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DESIGN OF EXPERIMENTS (DOE) ASSISTED CUBOSOMAL DRUG DELIVERY SYSTEMS FOR CANCER THERAPY: FORMULATION STRATEGIES, OPTIMIZATION, AND PHARMACEUTICAL APPLICATIONS

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ABSTRACT

Cancer remains a major global health challenge, creating a need for advanced drug delivery systems capable of improving therapeutic efficacy while minimizing systemic toxicity. Cubosomes, a class of lipid-based nanocarriers with bicontinuous cubic liquid crystalline structures, have gained significant attention in cancer therapy due to their high drug-loading capacity, biocompatibility, controlled release behavior, and ability to encapsulate both hydrophilic and hydrophobic drugs. These nanostructures enhance drug solubility, stability, cellular uptake, and tumor targeting, thereby improving the therapeutic performance of anticancer agents. However, optimization of cubosomal formulations is challenging because of the complex interactions among formulation variables and processing parameters. Design of Experiments (DOE) has emerged as an effective statistical and systematic approach for the development and optimization of pharmaceutical nanoformulations. DOE enables simultaneous evaluation of multiple variables, identification of critical factors, reduction in experimental runs, and generation of robust and reproducible formulations. Several DOE models, including factorial design, Box–Behnken design, central composite design, and response surface methodology, have been widely employed for cubosome optimization. This review highlights the fundamentals, composition, preparation techniques, characterization methods, and pharmaceutical applications of cubosomes in cancer therapy. Special emphasis is placed on DOE-assisted optimization strategies, critical quality attributes, and recent advancements in targeted and stimuli-responsive cubosomal systems. Additionally, Quality by Design (QbD), regulatory perspectives, current challenges, and future opportunities in cubosome-mediated cancer therapy are discussed. DOE-assisted cubosomal systems represent a promising platform for developing effective and targeted anticancer therapies with improved clinical outcomes.

KEYWORDS: Cubosomes, Cancer therapy, Design of Experiments (DOE), Nano-drug delivery Pharmaceutical optimization, Targeted drug delivery.

1. INTRODUCTION

1.1 GLOBAL BURDEN OF CANCER

Cancer is one of the leading causes of morbidity and mortality worldwide and continues to pose a major public health challenge. According to global health reports, the incidence of cancer is rapidly increasing due to factors such as population growth, aging, unhealthy lifestyles, environmental pollution, and genetic predisposition. Common types of cancer including breast cancer, lung cancer, colorectal cancer, prostate cancer, and liver cancer contribute significantly to global mortality. Despite remarkable advancements in diagnosis and treatment, effective management of cancer remains difficult because of tumor heterogeneity, multidrug resistance, metastasis, and severe adverse effects associated with conventional chemotherapy. Therefore, there is a growing need for advanced therapeutic strategies capable of improving treatment efficacy and patient outcomes.[1]

1.2 LIMITATIONS OF CONVENTIONAL CANCER THERAPY

Conventional cancer treatment approaches such as chemotherapy, radiotherapy, and surgery have been widely employed for cancer management. However, these therapeutic modalities possess several limitations, including poor selectivity, nonspecific drug distribution, systemic toxicity, rapid drug degradation, and inadequate accumulation of drugs at tumor sites. Chemotherapeutic agents often damage healthy tissues along with cancer cells, resulting in severe side effects such as immunosuppression, cardiotoxicity, nephrotoxicity, and gastrointestinal complications. Additionally, poor aqueous solubility and low bioavailability of many anticancer drugs further limit their therapeutic effectiveness. These challenges have encouraged researchers to explore novel drug delivery systems for targeted and controlled cancer therapy.

1.3 ROLE OF NANOTECHNOLOGY IN ONCOLOGY

Nanotechnology has emerged as a promising field in oncology for the development of advanced drug delivery systems with improved therapeutic performance. Nanocarriers such as liposomes, nanoparticles, dendrimers, nanoemulsions, solid lipid nanoparticles, and cubosomes have demonstrated the ability to enhance drug solubility, stability, bioavailability, and tumor targeting. Nanoscale drug delivery systems can exploit the enhanced permeability and retention (EPR) effect, enabling selective accumulation of therapeutic agents in tumor tissues. Furthermore, nanotechnology facilitates controlled drug release, reduced systemic toxicity, prolonged circulation time, and enhanced cellular uptake. Owing to these advantages, nanomedicine has become an important strategy in modern cancer therapy.

1.4 CUBOSOMES AS NOVEL LIPID NANOCARRIERS

Cubosomes are nanostructured lipid-based carriers composed of bicontinuous cubic liquid crystalline phases stabilized by surfactants. These nanocarriers possess a unique internal structure containing interconnected aqueous and lipid channels, allowing encapsulation of hydrophilic, hydrophobic, and amphiphilic drugs simultaneously. Cubosomes offer several advantages including high drug-loading capacity, biocompatibility, bioadhesion, controlled drug release, and structural stability. Their nanosized architecture enhances penetration into tumor tissues and improves intracellular drug delivery. In recent years, cubosomal formulations have attracted significant attention in cancer therapy for delivering chemotherapeutic agents, genes, proteins, and imaging agents. The versatility and multifunctional characteristics of cubosomes make them promising candidates for targeted anticancer drug delivery applications.

1.5 IMPORTANCE OF DOE IN PHARMACEUTICAL DEVELOPMENT

Design of Experiments (DOE) is a systematic statistical approach used for formulation and process optimization in pharmaceutical research and development. Unlike traditional one-factor-at-a-time methods, DOE enables simultaneous evaluation of multiple formulation variables and their interactions using a minimal number of experimental runs. DOE assists in identifying critical process parameters, optimizing formulation conditions, improving product quality, and ensuring reproducibility. Various experimental designs such as factorial design, Box–Behnken design, central composite design, and response surface methodology are extensively applied in nanoformulation development. In cubosomal systems, DOE plays a crucial role in optimizing variables such as lipid concentration, stabilizer concentration, homogenization speed, and processing conditions to achieve desired responses including particle size, entrapment efficiency, zeta potential, and drug release behavior.

1.6 AIM AND SCOPE OF THE REVIEW

The present review aims to provide a comprehensive overview of DOE-assisted cubosomal drug delivery systems for cancer therapy. The review discusses the structure, composition, preparation methods, characterization techniques, and pharmaceutical applications of cubosomes in oncology. Special emphasis is given to the role of DOE in cubosome optimization, critical formulation variables, and statistical approaches used in formulation development. Additionally, recent advances including targeted cubosomes, stimuli-responsive systems, Quality by Design (QbD)-based approaches, and artificial intelligence-assisted optimization strategies are highlighted. The review also addresses current challenges, regulatory perspectives, and future opportunities associated with cubosome-mediated cancer therapy.

Table 1: Comparison Between Conventional Cancer Therapy and Cubosomal Drug Delivery Systems.

Parameter	Conventional Cancer Therapy	Cubosomal Drug Delivery Systems
Drug distribution	Non-specific distribution to healthy and cancerous tissues	Enhanced tumor-targeted delivery
Drug solubility	Poor solubility of many anticancer drugs	Improved solubility of hydrophobic drugs
Drug release pattern	Rapid or uncontrolled drug release	Controlled and sustained drug release
Systemic toxicity	High systemic toxicity and adverse effects	Reduced systemic toxicity
Bioavailability	Limited bioavailability	Enhanced bioavailability
Cellular uptake	Limited intracellular uptake	Improved cellular internalization
Tumor targeting	Poor selectivity toward tumor tissues	Enhanced targeting through EPR effect
Drug stability	Susceptible to degradation	Improved protection against degradation
Encapsulation capability	Limited drug loading	Encapsulation of hydrophilic and hydrophobic drugs
Circulation time	Short plasma half-life	Prolonged circulation time
Dose frequency	Frequent administration required	Reduced dosing frequency
Patient compliance	Lower due to toxicity and frequent dosing	Improved patient compliance
Multidrug resistance	Limited effectiveness against resistant tumors	Potential to overcome multidrug resistance
Therapeutic efficacy	Moderate therapeutic response	Enhanced anticancer efficacy
Pharmaceutical versatility	Limited formulation flexibility	Suitable for multiple delivery routes and therapeutic agents

4. CUBOSOMES: STRUCTURE, COMPOSITION, AND PROPERTIES

4.1 STRUCTURE AND INTERNAL ARCHITECTURE OF CUBOSOMES

Cubosomes are nanostructured lipid carriers characterized by their unique bicontinuous cubic liquid crystalline structure. They are formed by the self-assembly of amphiphilic lipids in the presence of water, resulting in a three-dimensional honeycomb-like architecture consisting of interconnected aqueous channels separated by lipid bilayers.

This internal structure provides a large surface area and enables simultaneous encapsulation of hydrophilic, hydrophobic, and amphiphilic therapeutic agents. Cubosomes generally exist in different cubic phases such as primitive, double diamond, and gyroid structures, depending on lipid composition and environmental conditions. The nanosized dimensions and highly ordered internal arrangement of cubosomes contribute to improved drug loading, sustained release behavior, and enhanced interaction with biological membranes. Due to their structural similarity to biological membranes, cubosomes exhibit excellent biocompatibility and cellular uptake properties, making them promising carriers for cancer drug delivery.[2]

2.2 COMPONENTS OF CUBOSOMAL SYSTEMS

Cubosomal formulations are composed of lipids, stabilizers or surfactants, and aqueous phase components. The careful selection of these components significantly influences the stability, particle size, drug loading capacity, and release behavior of cubosomes.

2.2.1 Lipids

Lipids are the primary structural components responsible for the formation of cubic liquid crystalline phases. Glyceryl monooleate (GMO) and phytantriol are the most commonly used lipids in cubosome preparation due to their ability to spontaneously form stable cubic phases upon hydration. These lipids possess amphiphilic characteristics that facilitate self-assembly into nanostructured systems. Lipid concentration plays an important role in determining the internal architecture, viscosity, drug encapsulation efficiency, and release profile of cubosomes. In addition to GMO and phytantriol, other lipids such as monoacylglycerols and phospholipids may also be incorporated to modify the physicochemical properties of cubosomal formulations.

2.2.2 Stabilizers and Surfactants

Stabilizers and surfactants are incorporated into cubosomal systems to prevent particle aggregation and maintain colloidal stability. Poloxamer 407 is the most widely used stabilizer because of its excellent steric stabilization properties and biocompatibility. Surfactants reduce interfacial tension between lipid and aqueous phases, thereby facilitating the formation of stable nanosized particles. The concentration and type of stabilizer significantly influence particle size distribution, zeta potential, drug release characteristics, and physical stability of cubosomes. Other surfactants such as Tween 80, Span 80, and polyethylene glycol derivatives are also employed depending on formulation requirements.

2.2.3 Aqueous Phase Components

The aqueous phase serves as the hydration medium for cubic phase formation and contributes to the overall stability of cubosomal dispersions. Water is the most commonly used aqueous component, although buffers and co-solvents may also be incorporated to improve drug solubility and formulation stability. Hydrophilic drugs are mainly accommodated within the aqueous channels of cubosomes, while lipophilic drugs are incorporated into the lipid

bilayer regions. The pH and ionic strength of the aqueous phase may influence the structural organization and drug release behavior of cubosomal systems.

Table 2: Composition of Cubosomal Formulations and Their Functions.

Component Category	Examples	Function in Cubosomal System
Lipids	Glyceryl monooleate (GMO), Phytantriol	Formation of bicontinuous cubic phase structure
Stabilizers	Poloxamer 407, Poloxamer 188	Stabilization of cubosomal nanoparticles and prevention of aggregation
Surfactants	Tween 80, Span 80	Reduction of interfacial tension and improvement of dispersion stability
Aqueous phase	Purified water, phosphate buffer	Hydration medium for cubic phase formation
Hydrotropes/Co-solvents	Ethanol, Propylene glycol	Enhancement of lipid solubility and facilitation of cubosome formation
Anticancer drugs	Doxorubicin, Paclitaxel, 5-Fluorouracil	Therapeutic activity against cancer cells
Targeting ligands	Folic acid, Transferrin, Antibodies	Active targeting of tumor tissues and enhanced cellular uptake
Surface modifiers	Polyethylene glycol (PEG), Chitosan	Improved circulation time and surface functionality
Charge-inducing agents	Stearylamine, Dicetyl phosphate	Modification of surface charge and colloidal stability
Cryoprotectants	Mannitol, Trehalose	Protection during freeze-drying and storage
Preservatives	Methyl paraben, Propyl paraben	Prevention of microbial contamination
Antioxidants	Butylated hydroxytoluene (BHT), Vitamin E	Prevention of lipid oxidation and formulation degradation

2.3 Physicochemical Properties of Cubosomes

Cubosomes exhibit several unique physicochemical properties that make them attractive drug delivery carriers. They possess nanoscale particle size, large internal surface area, high drug-loading capacity, and structural stability. The bicontinuous cubic structure allows sustained and controlled release of encapsulated drugs. Cubosomes also demonstrate bioadhesive properties, which improve residence time at the site of administration and enhance drug absorption. Their ability to encapsulate molecules with varying solubility characteristics further broadens their pharmaceutical applications. Additionally, cubosomes exhibit thermodynamic stability, low toxicity, and compatibility with biological membranes. Important physicochemical parameters commonly evaluated include particle size, polydispersity index, zeta potential, entrapment efficiency, morphology, and drug release profile.[3]

2.4 Advantages of Cubosomes in Drug Delivery

Cubosomes offer numerous advantages in pharmaceutical and biomedical applications. Their unique internal structure enables efficient encapsulation of both hydrophilic and hydrophobic drugs. Cubosomal systems improve drug solubility, protect labile drugs from degradation, and provide controlled and sustained drug release. The nanosized particles enhance cellular uptake and facilitate passive targeting through the enhanced permeability and retention (EPR) effect in tumor tissues. Cubosomes also exhibit excellent biocompatibility and bioadhesion, which improve therapeutic efficacy and patient compliance. Furthermore, they can be administered through multiple routes including oral, topical, transdermal, ocular, and parenteral delivery. Their versatility makes them suitable carriers for anticancer drugs, peptides, proteins, genes, and vaccines.

2.5 Limitations and Challenges

Despite their promising advantages, cubosomes possess certain limitations and challenges that restrict their large-scale pharmaceutical application. One of the major challenges is the complexity associated with large-scale manufacturing and process reproducibility. Cubosomal dispersions may exhibit physical instability such as aggregation or phase separation during storage. High viscosity of cubic phases can also create difficulties during processing and administration. In some cases, low drug loading and drug leakage may affect formulation performance. Additionally, sterilization, scale-up, and long-term stability remain significant concerns for industrial production. Regulatory challenges and limited clinical data further hinder the commercialization of cubosome-based formulations. Therefore, systematic optimization approaches such as Design of Experiments (DOE) and Quality by Design (QbD) are essential to overcome these limitations and develop robust cubosomal drug delivery systems.

5. PREPARATION METHODS OF CUBOSOMES

Cubosomes can be prepared using various formulation techniques depending on the type of lipid, drug characteristics, desired particle size, and stability requirements. The preparation method significantly influences the internal structure, entrapment efficiency, particle size distribution, and drug release behavior of cubosomal systems. Commonly employed techniques include top-down and bottom-up approaches, high-pressure homogenization, solvent evaporation, melt dispersion, and microfluidization methods.[4]

5.1 Top-Down Technique

The top-down technique is one of the most widely used methods for cubosome preparation. In this method, a bulk cubic phase is initially formed by mixing lipids with water under controlled conditions. The highly viscous cubic gel obtained is subsequently fragmented into nanosized cubosomal particles using high-energy mechanical processes such as homogenization or sonication. Stabilizers such as Poloxamer 407 are incorporated to maintain colloidal stability and prevent particle aggregation.

This method offers good structural integrity and reproducibility of cubosomal systems. However, the high energy input required during fragmentation may lead to increased processing time and possible degradation of thermosensitive drugs. Despite these limitations, the top-down approach remains a commonly employed technique in pharmaceutical research due to its simplicity and effectiveness.

5.2 Bottom-Up Technique

The bottom-up technique, also known as the solvent dilution or hydrotrope method, involves the spontaneous formation of cubosomes from molecular building blocks. In this method, lipids are dissolved in a hydrotropic solvent or water-miscible solvent, followed by gradual addition of water under controlled conditions. Upon dilution, the lipid molecules self-assemble into cubic liquid crystalline nanoparticles.

Compared with the top-down approach, the bottom-up technique requires lower energy input and allows easier scale-up. This method also produces cubosomes with smaller particle sizes and narrow size distribution. However, the use of organic solvents or hydrotropes may create toxicity concerns and require additional purification steps. The bottom-up approach is particularly useful for the preparation of temperature-sensitive formulations.

5.3 High-Pressure Homogenization

High-pressure homogenization is a commonly used technique for reducing particle size and producing stable cubosomal dispersions. In this method, the coarse dispersion containing lipid, surfactant, drug, and aqueous phase is forced through a narrow gap under extremely high pressure. The intense shear forces, turbulence, and cavitation generated during the process break down larger particles into nanosized cubosomes.

This technique provides uniform particle size distribution, improved stability, and enhanced drug encapsulation efficiency. Parameters such as homogenization pressure, number of cycles, and temperature significantly influence the final characteristics of cubosomes. High-pressure homogenization is suitable for large-scale industrial production; however, repeated homogenization cycles may increase processing costs and affect sensitive therapeutic agents.

5.4 Solvent Evaporation Method

In the solvent evaporation method, lipids and drug molecules are first dissolved in a volatile organic solvent such as ethanol, chloroform, or methanol. The organic solvent is then removed under reduced pressure or controlled heating to form a thin lipid film. Subsequently, hydration with an aqueous phase containing stabilizers results in the formation of cubosomal dispersions.

This method is particularly advantageous for incorporating poorly water-soluble drugs into cubosomal systems. It also provides better control over drug loading and particle formation. However, the presence of residual organic solvents may affect formulation safety and stability. Therefore, complete solvent removal is essential during formulation development.

5.5 Melt Dispersion Technique

The melt dispersion technique involves melting the lipid phase at a temperature above its melting point followed by mixing with an aqueous surfactant solution under continuous stirring. The drug may be incorporated either into the molten lipid phase or aqueous phase depending on its solubility characteristics. Upon cooling, the lipid reorganizes into cubosomal nanoparticles.

This technique avoids the use of organic solvents and is considered relatively simple and cost-effective. It is suitable for thermally stable drugs and provides good drug encapsulation efficiency. However, high processing temperatures may not be appropriate for heat-sensitive compounds such as proteins and peptides.

5.6 Microfluidization Technique

Microfluidization is an advanced particle size reduction technique widely used for the preparation of nanosized cubosomes with uniform distribution. In this method, coarse dispersions are passed through microchannels at high velocity using specialized microfluidizer equipment. The collision of fluid streams under controlled pressure generates intense shear forces that reduce particle size efficiently.

Microfluidization produces cubosomes with narrow polydispersity index, improved stability, and enhanced reproducibility. This method is particularly advantageous for large-scale pharmaceutical manufacturing due to its scalability and consistent product quality. Nevertheless, specialized equipment and high operational costs may limit its widespread industrial application.

Table 3: Methods Used for Preparation of Cubosomes.

Preparation Method	Principle	Advantages	Limitations
Top-down technique	Fragmentation of bulk cubic phase into nanoparticles using high-energy processes	Simple and widely used method; good structural integrity	High energy consumption; time-consuming process
Bottom-up technique	Self-assembly of lipid molecules into cubosomes upon solvent dilution	Low energy requirement; smaller particle size	Use of solvents may require additional purification
High-pressure homogenization	Particle size reduction using high shear force and pressure	Uniform particle size; scalable for industrial production	Expensive equipment; possible thermal degradation
Solvent evaporation method	Formation of lipid film followed by hydration and cubosome formation	Suitable for poorly water-soluble drugs	Residual solvent concerns
Melt dispersion technique	Melting lipid phase followed by dispersion into aqueous phase	Solvent-free and cost-effective	Not suitable for thermolabile drugs
Microfluidization technique	Particle size reduction using collision of fluid streams at high pressure	Narrow particle size distribution; high reproducibility	High operational and equipment cost
Sonication method	Use of ultrasonic energy for particle size reduction	Simple and rapid method	Possible instability and heat generation
Spray drying technique	Drying cubosomal dispersions into powder form using hot air	Improved storage stability and handling	Thermal stress may affect sensitive compounds
Emulsification method	Formation of emulsion followed by conversion into cubosomes	Easy formulation process	Limited control over particle size
Hydrotrope dilution method	Cubosome formation through dilution of hydrotropic solvent systems	Reduced viscosity and easy processing	Hydrotrope removal may be necessary

6. FUNDAMENTALS OF DESIGN OF EXPERIMENTS (DOE)

Design of Experiments (DOE) is a systematic statistical approach widely employed in pharmaceutical research and development for formulation optimization, process improvement, and product quality enhancement. DOE enables simultaneous evaluation of multiple formulation and process variables to identify their individual and interactive effects on the desired responses. In nanopharmaceutical development, DOE has become an essential tool for developing robust and reproducible formulations while minimizing time, cost, and experimental workload. The application of DOE in cubosomal drug delivery systems assists in optimizing critical variables such as lipid concentration, surfactant concentration, homogenization speed, and processing conditions to achieve desired quality attributes including particle size, entrapment efficiency, zeta potential, and drug release behavior.[5]

6.1 Concept and Principles of DOE

DOE is a structured method used to plan, conduct, analyze, and interpret controlled experiments systematically. The primary objective of DOE is to establish relationships between independent variables (factors) and dependent variables (responses). Unlike conventional one-factor-at-a-time approaches, DOE investigates multiple variables simultaneously and evaluates possible interactions among them.

The fundamental principles of DOE include randomization, replication, and blocking. Randomization minimizes experimental bias by ensuring random allocation of experimental runs. Replication improves the reliability and reproducibility of experimental results by repeating experiments under identical conditions. Blocking is employed to

minimize variability arising from uncontrollable external factors. DOE also utilizes mathematical and statistical models to predict formulation behavior and optimize process conditions efficiently.

In pharmaceutical formulation development, DOE assists researchers in identifying critical formulation variables, reducing variability, and establishing optimized formulations with enhanced quality and performance characteristics.

6.2 Advantages of DOE over Conventional Methods

DOE offers several advantages compared with traditional trial-and-error or one-factor-at-a-time optimization methods. Conventional approaches evaluate one variable while keeping all other variables constant, which is time-consuming and incapable of identifying interaction effects between variables. In contrast, DOE simultaneously studies multiple factors and their interactions using fewer experimental runs.

The major advantages of DOE include reduced experimental workload, improved process understanding, efficient optimization, enhanced reproducibility, and cost-effectiveness. DOE also facilitates identification of critical process parameters and critical quality attributes affecting formulation performance. Statistical analysis provided by DOE improves data interpretation and enables prediction of optimized conditions with high accuracy.

In cubosomal formulation development, DOE assists in achieving optimized particle size, improved entrapment efficiency, controlled drug release, and enhanced stability. Additionally, DOE supports Quality by Design (QbD)-based pharmaceutical development by establishing design space and process control strategies.

6.3 Statistical Approaches in DOE

Various statistical approaches and experimental designs are employed in DOE depending on the formulation objectives and number of variables involved. Screening designs such as full factorial design and fractional factorial design are used to identify significant formulation and process variables. Optimization designs including Central Composite Design (CCD), Box–Behnken Design (BBD), and Response Surface Methodology (RSM) are employed to determine optimal formulation conditions and study nonlinear relationships between variables and responses.

Mathematical models generated through DOE provide polynomial equations that describe the relationship between independent variables and responses. Analysis of variance (ANOVA) is commonly used to evaluate the statistical significance of the models and determine the influence of formulation variables. Graphical tools such as contour plots, three-dimensional response surface plots, Pareto charts, and desirability functions assist in visualization and optimization of experimental outcomes.

These statistical approaches are extensively applied in cubosome formulation development to optimize formulation variables and achieve desired physicochemical characteristics and therapeutic performance.

6.4 Software Used in DOE Studies

Several statistical software packages are available for designing experiments, analyzing data, generating mathematical models, and optimizing pharmaceutical formulations. These software tools simplify experimental planning and statistical interpretation, making DOE applications more efficient and reliable in pharmaceutical research.

6.4.1 Design-Expert®

Design-Expert® is one of the most widely used software tools for DOE applications in pharmaceutical formulation development. The software provides user-friendly interfaces for constructing experimental designs, analyzing statistical models, generating response surface plots, and optimizing formulation variables. Design-Expert® supports various DOE models including factorial designs, Central Composite Design, Box–Behnken Design, mixture design, and response surface methodology. The software also provides desirability functions and numerical optimization tools for selecting optimized formulation conditions.

6.4.2 JMP®

JMP® is a statistical discovery software developed for advanced data analysis and experimental design applications. It offers interactive graphical visualization tools and robust statistical analysis capabilities for pharmaceutical optimization studies. JMP® supports screening designs, regression analysis, response surface methodology, and predictive modeling. The software is useful for identifying significant formulation variables and evaluating complex interactions among process parameters.

6.4.3 Minitab®

Minitab® is a widely used statistical software package for quality improvement and DOE analysis in pharmaceutical industries. The software provides tools for experimental design creation, regression analysis, ANOVA, optimization studies, and graphical interpretation of experimental data. Minitab® is commonly employed in Quality by Design (QbD)-based pharmaceutical development for process optimization and statistical quality control. Its simple interface and efficient statistical functions make it suitable for both academic and industrial research applications.

Table 4: DOE Models Commonly Used in Cubosome Optimization.

DOE Model	Purpose/Application	Advantages	Limitations
Full Factorial Design	Evaluation of main and interaction effects of formulation variables	Comprehensive analysis of variables and interactions	Requires large number of experimental runs
Fractional Factorial Design	Screening of significant variables using reduced experimental runs	Time- and cost-effective	Limited evaluation of higher-order interactions
Plackett–Burman Design	Identification of critical formulation and process variables	Efficient screening of multiple factors	Does not evaluate interaction effects
Central Composite Design (CCD)	Optimization of formulation variables and quadratic response analysis	Accurate prediction and response surface generation	More complex statistical analysis
Box–Behnken Design (BBD)	Response surface optimization with fewer experimental runs	Economical and efficient optimization	Not suitable for extreme variable combinations
Mixture Design	Optimization of formulation component proportions	Suitable for lipid and surfactant ratio optimization	Complex interpretation of mixture interactions
Response Surface Methodology (RSM)	Evaluation of relationships between variables and responses	Provides graphical optimization and predictive models	Requires statistical expertise
Taguchi Design	Robust process optimization with minimal experiments	Reduced variability and experimental cost	Limited interaction analysis
D-Optimal Design	Optimization of irregular or constrained experimental regions	Flexible experimental design	Computational complexity
Simplex Lattice Design	Optimization of multicomponent formulations	Effective for mixture-based formulations	Limited applicability to non-mixture systems

7. DOE MODELS USED IN CUBOSOME OPTIMIZATION

Various Design of Experiments (DOE) models are employed in cubosomal formulation development to identify critical variables, optimize formulation parameters, and improve product performance. Selection of an appropriate DOE model depends on the number of formulation variables, desired responses, experimental objectives, and complexity of the formulation system. DOE models assist researchers in understanding the interaction between formulation variables and achieving optimized cubosomal systems with improved particle size, entrapment efficiency, stability, and drug release characteristics.[6]

7.1 Full Factorial Design

Full factorial design is one of the most commonly used experimental designs in pharmaceutical optimization studies. In this design, all possible combinations of formulation variables and their levels are investigated systematically. It enables evaluation of both main effects and interaction effects among independent variables.

For example, in a two-factor, three-level factorial design, all possible experimental combinations are studied to determine the influence of variables such as lipid concentration and surfactant concentration on cubosomal responses.

Full factorial design provides detailed information regarding formulation behavior and variable interactions.

However, the number of experimental runs increases significantly with an increase in the number of variables and levels, making the design time-consuming and resource-intensive for complex formulations.

Despite this limitation, full factorial design is widely employed during preliminary optimization studies of cubosomal systems due to its accuracy and comprehensive statistical analysis.

7.2 Fractional Factorial Design

Fractional factorial design is a modified form of factorial design in which only a fraction of the total experimental runs is performed. This approach reduces the number of experiments while still providing valuable information regarding significant formulation variables and their interactions.

Fractional factorial designs are particularly useful during screening studies where a large number of formulation and process variables need to be evaluated efficiently. In cubosome optimization, this design helps identify critical factors such as lipid concentration, stabilizer concentration, homogenization speed, and sonication time with reduced experimental workload.

Although fractional factorial design minimizes time and cost, certain higher-order interactions may not be completely evaluated due to the reduced number of runs. Nevertheless, it remains an effective screening tool for early-stage pharmaceutical development.[7]

7.3 Plackett–Burman Design

Plackett–Burman design is a screening design primarily used for identifying the most influential variables among a large number of formulation and process parameters. This design evaluates main effects efficiently using a limited number of experimental runs without considering interaction effects between variables.

In cubosomal formulation development, Plackett–Burman design is employed to screen critical variables affecting particle size, zeta potential, drug loading, and entrapment efficiency. Variables identified as significant through this design are further optimized using advanced response surface designs such as CCD or BBD.

The major advantage of Plackett–Burman design is its ability to rapidly identify important formulation factors with minimal experimental effort. However, the inability to evaluate interaction effects represents a limitation of this design.

7.4 Central Composite Design (CCD)

Central Composite Design (CCD) is one of the most widely used response surface designs for formulation optimization in pharmaceutical research. CCD is particularly suitable for studying quadratic relationships between formulation variables and responses. The design consists of factorial points, axial points, and center points, allowing accurate estimation of curvature effects.

In cubosome optimization studies, CCD is extensively applied to optimize variables such as lipid concentration, surfactant concentration, homogenization speed, and processing temperature. Responses commonly evaluated include particle size, polydispersity index, zeta potential, entrapment efficiency, and drug release profile.

CCD generates polynomial equations and response surface plots that facilitate prediction of optimized formulation conditions. The design provides excellent optimization capability and detailed statistical analysis, making it highly suitable for nanoformulation development.

7.5 Box–Behnken Design (BBD)

Box–Behnken Design (BBD) is another widely employed response surface methodology used for pharmaceutical optimization studies. BBD requires fewer experimental runs compared with CCD and avoids experiments at extreme variable combinations, thereby reducing the risk of formulation instability.

The design evaluates the interaction and quadratic effects of independent variables on formulation responses. In cubosomal systems, BBD is commonly used to optimize formulation parameters affecting particle size, entrapment efficiency, and drug release behavior.

BBD provides efficient optimization with good prediction accuracy and reduced experimental cost. Due to its simplicity and effectiveness, it has become one of the preferred DOE models in cubosome formulation development.[8]

7.6 Mixture Design

Mixture design is specifically employed when formulation variables represent proportions of components in a mixture and the total composition remains constant. Unlike conventional factorial designs, the response in mixture design depends on the relative proportions of formulation components rather than their absolute quantities.

In cubosomal systems, mixture design is used to optimize the ratio of lipids, surfactants, stabilizers, and aqueous phase components. This design assists in determining the optimal composition required for achieving desired formulation characteristics such as particle size, stability, drug loading, and controlled drug release.

Mixture design is highly useful for complex lipid-based formulations where formulation composition significantly influences the physicochemical properties and performance of cubosomes.

7.7 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques used for modeling, analysis, and optimization of pharmaceutical formulations. RSM evaluates the relationship between multiple independent variables and one or more dependent responses through polynomial equations and graphical representations.

In cubosome optimization, RSM is extensively applied to study the combined effects of formulation variables and identify optimized formulation conditions. Graphical tools such as contour plots and three-dimensional response surface plots help visualize the influence of variables on responses and facilitate formulation optimization.

RSM enables accurate prediction of optimized formulations with minimal experimental runs and improved statistical reliability. Due to its flexibility and efficiency, RSM has become an essential optimization tool in DOE-assisted cubosomal drug delivery research.

8. CRITICAL FORMULATION VARIABLES AND OPTIMIZATION PARAMETERS

Optimization of cubosomal drug delivery systems requires careful evaluation of formulation and process variables that significantly influence the physicochemical properties and therapeutic performance of the final formulation. In Design of Experiments (DOE)-assisted optimization, formulation variables are considered independent variables or factors, while the measured outcomes are considered dependent variables or responses. Identification and optimization of these parameters are essential for developing stable, efficient, and reproducible cubosomal formulations for cancer therapy.[9]

8.1 Independent Variables (Factors)

Independent variables are formulation and process parameters intentionally varied during experimental studies to evaluate their influence on cubosomal characteristics. These factors play a crucial role in determining particle size, stability, entrapment efficiency, and drug release behavior of cubosomes.

8.1.1 Lipid Concentration

Lipid concentration is one of the most critical formulation variables in cubosome preparation because it directly affects the formation and stability of the cubic liquid crystalline structure. Commonly used lipids such as glyceryl monooleate (GMO) and phytantriol contribute to the internal architecture and drug encapsulation capacity of cubosomes.

An increase in lipid concentration generally leads to enhanced drug entrapment efficiency and prolonged drug release due to the formation of a more rigid lipid matrix. However, excessively high lipid concentrations may increase formulation viscosity and particle size, thereby affecting formulation stability and processability. Therefore, optimization of lipid concentration is essential for achieving desirable physicochemical characteristics and therapeutic performance.

8.1.2 Stabilizer Concentration

Stabilizers and surfactants are incorporated into cubosomal systems to reduce interfacial tension and prevent aggregation of nanoparticles. Poloxamer 407 is the most commonly used stabilizer in cubosome formulations because of its excellent steric stabilization properties and biocompatibility.

The concentration of stabilizer significantly influences particle size distribution, zeta potential, colloidal stability, and drug release behavior. Adequate stabilizer concentration promotes formation of stable nanosized particles with narrow size distribution. Conversely, insufficient stabilizer concentration may result in aggregation and instability, whereas excessive surfactant concentration may cause toxicity and affect drug encapsulation efficiency. Thus, optimization of stabilizer concentration is necessary for maintaining formulation stability and performance.

8.1.3 Homogenization Speed and Time

Homogenization speed and processing time are important process variables affecting particle size reduction and uniformity of cubosomal dispersions. High-pressure homogenization and sonication techniques are commonly employed during cubosome preparation to obtain nanosized particles.

An increase in homogenization speed and duration generally reduces particle size due to enhanced shear forces and turbulence. However, excessive processing may lead to instability, drug leakage, and degradation of thermosensitive compounds. Optimization of homogenization conditions is therefore essential for producing cubosomes with desirable particle size, improved stability, and enhanced drug delivery performance.

8.2 Dependent Variables (Responses)

Dependent variables or responses are measurable outcomes influenced by changes in independent variables. These responses are evaluated during DOE studies to determine optimized formulation conditions and assess formulation quality.

8.2.1 Particle Size

Particle size is one of the most important parameters influencing drug release, stability, cellular uptake, and tumor targeting efficiency of cubosomes. Nanosized cubosomes generally exhibit enhanced permeability and retention (EPR) effect, leading to improved accumulation in tumor tissues.

Smaller particle sizes provide larger surface area and enhanced cellular internalization, thereby improving therapeutic efficacy. Particle size is commonly measured using dynamic light scattering (DLS) techniques. Optimization studies aim to achieve uniform nanosized particles with improved stability and bioavailability.

8.2.2 Polydispersity Index

Polydispersity Index (PDI) indicates the uniformity of particle size distribution within the formulation. A lower PDI value reflects homogeneous particle distribution and better formulation stability.

Cubosomal formulations with low PDI values are preferred because they exhibit improved reproducibility, stability, and predictable drug release behavior. High PDI values indicate broad particle size distribution and possible aggregation, which may negatively affect formulation performance.

8.2.3 Zeta Potential

Zeta potential represents the surface charge of cubosomal particles and is an important indicator of colloidal stability. Higher absolute zeta potential values generally indicate stronger electrostatic repulsion between particles, thereby preventing aggregation.

Zeta potential also influences interaction of cubosomes with biological membranes and cellular uptake. Both positive and negative surface charges may affect biodistribution and therapeutic performance. Optimization of zeta potential is therefore important for improving physical stability and biological behavior of cubosomal systems.

8.2.4 Entrapment Efficiency

Entrapment efficiency refers to the percentage of drug successfully incorporated into cubosomal nanoparticles relative to the total amount of drug used during formulation. High entrapment efficiency is desirable because it improves therapeutic effectiveness and reduces drug wastage.

Entrapment efficiency is influenced by factors such as lipid composition, surfactant concentration, drug solubility, and processing conditions. Optimization studies aim to maximize drug loading while maintaining formulation stability and controlled drug release characteristics.

8.2.5 Drug Release Profile

Drug release profile is a critical response parameter used to evaluate the release behavior of therapeutic agents from cubosomal systems. Controlled and sustained drug release is highly desirable in cancer therapy because it maintains therapeutic drug concentrations for prolonged periods while minimizing systemic toxicity.

The release behavior of cubosomes is influenced by lipid composition, internal cubic structure, particle size, and formulation variables. In vitro drug release studies are commonly performed using diffusion-based techniques to assess release kinetics and mechanism of drug release. Optimization of formulation variables helps achieve sustained and targeted drug delivery with improved therapeutic outcomes.

9. CHARACTERIZATION OF CUBOSOMAL FORMULATIONS

Characterization of cubosomal formulations is an essential step in evaluating their physicochemical properties, structural integrity, stability, and drug delivery performance. Proper characterization helps in understanding the influence of formulation variables on cubosome quality and ensures reproducibility of optimized formulations. Various analytical techniques are employed to evaluate particle size, morphology, surface charge, drug encapsulation, thermal behavior, crystallinity, compatibility, drug release, and stability of cubosomal systems.[10]

9.1 Particle Size Analysis

Particle size analysis is one of the most important characterization parameters for cubosomal formulations because it significantly influences drug release behavior, stability, cellular uptake, and biodistribution. Nanosized cubosomes facilitate enhanced permeability and retention (EPR) effect, which improves accumulation of therapeutic agents in tumor tissues.

Particle size is commonly measured using Dynamic Light Scattering (DLS) techniques. Parameters such as average particle diameter and particle size distribution are determined to evaluate formulation uniformity. Smaller particle sizes generally provide improved drug absorption, enhanced cellular internalization, and better therapeutic efficacy.

Optimized cubosomal formulations usually exhibit particle sizes within the nanometer range with narrow size distribution.

9.2 Surface Charge Measurement

Surface charge measurement is performed to determine the zeta potential of cubosomal particles, which indicates the stability of colloidal dispersions. Zeta potential reflects the electrical charge present on the particle surface and influences particle aggregation, stability, and interaction with biological membranes.

Cubosomal formulations with high positive or negative zeta potential values exhibit improved physical stability due to electrostatic repulsion between particles. Zeta potential analysis is generally carried out using electrophoretic light scattering instruments. Surface charge also plays an important role in cellular uptake, biodistribution, and targeting efficiency of cubosomal systems.

9.3 Morphological Evaluation

Morphological evaluation is performed to examine the shape, surface characteristics, and internal structure of cubosomal formulations. Imaging techniques provide valuable information regarding particle architecture, aggregation behavior, and structural organization.

9.3.1 Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is widely used for evaluating the morphology and internal structure of cubosomes at high resolution. TEM images provide detailed information regarding particle size, shape, and cubic internal architecture. Cubosomes generally appear as discrete nanosized particles with well-defined cubic or spherical morphology. TEM analysis also assists in confirming successful formation of cubic liquid crystalline structures.

9.3.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is employed to analyze the surface morphology and topographical characteristics of cubosomal particles. SEM provides three-dimensional surface images that help evaluate particle shape, aggregation, and surface texture. SEM analysis is useful for assessing the uniformity and physical appearance of cubosomal dispersions.

9.4 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a thermal analysis technique used to study the thermal behavior and physical state of drugs and excipients in cubosomal formulations. DSC helps determine melting point, crystallinity, phase transition, and drug–excipient interactions.

Changes in thermal peaks may indicate successful drug incorporation into the cubosomal matrix or transformation of crystalline drugs into amorphous forms. DSC analysis is also useful for evaluating formulation stability and compatibility between formulation components.[11]

9.5 X-Ray Diffraction (XRD)

X-Ray Diffraction (XRD) analysis is performed to evaluate the crystalline or amorphous nature of drugs and lipids present in cubosomal systems. XRD patterns provide information regarding structural arrangement and phase behavior of formulation components.

Reduction or disappearance of characteristic crystalline peaks in optimized formulations indicates successful encapsulation of the drug within the lipid matrix and possible conversion into an amorphous state. Such transformations may improve drug solubility and bioavailability. XRD also assists in confirming the presence of cubic liquid crystalline phases.

9.6 FTIR Studies

Fourier Transform Infrared Spectroscopy (FTIR) is used to evaluate chemical compatibility and possible interactions between drugs and formulation excipients. FTIR spectra provide information regarding functional groups and molecular interactions within cubosomal systems.

Characteristic peaks corresponding to drugs and excipients are analyzed to identify any chemical changes or incompatibilities during formulation development. Absence of significant peak shifts or disappearance of characteristic peaks generally indicates compatibility between formulation components and successful incorporation of the drug into cubosomes.[12]

9.7 In-Vitro Drug Release Studies

In-vitro drug release studies are conducted to evaluate the release behavior of therapeutic agents from cubosomal formulations under simulated physiological conditions. Controlled and sustained drug release is one of the major advantages of cubosomal systems in cancer therapy.

Drug release studies are commonly performed using dialysis membrane diffusion techniques or dissolution apparatus.

Release data are analyzed using kinetic models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to understand the mechanism of drug release. Optimized cubosomal formulations generally exhibit prolonged and controlled release profiles, which improve therapeutic efficacy and reduce dosing frequency.

9.8 Stability Studies

Stability studies are essential for evaluating the physical, chemical, and functional stability of cubosomal formulations during storage. Stability testing helps determine shelf life, storage conditions, and formulation robustness.

Cubosomal formulations are typically stored under different temperature and humidity conditions according to International Council for Harmonisation (ICH) guidelines. Parameters such as particle size, zeta potential, entrapment efficiency, drug content, and physical appearance are periodically evaluated during storage. Stable cubosomal formulations should maintain their physicochemical properties and therapeutic performance without significant aggregation, drug leakage, or phase separation over time.

10. APPLICATIONS OF CUBOSOMES IN CANCER THERAPY

Cubosomes have emerged as promising nanocarriers in cancer therapy due to their unique bicontinuous cubic structure, high drug-loading capacity, biocompatibility, and controlled drug release characteristics. Their ability to encapsulate a wide range of therapeutic agents and improve drug targeting has attracted considerable attention in oncology research. Cubosomal systems enhance therapeutic efficacy while minimizing systemic toxicity, making them suitable candidates for advanced cancer treatment strategies.[13]

10.1 Targeted Drug Delivery

Targeted drug delivery is one of the most significant applications of cubosomes in cancer therapy. Conventional chemotherapy often results in nonspecific distribution of anticancer drugs, leading to severe toxicity in healthy tissues. Cubosomes overcome this limitation by facilitating selective delivery of therapeutic agents to tumor sites.

Due to their nanosized dimensions, cubosomes can exploit the enhanced permeability and retention (EPR) effect, enabling passive accumulation within tumor tissues. Surface modification of cubosomes with ligands such as antibodies, peptides, folic acid, and transferrin further enhances active targeting toward specific cancer cells. Targeted cubosomal systems improve intracellular drug uptake, increase therapeutic efficacy, and reduce adverse effects associated with conventional chemotherapy.

10.2 Controlled and Sustained Drug Release

Cubosomes possess a highly ordered bicontinuous cubic structure that allows controlled and sustained release of encapsulated drugs. The interconnected aqueous channels and lipid bilayers regulate drug diffusion and prolong drug release over extended periods.

Controlled drug release offers several therapeutic advantages, including maintenance of effective drug concentrations, reduction in dosing frequency, improved patient compliance, and minimization of systemic toxicity. Sustained release behavior is particularly beneficial in cancer therapy because it ensures prolonged exposure of tumor cells to anticancer agents, thereby enhancing therapeutic outcomes and reducing fluctuations in plasma drug concentrations.

10.3 Delivery of Hydrophilic and Hydrophobic Drugs

One of the major advantages of cubosomes is their ability to simultaneously encapsulate hydrophilic, hydrophobic, and amphiphilic therapeutic agents. Hydrophilic drugs are accommodated within the aqueous channels, while hydrophobic drugs are incorporated into the lipid bilayer regions of the cubosomal structure.

This dual drug-loading capability broadens the pharmaceutical applications of cubosomes and improves the solubility and stability of poorly water-soluble anticancer drugs. Cubosomal formulations also protect encapsulated drugs from chemical and enzymatic degradation, thereby enhancing bioavailability and therapeutic performance. Consequently, cubosomes serve as versatile carriers for a wide variety of anticancer compounds.

10.4 Cubosomes for Chemotherapeutic Agents

Cubosomes have been extensively investigated for delivery of various chemotherapeutic agents such as doxorubicin, paclitaxel, cisplatin, curcumin, docetaxel, and 5-fluorouracil. Encapsulation of chemotherapeutic drugs into cubosomal systems enhances drug solubility, stability, and tumor targeting efficiency while reducing systemic toxicity.

Cubosomal formulations improve cellular uptake of anticancer agents through endocytosis and facilitate sustained intracellular drug release. Several studies have demonstrated enhanced cytotoxicity and improved anticancer activity of cubosome-loaded chemotherapeutic agents compared with conventional drug formulations. Additionally, cubosomes may overcome multidrug resistance by improving intracellular drug accumulation within cancer cells.

10.5 Gene and siRNA Delivery

Gene therapy has emerged as a promising strategy for cancer treatment, and cubosomes have shown significant potential as carriers for nucleic acid delivery. Their lipid-based structure and biocompatibility facilitate efficient encapsulation and protection of genetic materials such as DNA, RNA, plasmids, and small interfering RNA (siRNA).[14]

Cubosomal systems enhance cellular uptake and intracellular transport of nucleic acids while protecting them from enzymatic degradation. siRNA-loaded cubosomes can selectively silence cancer-related genes and inhibit tumor growth. Moreover, surface-modified cubosomes improve targeted gene delivery and transfection efficiency. These properties make cubosomes attractive carriers for gene therapy and RNA-based cancer therapeutics.

10.6 Theranostic Applications

Theranostics refers to the combination of diagnostic and therapeutic functions within a single delivery system. Cubosomes have gained increasing attention in theranostic applications due to their ability to co-deliver imaging agents and therapeutic drugs simultaneously.

Cubosomal theranostic systems may incorporate fluorescent dyes, magnetic nanoparticles, or contrast agents along with anticancer drugs to enable simultaneous tumor imaging, diagnosis, and therapy. Such multifunctional systems facilitate real-time monitoring of drug distribution, treatment response, and disease progression. Theranostic cubosomes represent an advanced and personalized approach to cancer management with improved diagnostic accuracy and therapeutic efficiency.

11. RECENT ADVANCES IN DOE-ASSISTED CUBOSOMAL SYSTEMS

Recent advancements in cubosomal drug delivery systems have significantly improved their therapeutic potential in cancer treatment. Integration of Design of Experiments (DOE) with modern nanotechnological approaches has enabled systematic optimization of formulation variables, improved targeting efficiency, enhanced drug loading, and better control over drug release behavior. Emerging innovations such as surface modification, ligand conjugation, stimuli-responsive systems, dual drug loading, artificial intelligence-assisted optimization, and personalized nanomedicine have expanded the pharmaceutical applications of cubosomes in oncology.[15]

11.1 Surface-Modified Cubosomes

Surface modification of cubosomes has emerged as an effective strategy for improving stability, circulation time, targeting efficiency, and cellular uptake. Surface-functionalized cubosomes are developed by incorporating polymers, surfactants, or biocompatible coatings onto the nanoparticle surface.

Polyethylene glycol (PEG) coating is one of the most commonly employed surface modification techniques because it enhances steric stabilization and prolongs systemic circulation by reducing opsonization and macrophage uptake. Surface-modified cubosomes also improve mucoadhesion, bioavailability, and tumor penetration. DOE-assisted

optimization plays an important role in determining the ideal concentration of surface modifiers and processing parameters to achieve desired physicochemical characteristics and therapeutic performance.

11.2 Ligand-Conjugated Cubosomes

Ligand-conjugated cubosomes are advanced targeted nanocarriers designed to selectively deliver therapeutic agents to cancer cells. Surface conjugation of ligands such as folic acid, antibodies, peptides, transferrin, and aptamers enhances receptor-mediated uptake of cubosomes by tumor tissues.

These targeted systems improve drug accumulation at the tumor site while reducing off-target toxicity and systemic adverse effects. DOE approaches are extensively used to optimize ligand concentration, conjugation efficiency, particle size, and drug release behavior. Ligand-targeted cubosomes have shown enhanced anticancer activity, increased cellular internalization, and improved therapeutic efficacy in various cancer models.

11.3 Stimuli-Responsive Cubosomes

Stimuli-responsive cubosomes, also known as smart cubosomes, are designed to release drugs in response to specific internal or external stimuli such as pH, temperature, enzymes, magnetic fields, or light. Tumor microenvironments often exhibit acidic pH and abnormal enzymatic activity, which can be exploited for site-specific drug release.

pH-sensitive cubosomes release anticancer drugs preferentially in acidic tumor tissues, thereby improving targeting efficiency and minimizing damage to healthy cells. Thermoresponsive and photoresponsive cubosomal systems also enable controlled release under external stimuli. DOE-assisted optimization helps identify suitable formulation compositions and process parameters required for developing efficient stimuli-responsive delivery systems with predictable release behavior.

11.4 Dual Drug-Loaded Cubosomes

Dual drug-loaded cubosomes have gained significant attention for combination cancer therapy. These systems simultaneously encapsulate two therapeutic agents with different mechanisms of action, thereby enhancing synergistic anticancer effects and overcoming multidrug resistance.

Cubosomes can effectively co-deliver hydrophilic and hydrophobic drugs due to their unique bicontinuous cubic structure. Combination therapy using dual drug-loaded cubosomes improves therapeutic efficacy, reduces required drug doses, and minimizes systemic toxicity. DOE techniques are employed to optimize drug ratios, lipid composition, entrapment efficiency, and release kinetics to achieve balanced and controlled delivery of multiple therapeutic agents.

11.5 AI and Machine Learning-Assisted Optimization

Artificial intelligence (AI) and machine learning (ML) technologies are increasingly being integrated with DOE for advanced pharmaceutical optimization. AI-assisted optimization enables rapid prediction of formulation behavior, identification of critical variables, and reduction in experimental workload.

Machine learning algorithms analyze large experimental datasets to establish predictive models for particle size, entrapment efficiency, stability, and drug release characteristics. Integration of AI with DOE enhances formulation accuracy, minimizes experimental errors, and accelerates nanoformulation development. These advanced

computational approaches are expected to revolutionize cubosomal drug delivery research by enabling intelligent and data-driven formulation optimization.

11.6 Personalized Nanomedicine Approaches

Personalized nanomedicine aims to develop patient-specific therapeutic systems based on individual genetic, molecular, and physiological characteristics. Cubosomes provide an adaptable platform for personalized cancer therapy because of their ability to encapsulate multiple therapeutic agents and targeting ligands.

DOE-assisted formulation development enables customization of cubosomal systems according to patient-specific treatment requirements. Personalized cubosomal therapies may improve therapeutic response, reduce adverse effects, and enhance treatment precision. Recent advances in genomics, biomarker identification, and computational modeling have further accelerated the development of individualized nanomedicine approaches for cancer management.

12. QUALITY BY DESIGN (QBD) AND REGULATORY PERSPECTIVES

Quality by Design (QbD) has emerged as a systematic and science-based approach for the development of safe, effective, and high-quality pharmaceutical products. In nanomedicine, particularly in cubosomal drug delivery systems, QbD plays an essential role in understanding formulation variables, optimizing process parameters, and ensuring consistent product quality throughout the product lifecycle. Integration of Design of Experiments (DOE) with QbD principles facilitates identification of critical variables, establishment of design space, and development of robust cubosomal formulations for cancer therapy. Regulatory agencies such as the United States Food and Drug Administration (FDA) and the International Council for Harmonisation (ICH) strongly encourage the implementation of QbD approaches in pharmaceutical development to improve product quality, safety, and manufacturing efficiency.[16]

12.1 QbD Principles in Nanomedicine

QbD is defined as a systematic approach to pharmaceutical development that begins with predefined objectives and emphasizes product and process understanding based on scientific principles and risk management. The fundamental concept of QbD is to build quality into the product during formulation and process development rather than relying solely on final product testing.

In nanomedicine, QbD involves identification of formulation variables and process parameters that significantly influence the physicochemical properties and therapeutic performance of nanosystems. Application of QbD in cubosomal formulation development helps achieve consistent particle size, entrapment efficiency, zeta potential, stability, and controlled drug release behavior.

The QbD framework generally includes defining the Quality Target Product Profile (QTPP), identifying Critical Quality Attributes (CQAs), performing risk assessment, establishing design space, and implementing control strategies. DOE is widely employed within the QbD framework to optimize formulation variables and ensure reproducible product quality.

12.2 Critical Quality Attributes (CQAs)

Critical Quality Attributes (CQAs) are physical, chemical, biological, or microbiological properties that must be controlled within specified limits to ensure product quality, safety, and efficacy. Identification of CQAs is a key step in QbD-based pharmaceutical development.

For cubosomal drug delivery systems, important CQAs commonly include particle size, polydispersity index, zeta potential, entrapment efficiency, drug loading capacity, morphology, drug release profile, and physical stability.

These attributes directly influence biodistribution, cellular uptake, targeting efficiency, therapeutic efficacy, and shelf life of the formulation.

DOE-assisted optimization helps establish relationships between formulation variables and CQAs, enabling researchers to identify critical process parameters that significantly impact formulation quality. Maintaining CQAs within acceptable ranges is essential for achieving robust and reproducible cubosomal systems suitable for clinical application.

12.3 Risk Assessment and Design Space

Risk assessment is an important component of QbD that involves systematic identification, analysis, and control of factors that may affect product quality and performance. Risk assessment tools such as Failure Mode and Effects Analysis (FMEA), Ishikawa diagrams, and risk ranking methods are commonly used in pharmaceutical development.

In cubosomal systems, risk assessment assists in identifying formulation variables and process parameters that significantly influence CQAs. Parameters such as lipid concentration, surfactant concentration, homogenization speed, sonication time, and temperature are evaluated to determine their impact on formulation performance.

Design space refers to the multidimensional combination of formulation variables and process parameters that consistently produce products meeting predefined quality criteria. DOE plays a critical role in establishing design space by generating mathematical models and response surface plots that predict optimized formulation conditions.

Operating within the approved design space ensures consistent product quality and provides greater manufacturing flexibility.

12.4 FDA and ICH Regulatory Considerations

Regulatory agencies including the FDA and ICH have established guidelines encouraging implementation of QbD principles in pharmaceutical development and manufacturing. These guidelines aim to improve product quality, process understanding, risk management, and regulatory flexibility.

ICH Q8 guideline focuses on pharmaceutical development and emphasizes scientific understanding of formulation and manufacturing processes. ICH Q9 addresses quality risk management and provides systematic approaches for identifying and controlling potential risks affecting product quality. ICH Q10 describes pharmaceutical quality systems that support continuous process improvement and lifecycle management.

For nanomedicine and cubosomal formulations, regulatory evaluation includes assessment of physicochemical characterization, stability, safety, toxicity, manufacturing reproducibility, and therapeutic performance. Due to the

complexity of nanosystems, regulatory approval of nanomedicines often requires comprehensive characterization and robust quality control strategies.

Implementation of QbD and DOE approaches facilitates regulatory acceptance by demonstrating scientific understanding, process robustness, and consistent product quality. Consequently, QbD-based cubosomal systems represent a promising strategy for the successful development and commercialization of advanced cancer therapeutics.

13. CHALLENGES AND LIMITATIONS

Despite the promising potential of cubosomal drug delivery systems in cancer therapy, several challenges and limitations hinder their large-scale development and clinical translation. Factors related to manufacturing complexity, physical stability, toxicity, regulatory approval, and commercialization remain major concerns in the development of cubosome-based nanomedicines. Addressing these limitations through advanced optimization strategies such as Design of Experiments (DOE) and Quality by Design (QbD) is essential for successful industrial and clinical applications.[17]

13.1 Scale-Up and Manufacturing Challenges

Large-scale production of cubosomal formulations remains one of the major obstacles in pharmaceutical manufacturing. Preparation methods such as high-pressure homogenization, microfluidization, and sonication require specialized equipment and strict process control to achieve consistent product quality. Variations in process parameters such as homogenization pressure, temperature, mixing speed, and processing time can significantly affect particle size, entrapment efficiency, and stability of cubosomal systems.

The highly viscous nature of cubic liquid crystalline phases also creates difficulties during handling, processing, and large-scale production. Reproducibility between laboratory-scale and industrial-scale batches remains challenging because minor process variations may alter the physicochemical properties of cubosomes. In addition, high production costs and complex manufacturing procedures may limit commercial feasibility. Therefore, systematic optimization and process standardization are necessary to improve scalability and industrial applicability of cubosomal systems.

13.2 Stability Issues

Physical and chemical stability is a critical concern in cubosomal formulation development. Cubosomal dispersions may undergo aggregation, fusion, phase separation, or structural transformation during storage, which can adversely affect therapeutic performance and shelf life. Factors such as temperature, pH, ionic strength, and storage conditions significantly influence formulation stability.

Drug leakage from cubosomal systems during storage is another important limitation that may reduce drug loading efficiency and therapeutic efficacy. Furthermore, oxidation and hydrolysis of lipid components can lead to degradation of the formulation over time. Maintaining long-term stability while preserving nanoscale particle size and internal cubic structure remains a major challenge in cubosome development.

Appropriate selection of stabilizers, optimization of formulation variables, and implementation of controlled storage conditions are essential for improving stability and extending shelf life of cubosomal formulations.

13.3 Toxicity and Biocompatibility Concerns

Although cubosomes are generally considered biocompatible due to their lipid-based composition, potential toxicity associated with formulation components and nanoscale properties cannot be completely ignored. Surfactants and stabilizers used in cubosome preparation may exhibit cytotoxicity at higher concentrations and affect cellular integrity.

Nanoparticles may also interact with biological systems in unpredictable ways, leading to immunogenicity, oxidative stress, inflammation, or accumulation in non-target organs. Surface properties, particle size, charge, and composition significantly influence the biological behavior and safety profile of cubosomal systems.

Comprehensive in-vitro and in-vivo toxicity evaluations are therefore necessary to assess biocompatibility, biodistribution, pharmacokinetics, and long-term safety of cubosomal formulations. Optimization of formulation composition and surface characteristics is essential for minimizing toxicity and improving therapeutic safety.

13.4 Regulatory and Commercialization Challenges

Regulatory approval of nanomedicines remains a complex and evolving process due to the unique physicochemical characteristics and biological behavior of nanosystems. Lack of standardized regulatory guidelines specific to cubosomal formulations creates challenges in product evaluation, quality assessment, and approval procedures.

Regulatory agencies require extensive characterization of nanoparticle properties, manufacturing reproducibility, stability, toxicity, pharmacokinetics, and therapeutic efficacy before clinical approval. The complexity associated with characterization techniques and quality control of cubosomal systems further complicates regulatory submissions.

Commercialization of cubosome-based products is also limited by high development costs, manufacturing complexity, limited clinical studies, and scalability issues. Intellectual property concerns and market competition may additionally affect commercial success. Therefore, collaborative efforts among researchers, pharmaceutical industries, and regulatory agencies are required to establish standardized guidelines and facilitate successful translation of cubosomal technologies from laboratory research to clinical and commercial applications.

Table 6: Regulatory Guidelines Related to Nanomedicine and DOE.

Guideline/Regulatory Framework	Regulatory Agency	Scope and Relevance
ICH Q8 (Pharmaceutical Development)	ICH	Emphasizes Quality by Design (QbD) and systematic pharmaceutical development
ICH Q9 (Quality Risk Management)	ICH	Provides principles for risk assessment and quality management
ICH Q10 (Pharmaceutical Quality System)	ICH	Focuses on lifecycle quality management and continuous improvement
ICH Q11 (Development and Manufacture of Drug Substances)	ICH	Guidance on development and manufacturing process control
FDA Guidance on Nanotechnology	FDA	Evaluation and regulation of nanotechnology-based pharmaceutical products
FDA Quality by Design Guidance	FDA	Encourages DOE-based optimization and process understanding
EMA Reflection Paper on Nanomedicines	EMA	Regulatory considerations for nanotechnology-based medicinal products

OECD Guidelines for Nanomaterials	OECD	Safety evaluation and toxicological assessment of nanomaterials
ISO Standards for Nanotechnology	ISO	Standardization of characterization and terminology in nanotechnology
GMP Guidelines	WHO/FDA/EMA	Ensures quality, safety, and consistency in pharmaceutical manufacturing
SUPAC Guidelines	FDA	Scale-up and post-approval changes in pharmaceutical manufacturing
PAT (Process Analytical Technology) Guidance	FDA	Real-time monitoring and control of pharmaceutical

14. FUTURE PERSPECTIVES AND EMERGING TRENDS

Cubosomal drug delivery systems have demonstrated significant potential in cancer therapy owing to their unique nanostructure, high drug-loading capacity, controlled release characteristics, and targeting capabilities. Continuous advancements in nanotechnology, pharmaceutical sciences, and computational approaches are expected to further expand the applications of cubosomes in modern oncology. Future research is primarily focused on developing multifunctional cubosomal systems with improved targeting efficiency, enhanced therapeutic performance, and reduced systemic toxicity.[18]

One of the most promising emerging trends involves the integration of artificial intelligence (AI), machine learning (ML), and computational modeling with Design of Experiments (DOE) for intelligent formulation optimization. These advanced computational tools can predict formulation behavior, identify critical process parameters, and accelerate development of optimized cubosomal systems with minimal experimental workload. AI-assisted optimization is expected to improve reproducibility, reduce formulation development time, and enhance manufacturing efficiency.

Surface-engineered and ligand-targeted cubosomes are also gaining considerable attention for site-specific drug delivery. Functionalization with antibodies, peptides, aptamers, folic acid, and other targeting ligands can significantly improve selective accumulation of therapeutic agents in tumor tissues while minimizing damage to healthy cells. Similarly, stimuli-responsive or smart cubosomes capable of responding to pH, temperature, enzymes, magnetic fields, or light represent an advanced strategy for controlled and on-demand drug release.

The development of theranostic cubosomes combining therapeutic and diagnostic functions within a single platform is another emerging area in personalized cancer management. These multifunctional systems enable simultaneous imaging, monitoring, and treatment of tumors, thereby improving diagnostic accuracy and therapeutic outcomes.

Future research is also expected to focus on gene delivery, RNA therapeutics, immunotherapy, and combination therapy using cubosomal platforms. Advances in Quality by Design (QbD), continuous manufacturing, and scalable production technologies may facilitate industrial translation and commercialization of cubosome-based formulations. Furthermore, establishment of harmonized regulatory guidelines and comprehensive clinical studies will be essential for successful clinical application of cubosomal nanomedicines.

Overall, DOE-assisted cubosomal systems are anticipated to play a significant role in the future of precision medicine and targeted cancer therapy due to their versatility, adaptability, and therapeutic potential.

15. CONCLUSION

Cubosomes have emerged as highly promising lipid-based nanocarriers for cancer therapy because of their unique bicontinuous cubic structure, excellent biocompatibility, high drug-loading capacity, and controlled drug release properties. Their ability to encapsulate hydrophilic, hydrophobic, and amphiphilic therapeutic agents makes them versatile carriers for a wide range of anticancer applications. Cubosomal systems improve drug solubility, enhance tumor targeting, prolong circulation time, and reduce systemic toxicity associated with conventional chemotherapy.

Design of Experiments (DOE) has become an essential optimization tool in cubosomal formulation development by enabling systematic evaluation of formulation and process variables. DOE-assisted approaches facilitate identification of critical factors, optimization of physicochemical properties, minimization of experimental runs, and development of robust and reproducible cubosomal systems. Various experimental designs such as factorial design, Box–Behnken design, Central Composite Design, and Response Surface Methodology have been successfully applied in cubosome optimization studies.[19]

Recent advancements including ligand-targeted cubosomes, surface-modified systems, stimuli-responsive formulations, dual drug-loaded carriers, and AI-assisted optimization have significantly expanded the pharmaceutical applications of cubosomes in oncology. Integration of Quality by Design (QbD) principles further improves process understanding, product quality, and regulatory compliance.

Despite several advantages, challenges related to large-scale manufacturing, stability, toxicity evaluation, and regulatory approval still remain. Continued research focusing on advanced optimization strategies, scalable production methods, and clinical translation is necessary to overcome these limitations.

In conclusion, DOE-assisted cubosomal drug delivery systems represent a promising and innovative platform for targeted cancer therapy with the potential to improve therapeutic efficacy, patient compliance, and clinical outcomes in future oncological treatments.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this review article.

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