ijlpr 2020; doi 10.22376/ijpbs/lpr.2021.11.1.P124-135

International Journal of Life science and Pharma Research

Review Article

Industrial Pharmacy

Past Decade Work Done on Cubosomes and its Factorial Design: A Fast Track Information for Researchers

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Abstract: The objective of this review is to explore the past work done on Cubosomes as drug delivery systems by factorial design. Cubosomes are Nanoparticulate systems made of amphiphilic lipids at a certain percentage, known as liquid crystals. They have tightly packed honeycomb structures twisted into 3D bilayers. Cubosomes can capture all categories of the lipophilic, hydrophilic, and amphiphilic substances irrespective of their affinity, by that they fit for delivering all range of drugs with ease. Many works in recent days are concentrating on Cubosomes for drug delivery, as it suits all ranges of drugs without much difficulty. Cubosomes acts as a carrier in drug delivery for a wide range of drugs and protects them from degradation issues like hydrolysis, oxidation, and others. Moreover, numerous studies have established the benefits of Cubosomes in nanotechnology, prolonged-release, and also enhanced bioavailability. This article reviews about the past work done on Cubosomes using factorial design. Additionally, many studies need to be performed for the optimization of Cubosomes for artificial cells, and biosensors, etc. Moreover, the rational design of Cubosomes for biomedical applications need to be developed. A widespread literature assessment revealed that many reviews and research attempts were made on Cubosomes, but no review article is still available in bringing the attempts made on Cubosomes by factorial design on a single platform. The factorial approach is used to optimize the formulation, which is acceptable and used in the current scenario in optimizing the formulations. So, the authors made widespread work by referring to peer-review journals, periodicals, magazines, and succeeded in bringing work done on Cubosomes in the last ten years by using factorial design. The study concludes and gives a quick reference to the young researchers to get literature on earlier successive attempts done on Cubosomes by factorial design.

Keywords: Cubosomes, delivery, factorial, review, design

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Recieved On 31 March 2020
Revised On 24 October 2020
Accepted On 30 October 2020
Published On 04 January 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Shravani Y, Hindustan Abdul Ahad, Chinthaginjala Haranath, Bhumireddy gari Poojitha, Syed Rahamathulla, Aswarthanarayana Rupasree , Past Decade Work Done On Cubosomes Using Factorial Design: A Fast Track Information for Researchers..(2021).Int. J. Life Sci. Pharma Res.11(1), P124-135 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.1.P124-135

1. INTRODUCTION

Cubosomes are structurally similar to nanoparticles composed of amphiphilic lipids as bicubic crystals with the liquid phase. Hydrating a polar lipid or surface-active agent which produces cubic phase, later disbanding a solid phase into finer subdivisions forms a Cubosomes.[1] Cubosomes have solid-like consistency with distinct features for drug loading. Cubosomes are thermodynamically steady, and they have honeycomb assembly that is firmly crowded warped into 3D bilayers (Fig.1).[2] The Cubosome structure makes more drug loading capacity.[3] Cubosome dispersions are bioadhesive and biocompatible. Owing to these characteristics, Cubosomes are multipurpose structures, administered by various routes. Amphiphilic drug structures arrangements under some special circumstances and produce a great drug engulfing moieties that can be adopted in drug delivery systems. This approach has more success rates in vitro, but protecting the drug loading activitiesin *invivo* is a challenging one. These issues are overwhelmed by formulating these drugs as Cubosomes.

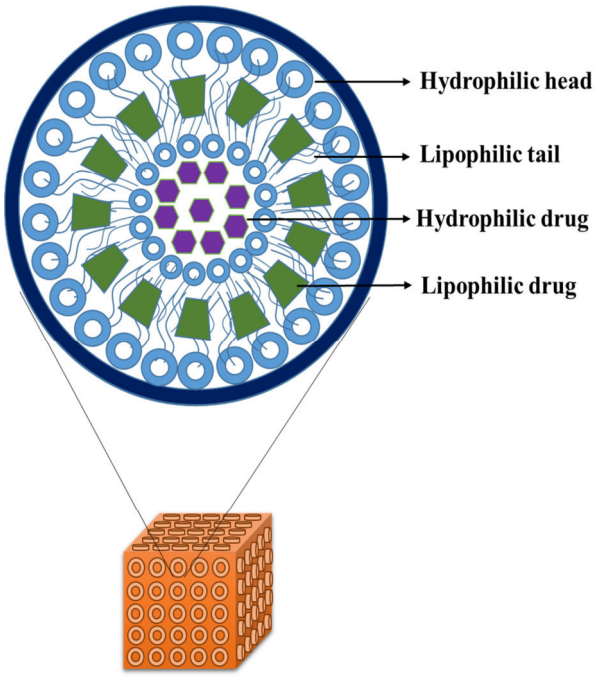

Fig.1. Assembly of cubosomes[4]

1.1. ADVANTAGES

The merits of Cubosomes are illustrated below.[4]

- Economical.
- Non-toxic
- Biocompatible
- Simple to prepare
- Brilliant bioadhesive properties
- Skin permeation enhancement activity
- Thermodynamically stable
- Encapsulating hydrophilic, lipophilic, and amphiphilic drugs
- Targeted and controlled release
- High drug loading.

1.2. DISADVANTAGES

The demerits with Cubosomes are explained as follows.[5]

- A low trap of polar drugs
- The high viscosity limits its manufacturing in bulk scale.

2. METHODS OF PREPARATION OF CUBOSOMES

Cubosomes can be prepared by two diverse techniques namely top-down technique and bottom-up techniques[6]. In the former approach, lipid and stabilizer were dispersed in the aqueous phase, then agitated (by sonication/ high-pressure homogenization/ spontaneous emulsification/ spray drying) at high shear. Whereas in the latter approach, for a lipid and stabilizer mixture a hydrotrope (E.g., Ethanol, glycerin, propylene glycol, polyethylene glycol, and toluene sulphonate, etc.) is added, later dispersed in aqueous phase by low-speed agitation methods same as in the former technique.

3. EVALUATION OF CUBOSOMES

The following parameters to be studied for Cubosomal formulation (CF) to assure the quality of cubosomes.

3.1. VISUAL SCRUTINY

The CF are visually judged using a microscope for ocular look viz., colour, turbidity, foreign particles, and homogeneity, etc. This scrutiny assessment helps in excluding poor/ruptured dispersions for auxiliary studies. Normally these dispersions visually are observed as a milky white mixture[7].

3.2. SHAPE AND SURFACE MORPHOLOGY

Simple microscopy gives a basic outline of Cubosomes. The surface and deep morphology should be studied by sophisticated instruments and tactics. Scanning and Transmission electron microscopy was espoused to judge the shape and surface of the Cubosomes. Cubosomes were kept

in brass pins and freeze in propane. Later the sample was sublimed from -140 to -90°C, coated with platinum, and viewed at 3 kV. Transmission Electron Microscopy at high resolution helps in finding the morphology and channels of Cubosomes, which need negative staining and the formulation lattice was dried and noticed under a transmission electron microscope[8]. The images at various magnifications were taken at room temperature.

3.3. PARTICLE SIZE DISTRIBUTION

Uniformity in particle size of Cubosomes is vital in syringeability for their administration. Laser light scattering using Zeta sizer was utilized for this purpose. In this, the CF was diluted with a suitable solvent, attuned to light scattering intensity of ~300 Hz and measured at room temperature, and the zeta potential and polydispersity index (PDI) were assessed[9]. The particle sizes were assessed before and after ultracentrifugation. These experiments to be conducted multiple times to get a mean size value. The mean diameter of Cubosomes must be ranged from 0.3-10µm, and PDI values < 1 are said to be desirable for a stable CF.

3.4. ZETA POTENTIAL

The zeta potential (ZP) of Cubosomes plays a vivacious role in envisaging the long term stability of the Cubosome formulation. The degree of ZP designates the degree of electronic repulsion between dispersed particles. ZP measurements help in finding and confirming a negative charge on the surface of the Cubosomes. High ZP values of Cubosome particles make them electrically deter each other without particle accretion[10]. The more the repulsion between the particles less will be the chances of clumping. The ZP value for Cubosomes which can be anticipated is ≤-30mV.

3.5. ENTRAPMENT EFFICIENCY

Ultrafiltration technique or dialysis method is adopted for determining the entrapment efficiency (EE) of Cubosomes, and the un-entrapped drug concentration can be measured using a spectrophotometer. The CF was kept in a dialysis bag, then it kept in a flask containing water. The complete set is rotated in an incubator shaker (100 rpm at 37°C). After 4-6 h, the drug released was analysed and the EE of the CF using the formula[11].

$$EE\ \% = \frac{Total\ drug - Free\ drug}{Total\ drug} X100\%$$

3.6. MEASUREMENT OF DRUG RELEASE

A pressure ultrafiltration tactic is adopted, which involves using a stirred pressure ultrafiltration cell fitted with a cellulose membrane at equipment environment temperature. In this tactic, the drug release rate was determined after the separation of free drug from CF. The CF kept in a dialysis tubing for 15-30 min containing a buffer (pH 7.4) at 37±0.5°C (stirred at 50 rpm). The samples were introverted periodically and analysed for drug content[12]. Sink conditions to be upheld during the operation.

3.7. STABILITY STUDIES

The morphological and drug content of the CF were estimated after stressed storage conditions as per ICH guidelines, which confirms the stability of the formulation. Normally, the CF (filled in vials) and kept in stability chambers and 40±2°C temperature, and 75±5% RH was maintained for 6 months. At regular intervals, the drug content and other restraints were judged[13]. The CF should hold all the morphological and chemical constraints even after storing at elevated environmental conditions. These tests also performed at 25±2°C temperature and 60±5% RH (long term) or 30±2°C temperature and 65±5% RH (intermediate).

4. LATEST FEAT IN CUBOSOMS

The latest updates on CF were itemized as below.

4.1. STABILIZERS FOR CUBOSOMES

The leakage and aggregation of CF is a major obstacle in their stability. This issue can be overcome with the inclusion of stabilizers. Studies divulge that surfactants act as stabilizers for refining stability of CF, as they foil accretion of particles into a bulk cubic phase. The selection of a stabilizing/dispersing agent is decisive and should contribute in the lipid–water muster deprived of troublesome in the cubic liquid crystallinity of the structure. Among them popularly lamellar forming agents like Poloxamers (F-188, F-338 & F-407), Propylene glycol, polyethylene glycol-400 & 2000, and Polysorbate 80; on the other hand sponge phase producing water-miscible solvent i.e., 2-meth-yl-2, 4-pentanediol were proved as stabilizers[14].

4.2. TOXICITY STUDIES

Some studies revealed that the CF containing stabilizers viz., Pluronics F108 and F127, and lipid PEG derivatives may cause cytotoxicity. These studies appraised using MTT assay/hemolytic assay/Alamar Blue cell viability assay[15, 16, 17, 18, 19]. Among the Poloxamers, Poloxamer F-127 stands on the first row in causing cell toxicity on cell lines when given >25 µg/ml (on Chinese hamster ovary and alveolar basal epithelial cells), >40 µg/ml (on human embryonic kidney cells), >70 µg/ml (on fibroblast cells) and haemolysis >1 µg/ml (on RBC). Even Poloxamer F-108 has also reported toxicity at >80 µg/ml (on cervical and embryonic kidney cells). Likewise, PEG-3000/4000/5000 were also not safe as >25 µg/ml will be lethal for alveolar basal epithelial cells and adeno carcinogenic cells. Overzealous addition stabilizers in CF would certainly have an effect and cause cytotoxicity and henceforth formulation optimization and toxicity studies are essential to be accomplished for every formulation.

4.3. LIPIDS FOR MAKING CUBOSOMES

Lipid is the main component in making CF, Numerous

Synthetic lipids have been examined, but only a few accomplished of creating stable upturned mesophases. Initially, monoolein and oleic acid were found well at the lab, whereas they suffer esterase catalysis by hydrolysis *in vivo*, which limits their application. Further searching of lipids to increase the selection of lipids that form stable non-lamellar mesophases in water like monoolein, an additive used in cosmetics for improving moisture retention i.e., phytantriol discovered. In water phytantriol display cubic phase morphology from room temperature (RT) to 80°C, whereas monoolein from RT to 43°C. Both lipids are previously well-characterized, biocompatible, and have the authorization to use in bio-systems. Among lipids, phytantriol and monoolein are usually adopted[20].

4.4. MECHANISMS FOR LOADING AND RELEASE

CF load drugs by 3 mechanisms viz., a drug can be loaded within the lipid membrane/tied to the lipid membrane/restricted within the water straits of CF. CF can be encumbered in lipid film either pre-dispersion by co-lyophilizing the drug molecules or post-dispersion by loading the CF through incubation. The drugs which are loaded in CF are of proteins or small molecules within the lipid membrane and primarily use single or binary lipid configurations based on monoolein or phytantriol. The exact mechanism of drug release from CF is not known, the probable mechanisms are by burst/diffusion/partition. Lipophilic drugs like Diazepam, Griseofulvin, Propofol, and Rifampicin from monoolein CF by partition. The release of hydrophilic drug molecules is assumed to be purely by diffusion. Whereas the burst release mechanism for all kinds of drugs. The release is also exaggerated by pH, temperature, ultrasound, and electrostatics [21,22].

4.5. APPLICATIONS

Partial solubility and penetrability of many drugs can be attained by using lipid carriers for drug Administration. CF has been scrutinized for dealing with fungal infections, treat pneumonia, and diseased wounds. Wide work has been done on CF since the past decade, yet no FDA-approved CF existing in the market due to lacking sufficient in vivo data. So, an extra afford to be kept by the researchers for a strong in vivo data. CF are successfully tried as dosage forms for antineoplastic/ vaccines/ topical treatment/small drug molecules delivery/ nano-carriers for bioactive lipids/ transfection/biosensors[23,24].

4.6. CUBOSOMES FOR PARENTERAL DRUG DELIVERY

The unique solubilization, an efficient encapsulation, extended-release, in –vivo stability, minimal viscosity, good syringeability, and lesser depletion of solvent while formulation makes CF attractive in parenteral formulations. Further, CF has a lower viscosity than the liquid crystalline phase and still keeps the stuff of controlled release. Furthermore, CF is a necessary replacement of the normal microsphere and implant, due to its good syringeability and low ingesting of solvent while their making. Some literature testified the self-amassed contents of monoglyceride and glycerylmonooleate (GMO) could persuade hemolysis in vivo for intravenous administration, thus parenteral injection of CF was delimited. CF encompassing interior liquid crystal constructions of bent lipid films are applied to solubilize encapsulate and conveyance drugs to aimed regions. However, emulsions type CF proved as intravenous carriers in medicaments. CF elevates contents of peptides/proteins/insoluble small-sized drugs that can be made as formulation, which can be given as injections [25-26].

4.7. CUBOSOMES FOR MUCOSAL AND TRANSDERMAL DRUG DELIVERY

The distinct morphology, particle size, and matching with human cell linings, and elegant permeability makes CF attractive for mucosal and transdermal drug delivery. CF possesses superior specific surface area and their dispersals have much lower viscosity in contrast to the bulk cubic phase. Maximum concentrated surfactants that form CF mislay these phases to micelle creation at higher dilutions, owing to ideal water insolvability. CF exists in equipoise with surplus water and can be distributed to form spheres. CF are made by high-energy dispersion of bulk phase, stabilized using surfactants. The emulsification of cubic lipid phases in water results in the production of CF, which looks like a nanoparticle disperse systems with elevated compatibility and availability in biofluids. CF of lipids, surfactants, and polymer possesses polar and non-polar constituents. The water affectionate power drives amphiphilic drugs in to in polar solvents to spontaneously self-assembling into an array of thermodynamically stable liquid crystalline phases of nano-size. CF are a bi-continuous liquid phase enclosing 2 separate areas of water separated by surfactant mediated bilayers. CF are optically isotropic, highly viscous, and solid like liquid crystalline substance having cubic crystallographic symmetry. CF covering interior liquid crystal structures of curved lipid membranes are used to solubilize condense and transport drugs to the infected site. CF, as they have an affinity and encapsulating both lipid and water-soluble drugs, heavily loaded peptides, proteins, and various insoluble small drug molecules, CF are flawless transferors for injectable[27, 28].

4.8. CUBOSOMES FOR ORAL DELIVERY

The lipophilic drug solubility is a major hurdle in oral dosage forms and this can be eased by the cubosomal approach[29, 30]. CF are the choice for overcoming diverse tasks in oral delivery of plentiful auspicious drugs viz., poor aqueous solubility, improper absorption, and heavy molecular size. In CF both liquid and encapsulated powder drugs can be incorporated with ease. Additionally, gastro sensitive drugs/large proteins can be encapsulated to bypass the stomach and achieving good availability in an alkaline environment. Moreover, CF can be used for controlled discharge and targeting possessions. The unique liquid crystalline structure of CF provides a shield to the encapsulated drug when bared to the punitive environmental circumstances including enzymes in the gut. Moreover, the CF with GMO elevates gastric absorption of drugs. GMO forms micelle/solution/emulsion that boost the bioavailability. GMO is a harmless and inexpensive lipid diluent and is stared an improved substitute for oral administration, equated alongside other lipid diluents. The drug discharge form CF are controlled and allows an effective in vivo distribution of the drugs. CF approach also releases the drug at different absorption sites with varied pH ranges starting from the stomach, duodenum till the large intestine. SO, CF is best suitable for those drugs having a narrow absorption window. CF made with surfactants, that arrange itself into a complex cubic design by the self-alignment into bilayers around bi-

continuous non-crossing water channels and these structures can be yoked to summarize all ranges of drugs irrespective of their affinity. These lipids based CF are measured to be one of the most fruitful schemes to augment the dissolution and perviousness of drugs. In CF, the drug can be dissolved/entrapped/encapsulated/attached to a matrix of nano-size thus strangely swaying release outlines of drugs. CF are distinctive and fascinating self-amassed formulations with huge latent in varied areas of drug administration. Additionally, they have outstanding loading assets, expedite absorption, and propose a shelter for drugs from squalor, hence making them an outstanding option orally given poorly soluble drugs.

5. FACTORIAL DESIGN (FD)

Traditional research approaches, usually study the sway of one variable at a time, due to its feasibility to manipulate statistically, and only one factor can be studied each time. It 2 factors are tried, they will be inter-reliant, and false results will arise[31]. The Design of experiments (DOE) is an essential part of multivariate analysis. However, DOE is understood to a treaty with a partial numeral of factors. The objectives of DOE are screening response and optimization. In an FD, all conceivable amalgamations of the levels of the factors are explored in each imitation. In FD the levels are designated as `high' (+1) and `low' (-1), and all the input factors are called an FD in two levels. For a 2 level design, the factors maybe 2, 3, 4, 5, 6, 7…, the integer of runs will be 4, 8, 16, 32, 64, and 128 respectively. Above 5 factors the numeral of runs will be drastically increased, so fractional FD or Plackett-Burman design (PBD) is preferred[32]. For 2-4 factors the screening goal is FD, and the response surface goals are Central composite (CCD) or Box-Behnken design (BBD). And for 5 and more factors, the screening goal is FD or PBD, and the response surface goals are suitable screening[33]. The popularly used software for FD is are Design-expert/STATISTICA/Minitab. These designs were created by entering a controllable independent variable to get a desirable output called the dependent variable.

5.1. MERITS OF FD

The merits of CF as summarized[34]

- Extra proficient than one-factor-at-a-time
- FD is obligatory when interactions may be present to evade deceptive assumptions.
- The conclusions that are effective over an array of investigational circumstances.
- Saves time and finance as it has minimal failures
- FD is a broad methodology to issue resolving
- FD allows the possessions of a factor to be appraised at several levels of the added factors
- FD is a more powerful tool for getting reduced errors and alteration in the experiment
- FD approach is cost-effective and done in a flash time

5.2. DE MERITS OF FACTORIAL DESIGN

CF have few demerits as briefed below[35]

- The size of the trial will upsurge with the numbers of factors
- Tough to make surefire the investigational units are consistent if the quantities of treatments are huge.
- Problematic to understand the large size of the factorial experiment chiefly when the interface among factors exists.
- Need an extra time, acquiescence, and supervision of smearing two dealings at the equivalent spell
- Data analysis and haphazard may be an additional effort

Factorial designs are gaining attraction from many researchers as they are true experiment design models, which involves controlling multiple factors (independent variables) that can be manipulated/varied to examine the main effects on final product parameters (dependent variables). Factorial designing helps in detecting the interactions among the variables by that a product of definite quality can be produced. The popularly used designs were listed in Table 1[36].

Table 1: Various designs for optimizing the formulations

Design	Purpose	Variables
Box-Behnken Design	When the optimum response is not positioned at the immoderations of the experimental area and when earlier results from a factorial design are not accessible.	3-6
Central Composite Design	Used when all design variables are continuous	2-6
D-Optimal Design	Used when design variables and have multi linear restraints, and not orthogonal.	2-9
Fractional Factorial design	Used to study the effect of a lower number of design variables independently from each other	2-9
Fractional FFD	Used to find the most important main effects among many factors	3-13
Mixture (Axial) Design	Contains mixture variables only	3-30
Palackett Burman Design	Used to study the main effects only	8-35
Simplex Centroid Design	Contains mixture variables	3-6
Simplex-Lattice Design	Contains mixture variables only	3-6

6. FULL FACTORIAL DESIGNS IN TWO LEVELS

It is a simple design with all contribution factors fixed at 2 levels individually. These levels are termed as 'high' (+1) and 'low' (-1). A design with all likely high/low mishmashes of all the input factors is called a full FD in 2 levels. When there are k factors, each at 2 levels, a full FD has 2^k runs[37].

Table 2: Number of Runs for factors in FD[38']	
Number of factors	Number of runs
2	4
3	8
4	16
5	32
6	64
7	128

As expressed in table 2, when the number of factors is 5 and more, which need a large number of experiments to be assessed and is not very effectual. In such cases, PBD is a better choice. A 2 level 3 factor (2^3) design is as exemplified in fig. 2 and Table 3.

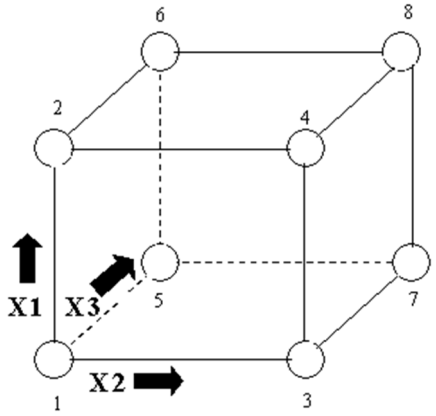

Fig. 2. A 2^3 design with factors[38] X1, X2, X3

Table 3: A 2^3 full factorial design indicating the runs[38]			
Run	X1	X2	X3
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1

7. FRACTIONAL FD (FFD)

In this alchemist accomplish only a designated subdivision or "fraction" of the analyses in the full FD. FFD is a good option when resources are inadequate or the number of factors in the plan is huge since they use rarer cycles than full FD. An FFD uses a subsection of a full FD, so some of the chief belongings and two-way connections are unclear and cannot be unglued from the effects of other higher-order connections. In general, alchemists are eager to undertake that higher-order belongings are insignificant to gain insight into major effects and lower-order interactions with fewer implementations[39].

8. PLACKETT-BURMAN DESIGN (PBD)

These designs are very capable tactics when wholly only key properties are of attention. PBD is used for screening experiments because, in this, the main possessions are, in general, heavily perplexed with two-factor interactions. It is a small two-level FFD planned to identify critical physicochemical strictures from the N number of variables in N + 1 experiments without resorting to the interaction effects between the variables. Since the sample size is conventionally small, the interaction effects are entirely enclosed in the main effects. Therefore, PBD simply filters the design space to detect major effects. The selected restrictions are further optimized by a suitable design method of a Response Surface Method (RSM). This method is a collection of statistical techniques that uses the design of experiments to build models, evaluate the effect of factors, and predict optimal conditions for factors[40].

9. CENTRAL COMPOSITE (CCD)

A central composite design always covers twice as numerous star points (SP) as there are factors in the design[38]. The SP signifies new thrilling values (low and high) for every factor in the design. They are further classified as follows (Fig.3)

9.1. CIRCUMSCRIBED (CCCD)

- They are the original form of the CCD
- The SP are at some distance alpha from the center based on the possessions anticipated for the design and the number of factors in the design
- The SP create new excesses for the low and high sceneries for all factors

- These designs have circular/spherical/hyperspherical regularity and require 5 levels for each factor
- Boosting a current factorial/resolution of FFD with SP can generate this design.

9.2. FREE CENTERED (FCCD)

- In this design, the SP are at the center of each face of the factorial space (α=±1)
- This variety necessitates 3 levels of each factor
- Extending prevailing factorial /resolution designs with suitable SP can also yield this design.

9.3. INSCRIBED (ICCD)

- It is a scaled-down CCC design with each factor level of the CCC design divided by α to generate the CCI design
- CCI design necessitates 5 levels of each factor.

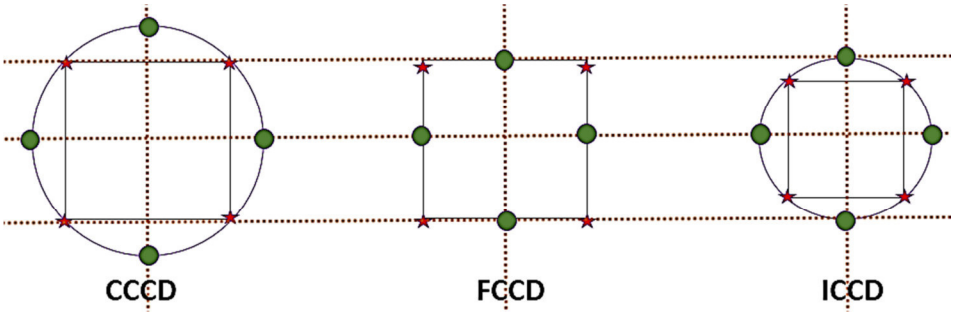

Fig. 3. Different central composite designs[41]

10. BOX-BEHNKEN DESIGN (BBD)

- The BBD is an independent quadratic design
- It does not cover an entrenched factorial/fractional factorial design
- In this, the treatment groupings are at the midpoints of edges of the progression space and at the center
- These designs are rotatable (or near rotatable) and necessitate 3 levels of apiece factor (Fig.4)
- It has limited capability for orthogonal blocking equated to the CCD

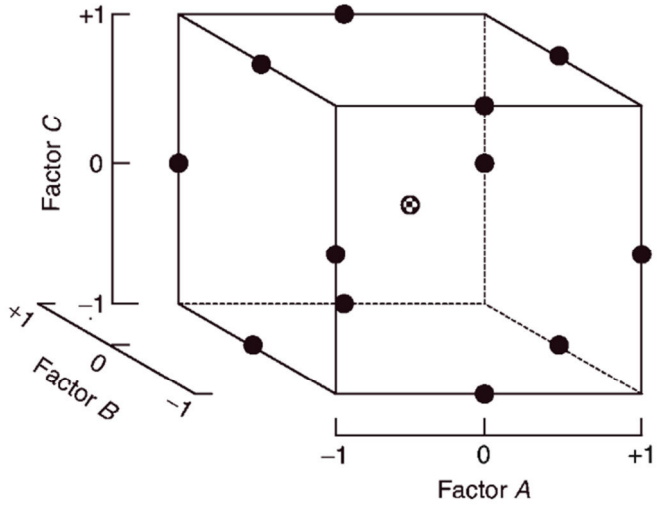

Fig. 4. BBD with 3 factors[42]

11. PAST WORK ON CUBOSOMES

The optimization procedure is facilitated by applying factorial designs and by the fitting of an empirical polynomial equivalence to the investigational results. The decade work done in the optimization of Cubosomes using the factorial design is illustrated in table 4.

Table 42: Drugs and design parameters in the optimization of Cubosomes by factorial design

Name of the Drug	Polymer Used	Design	Independent Variable	Dependent Variable
Clopidogrel bisulphate[43]	Polyvinyl alcohol(PVA), and Poloxamer 407	3^3Full Factorial Design (FFD)	Concentration of PVA, Poloxamer 407, and ratio of drug to disperse	% Entrapment efficiency (EE), and in vitro release at

			phase	15min
Timolol maleate[44]	Poloxamer 407, and glycerol monooleate (GMO)	3^2 FFD	Concentration of GMO and Poloxamer 407	Particle size (PS), % EE and in-vitro drug release
Dapsone[45]	Glyceryl monooleate (GMO), and poloxamer 407	CCD	Drug concentration	PS, Surface morphology, Zeta potential (ZP), % EE, and *in vitro* release
Rebamipide[46]	GMO, Poloxamer 407	Solvent dilution method	Drug concentration	PS, Polydispersity index (PDI), ZP, and Drug crystallinity
Sildenafil citrate[47]	GMO, PVA, Chitosan, acetonitrile, methanol, and diethyl ether	4^2 FFD	Poloxamer 407, and PVA	% EE, PS, and drug release in 8 h
Clonazepam[48]	GMO, and Pluronic-F127	2^3 Actual Statistical Design	Poloxamer 127, and GMO concentration	% EE, and PS
Dapoxetine[49]	Phosphatidylcholine (PC), Pluronic F-123, and Pluronic F-127	3^2 FFD	Binary Pluronics mixture, the concentration of PC, and the concentration of Tween 80	% EE, PS, PDI, ZP, and drug payload
Glibenclamide[50]	Glyceryl mono stearate (GMS), Poloxamer 188	3^2 FFD	Poloxamer 188 and GMS	% EE, PS, % drug release at 8h, and 24 h
Resveratrol[51]	GMO, and Lutrol F127	3^2 FFD	Concentration of GMO, and Lutrol F127	PS, and EE
Ketoconazol[52]	GMO, and Poloxamer 407	3^2 FFD	Concentration of GMO, and Poloxamer 407	PS, and EE
Tropicamide[53]	Monoolein, Pluronic F127,	Central Composite Design (CCD)	Sonication time, amplitude, sonication depth, and premixing time	PS, and PDI
Fluconazole[54]	HPMC K4M, and PVP	3^2 FFD	HPMC K4M, and PVP concentration	Drug release at 1h, and cumulative % release at 12h
Ketorolac[55]	GMO, and Poloxamer 407	3^2 FFD	Concentration of GMO, and Poloxamer 407	PS, % EE
Clotrimazole[56]	Pluronic F127, and Chitosan	2^3 CCD	% Pluronic F127, and chitosan	PS, and Mucin Binding
Miconazole nitrate[57]	GMO, and Poloxamer 407, PVA, PEG 400, PG, and HPMC15000	3^2 FFD	Concentration of Monoolein, and Poloxamer 407	%DR
Capsaicin[58]	GMO, Poloxamer 407, and Phytantriol	3^2 FFD	Phytantriol, GMO, and Poloxamer 407	DR
Silver sulfadiazine[59]	GMO, PVA, Carbopol 934, and Poloxamer 407	3^2 FFD	Chitosan, and Carbopol	bioadhesive force
Ropinirole hydrochloride[60]	Pluronic F-68, and Stearylamine	2^3 FFD	Pluronic F-68, and concentration of stearylamine	PS, ZP, and EE
Amphotericin B[61]	Phytantriol, and Poloxamer 407	3^2 FFD	bioavailability, and tissue distribution	PS, EE, % drug release
Acyclovir[62]	Poly(lactic-co-glycolic acid) (PLGA), Polycarbophil and Pluronic F68	2^3 FFD	Amount of PLGA, Pluronic F68, and Polycarbophil	PS, EE, and % DR in 12 h
Flurbiprofen[63]	GMO, Poloxamer 407	2^3 FFD	Concentration of GMO, Poloxamer 407	PS, PDI, and ZP
Dicarbazine[64]	GMO, Poloxamer 407, and Pluronic F127	3^3 Box Behnken Design	Homogenization Speed, Duration, and Temperature	PS, and EE
Indomethacin[65]	PLGA, and methacrylic acid copolymer	3^2 FFD	The amount of PLGA, and methacrylic acid	% EE, and yield

The cubosomes are fundamentally similar to nanoparticles in which both hydrophilic and lipophilic drugs can be loaded. CF are gaining attraction as they are biocompatible, simple, stable, non-toxic, and high loading[66]. CF can be made by top-down[67] and bottom-up techniques[68]. Once the Cubosomes are formulated they have to assess for outlook, surface morphology, particle size, ZP, EE, drug release, and stability by accelerated stability studies[69, 70]. The CF can be stabilized by surfactants like Polysorbate 80, PEG-2000, Poloxamer F-188, F-338, and F-407 etc[71, 72, 73]. Phytantriol and monoolein are frequently embraced lipids for making CF[74]. Studies revealed that the drug release from CF is by burst/diffusion/partition mechanisms[75]. A wide range of drug and dosages viz., antineoplastic, vaccines, topical preparations, can be formulated as CF[76, 77]. CF were successfully made for a parenteral, transdermal, and a wide range of oral delivery[78, 79, 80]. As traditional research approaches, has pitfalls like only one variable can be checked once, ease of manipulation, and more chances of getting false results DOE is emerged for formulating and optimization. In the FD, the relation between 2 and more factors with different levels and their impact on the final product can be inter related[81]. In FD the levels are designated as `high' (+1) and `low' (-1), and all the input factors are called an FD in two levels. For a 2 level design, the factors maybe 2, 3, 4, 5, 6, 7…, the integer of runs will be 4, 8, 16, 32, 64, and 128 respectively. Above 5 factors the numeral of runs will be drastically increased, so fractional FD or Plackett-Burman design (PBD) are preferred[82]. The popularly used software for FD is are Design-expert/STATISTICA/Minitab. Various approaches in making CF using FD with their independent variables (IV) and dependent variables (DV) reveals that these approaches are better in the formulation and optimization of foliations[83]. Various IV like the amount of polymer concentrations (E.g., PVA, Poloxamers, Chitosan, Carbopol, PLGA), the impact of surfactant, sonication time, mixing time, temperature et on DV like % EE, drug release, PS, surface morphology, ZP, PDI, drug-polymer load, binding, yield were studied.

12. CONCLUSION

The study concludes that the Cubosomes are getting attracted to their good drug loading capability to all ranges of drugs irrespective of their polar and nonpolar nature. The Cubosome methodology has more success rates in vitro and in vivo, as these systems have good drug loading and release rates. This review will help new researchers in knowing past attempts made on these systems by factorial design.

13. AUTHORS CONTRIBUTION STATEMENT

Shravani Y, Hindustan Abdul Ahad, Chinthaginjala Haranath, Bhumireddy gari Poojitha, Syed Rahamathulla, Aswarthanarayana Rupasree, all authors were involved in the developed of the theory, performed the computations, and verified the manuscript.

14. CONFLICT OF INTEREST

Conflict of interest declared none.

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