipid map of the mammalian cell, J. Cell Sci. 124 (1) (2011 Jan 1) 5–8 [Internet]. The Company of Biologists.
21. G. Garg, S. Saraf, S. Saraf, Cubosomes: an overview, Biol. Pharm. Bull. 30 (2) (2007) 350–353 [Internet]. Pharmaceutical Society of Japan.
22. V. Luzzati, R. Vargas, P. Mariani, A. Gulik, H. Delacroix, Cubic phases of lipidcontaining systems, J. Mol. Biol. 229 (2) (1993 Jan) 540–551.
23. H.M.G. Barriga, M.N. Holme, M.M. Stevens, Cubosomes: the next generation of smart lipid nanoparticles? Angew. Chem. Int. Ed. 58 (10) (2019 Mar 4) 2958–2978 [Internet]. Wiley
24. J. Barauskas, T. Landh, Phase behavior of the phytantriol/water system, Langmuir 19 (23) (2003 Oct 8) 9562–9565 [Internet]. American Chemical Society (ACS).
25. J.Y.T. Chong, X. Mulet, L.J. Waddington, B.J. Boyd, C.J. Drummond, Steric stabilisation of self-assembled cubic lyotropic liquid crystalline nanoparticles: high throughput evaluation of triblock polyethylene oxide-polypropylene oxidepolyethylene oxide copolymers, Soft Matter 7 (10) (2011) 4768 [Internet]. Royal Society of Chemistry (RSC).
26. S.B. Rizwan, B.J. Boyd, Cubosomes: structure, preparation and use as an antigen delivery system, Subunit Vacc. Deliv. (2014 Nov 1) 125–140 [Internet]. Springer New York. Available from: https://doi.org/10.1007/978-1-4939-1417-3_7.
27. K. Larsson, Aqueous dispersions of cubic lipid–water phases, Curr. Opin. Colloid Interface Sci. 5 (1–2) (2000 Mar) 64–69 [Internet]. Elsevier BV. Available from: https://doi.org/10.1016/s1359-0294(00)00040-6. [16] T.
28. Madheswaran, M. Kandasamy, R.J. Bose, V. Karuppagounder, Current potential and challenges in the advances of liquid crystalline nanoparticles as drug delivery systems, Drug Discov. Today 24 (7) (2019 Jul) 1405–1412 [Internet]. Elsevier BV.
29. B. Siekmann, H. Bunjes, M.H. Koch, K. Westesen, Preparation and structural investigations of colloidal dispersions prepared from cubic monoglyceride–water phases, Int. J. Pharm. 244 (1–2) (2002 Sep) 33–43 [Internet]. Elsevier BV.
30. M. Thadanki, P.S. Kumari, K.S. Prabha, Overview of cubosomes: a nano particle, Int. J. Res. Pharm. Chem. 1 (3) (2011) 535–541. [19]
31. P.T. Spicer, W.B. Small, M.L. Lynch, J.L. Burns, Dry powder precursors of cubic liquid crystalline nanoparticles (cubosomes), J. Nanopart. Res. 4 (4) (2002) 297–311.
32. J. Barauskas, M. Johnsson, F. Joabsson, F. Tiberg, Cubic phase nanoparticles (cubosome): principles for controlling size, structure, and stability, Langmuir 21 (6) (2005 Feb 17) 2569–2577 [Internet]. American Chemical Society (ACS). e, http s://doi.org/10.1021/la047590p. [21]

33. P.T. Spicer, K.L. Hayden, M.L. Lynch, A. Ofori-Boateng, J.L. Burns, Novel process for producing cubic liquid crystalline nanoparticles (cubosomes), Langmuir 17 (19) (2001 Aug 23) 5748–5756 [Internet]. American Chemical Society (ACS).
34. Y. Elnaggar, S. Etman, D. Abdelmonsif, O. Abdallah, Novel piperine-loaded Tweenintegratedmonooleincubosomes as brain-targeted oral nanomedicine in Alzheimer's disease: pharmaceutical, biological, and toxicological studies, Int. J. Nanomedicine (2015 Aug) 5459 [Internet]. Informa UK Limited. Available from: https://doi.org/10.2147/ijn.s87336.
35. C. Caltagirone, A.M. Falchi, S. Lampis, V. Lippolis, V. Meli, M. Monduzzi, et al., Cancer-cell-targeted theranosticcubosomes, Langmuir 30 (21) (2014 May 21) 6228–6236 [Internet]. American Chemical Society (ACS).
36. V. Meli, C. Caltagirone, A.M. Falchi, S.T. Hyde, V. Lippolis, M. Monduzzi, et al., Docetaxel-loaded fluorescent liquid-crystalline nanoparticles for cancer theranostics, Langmuir 31 (35) (2015 Aug 27) 9566–9575 [Internet]. American Chemical Society (ACS). Available from: https://doi.org/10.1021/acs.langmui r.5b02101.
37. E. Nazaruk, E. Gorecka, ´ Y.M. Osornio, E.M. Landau, R. Bilewicz, Charged additives modify drug release rates from lipidic cubic phase carriers by modulating electrostatic interactions, J. Electroanal. Chem. 819 (2018 Jun) 269–274 [Internet]. Elsevier BV
38.] F. Bray, M. Laversanne, E. Weiderpass, I. Soerjomataram, The ever-increasing importance of cancer as a leading cause of premature death worldwide, Cancer 127 (16) (2021 Jun 4) 3029–3030 [Internet]. Wiley. Available from: https://doi.org /10.1002/cncr.33587.
39. H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. 71 (3) (2021 Feb 4) 209–249 [Internet]. Wiley. A
40.] Q. Guo, C. Jiang, Delivery strategies for macromolecular drugs in cancer therapy, Acta Pharm. Sin. B 10 (6) (2020 Jun) 979–986 [Internet]. Elsevier BV.
41. B. Qi, C. Wang, J. Ding, W. Tao, Editorial: applications of nanobiotechnology in pharmacology, Front. Pharmacol. 10 (2019 Dec 4)
42. C.-Y. Huang, D.-T. Ju, C.-F. Chang, P. Muralidhar Reddy, B.K. Velmurugan, A review on the effects of current chemotherapy drugs and natural agents in treating non–small cell lung cancer, BioMedicine 7 (4) (2017 Nov 24) 23 [Internet]. China Medical University
43. L. Zhang, J. Li, D. Tian, L. Sun, X. Wang, M. Tian, Theranostic combinatorial drugloaded coated cubosomes for enhanced targeting and efficacy against cancer cells, Cell Death Dis. 11 (1) (2020 Jan)
44. A.Y. Chang, K. Kim, J. Glick, T. Anderson, D. Karp, D. Johnson, Phase II study of taxol, merbarone, and piroxantrone in stage IV non-small-cell lung cancer: the eastern cooperative oncology group results, JNCI J. Nat. Cancer Instit. 85 (5) (1993 Mar 3) 388–394 [Internet]. Oxford University Press (OUP)
45.] J. Zhai, R.B. Luwor, N. Ahmed, R. Escalona, F.H. Tan, C. Fong, et al., Paclitaxelloaded self-assembled lipid nanoparticles as targeted drug delivery systems for the treatment of aggressive ovarian cancer, ACS Appl. Mater. Interfaces 10 (30) (2018 Jul 2) 25174–25185 [Internet]. American Chemical Society (ACS).

46.] Y. Tian, J. Li, J. Zhu, N. Zhu, H. Zhang, L. Liang, et al., Folic acid-targeted etoposidecubosomes for theranostic application of cancer cell imaging and therapy, Med. Sci. Monit. 23 (2017 May 22) 2426–2435 [Internet]. International Scientific Information, Inc
47.] A. Cytryniak, E. Nazaruk, R. Bilewicz, E. Gorzy ´ nska, ´ K. Zelechowska-Matysiak, ˙ R. Walczak, et al., Lipidic cubic-phase nanoparticles (cubosomes) loaded with doxorubicin and labeled with 177Lu as a potential tool for combined chemo and internal radiotherapy for cancers, Nanomaterials 10 (11) (2020 Nov 16) 2272 [Internet]. MDPI AG
48. N. Han, B. Zhang, X. Wei, L. Yu, The inhibitory function of icariin in cell model of benign prostatic hyperplasia by upregulation of miR-7, BioFactors (2019 Nov 29), https://doi.org/10.1002/biof.1591 [Internet]. Wiley. Available from:.
49. U.A. Fahmy, O. Fahmy, N.A. Alhakamy, Optimized icariincubosomes exhibit augmented cytotoxicity against SKOV-3 ovarian cancer cells, Pharmaceutics 13 (1) (2020 Dec 24) 20 [Internet]. MDPI AG. A
50. Y. Tian, J. Li, J. Zhu, N. Zhu, H. Zhang, L. Liang, et al., Folic acid-targeted etoposidecubosomes for theranostic application of cancer cell imaging and therapy, Med. Sci. Monit. 23 (2017 May 22) 2426–2435 [Internet]. International Scientific Information, Inc
51.] A. Archana, K. Vijayasri, M. Madhurim, C. Kumar, Curcumin loaded nanocubosomal hydrogel: .
52.] Y.S. Tu, J.W. Fu, D.M. Sun, J.J. Zhang, N. Yao, D.E. Huang, et al., Preparation, characterisation and evaluation of curcumin with piperine-loaded cubosome nanoparticles, J. Microencapsul. 31 (6) (2014 Mar 18) 551–559 [Internet]. Informa UK Limitedpreparation, in vitro characterization and antibacterial activity, Chem. Sci. Trans. 4 (2015) 75–80
53. C. Chang, T.G. Meikle, C.J. Drummond, Y. Yang, C.E. Conn, Comparison of cubosomes and liposomes for the encapsulation and delivery of curcumin, Soft Matter 17 (12) (2021) 3306–3313 [Internet]. Royal Society of Chemistry (RSC). Available from: https://doi.org/10.1039/d0sm01655a.
54. D.K. Flak, V. Adamski, G. Nowaczyk, K. Szutkowski, M. Synowitz, S. Jurga, et al., T101-Loaded cubosomes as an alternative for improved glioblastoma therapy, Int. J. Nanomedicine 15 (2020 Oct) 7415–7431 [Internet]. Informa UK Limited
55. S. Manivannan, S. Nagaraj, S. Narayan, A reflection on the mechanism of the role of nanoparticles in increasing the efficacy of anti-tumour properties of docetaxel, Curr. Pathobiol. Rep. 9 (3) (2021 May 3) 79–91 [Internet]. Springer Science and Business Media LLC. A
56.] N.R. Rarokar, S.D. Saoji, N.A. Raut, J.B. Taksande, P.B. Khedekar, V.S. Dave, Nanostructured cubosomes in a thermoresponsive depot system: an alternative approach for the controlled delivery of docetaxel, AAPS PharmSciTech 17 (2) (2015 Jul 25) 436–445 [Internet]. Springer Science and Business Media LLC. Available from: https://doi.org/10.1208/s12249-015-0369-y.
57. K. Janakiraman, V. Krishnaswami, V. Sethuraman, V. Rajendran, R. Kandasamy, Development of methotrexate-loaded cubosomes with improved skin permeation for the topical treatment of rheumatoid arthritis, Appl. Nanosci. 9 (8) (2019 Mar 6) 1781–1796 [Internet]. Springer Science and Business Media LLC.
58. H.A. Abo El-Enin, Development of nanostructured liquid crystalline formulation of anti-cancer drug as a new drug delivery system, J. Pharm. Innov. 15 (1) (2019 Jan 19) 80–93 [Internet]. Springer Science and Business Media LLC. A

59. Chircov, C.; Grumezescu, A.M. Basics in nanoarchitectonics. In Nanoarchitectonics in Biomedicine; Elsevier: Amsterdam, The Netherlands, 2019; pp. 1–21.
60. . Zhang, L.; Li, J.; Tian, D.; Sun, L.; Wang, X.; Tian, M. Theranostic combinatorial drug-loaded coated cubosomes for enhanced targeting and efficacy against cancer cells. Cell Death Dis. 2020, 11, 1
61. 9. Tian, Y.; Li, J.-C.; Zhu, J.-X.; Zhu, N.; Zhang, H.-M.; Liang, L.; Sun, L. Folic acid-targeted etoposidecubosomes for theranostic application of cancer cell imaging and therapy. Med. Sci. Monit. 2017, 23, 2426
62. Park, D.; Park, S.H.; Kim, J.-C.J.B.; Engineering, B. In vitro Anti-cancer Efficacy and Cellular Interaction of Cubic Phases Containing Cinnamic Acid, Poly (ethyleneimine), and Doxorubicin. Biotechnol. Bioprocess Eng. 2020, 25, 235–245. [CrossRef]
63. Godlewska, M.; Majkowska-Pilip, A.; Stachurska, A.; Biernat, J.F.; Gaweł, D.; Nazaruk, E. Voltammetric and biological studies of folate-targeted non-lamellar lipid mesophases. Electrochim. Acta 2019, 299, 1–11.