

Cubosomes: composition, preparation, and drug delivery applications.

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Abstract

Cubosomes can be considered as a novel lipid-based nanosystems similar to well-known vesicular systems such as liposomes and niosomes. Cubosomes have been widely formulated using certain amphiphilic lipids (e.g. glyceryl monooleate and phytantriol) in the presence of a suitable stabilizer. They can represent a novel drug delivery system which could be loaded with hydrophilic, lipophilic and amphiphilic drug molecules. They are widely used for various drug delivery applications such as oral, ocular, transdermal and chemotherapy drug delivery. In this review, the pertinent literature of cubosomes with emphasis on theories of self-assembling, the composition of cubosomes, methods of preparation and drug delivery applications will be critically reviewed.

Key words

Cubosomes, glycerol monooleate (GMO), phytantriol (PHYT), poloxamer 407, theories of self-assembling

1. Introduction

Cubosomes are distinct, nanovesicles of bicontinuous cubic structures which are formulated by dispersion of liquid crystalline cubic aggregates in aqueous media and they are characterized by high surface area and identical microstructure to its parent cubic aggregates [1]. They are formulated by certain amphiphilic lipids as glycerol monooleate (GMO) and phytantriol (PHYT), which have the ability to self-assemble in water to form cubosomes. They encompass a structure similar to honeycomb (cavernous) structures with a size range of 100-500 nm [2]. Cubosomes are gaining special interest as a unique drug delivery system and recently they have been employed in ocular, dermatological, oral and cancer therapy [3]. Actually, there is a structural similarity between cubosomes and polymeric micelles and both are commonly used in various drug delivery applications. However, polymeric micelles are formed by an amphiphilic polymer which self-assembled in water into core-shell structure when its concentration is above the critical micelle concentration. When a hydrophobic drug is added, it can be incorporated within the hydrophobic core of micelle while hydrophilic bioactive molecule is incorporated in the outer hydrophilic shell of the micelles [4, 5]. Cubosomes as drug delivery system have certain advantages reported as the following:

- They are able to encapsulate different drug molecules with hydrophilic, hydrophobic and amphiphilic properties [6].
- providing an optimistic approach for bioavailability enhancement for poorly-water soluble drugs [7, 8].
- They can be prepared by simple techniques [9].
- They are composed of biodegradable lipid [10].

- Protect the incorporated drug from physical and chemical degradation [1]
- The nanovesicles forming lipid (GMO) provide a permeation enhancer during cubosomes penetration through corneal and skin layers [11].
- They have high drug loads due to their high internal surface [12].
- They render the bioactive drug molecules with targeted and controlled release [9, 13, 14].

In addition to those interesting advantages of cubosomes, however, few drawbacks of cubosomes exist:

- Challenging in large scale production due to the high viscosity of cubic phase [15].
- They have low entrapment efficiency for water-soluble drug molecules due to their high water content inside their structure [1].

2. Theories of self-assembling of amphiphilic lipids

Certain amphiphilic surfactants with polar and non-polar components are able to self-assemble in aqueous media into highly ordered aggregates (structures) [16], with long-range order in one, two or three dimensions and short-range order at atomic distance [17]. The resultant surfactant structures could be micelles, open lipid bilayer, closed lipid bilayer and inverted micelles. There are two main theories tried to explain how these surfactant can be self-assembled into especial structure.

2.1. The principle of opposing forces

Amphiphilic molecules when exposed to a polar solvent they are arranged in such a way to minimize their free energy, where the polar solvent penetrates through the amphiphilic molecules exposing the hydrophilic portions to the aqueous environment

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while the hydrophobic portions of the amphiphiles are shielded from the solvent [17, 18]. Thus opposing forces were developed as a result of hydrophobic interactions occurred between the hydrophobic hydrocarbon tails and the hydrophilic head group of the amphiphilic molecules, these interactions compete together at the interfacial area. The first drives the association of molecules while the latter induces the opposite. This is commonly referred to as the hydrophobic effect phenomenon [19]. The hydrophobic bonding actually differs from van der Waals interaction forces, as they result from different interactions. The hydrophobic bonding as previously mentioned occurs between the hydrophobic hydrocarbon tails which induce molecular association together to hide from water. It is stronger than the weak intermolecular van der Waals force (as hydrogen bond) which occurred between atoms as a result of the oppositely polarized electron clouds [20].

2.2. The principle of packing parameter

The lipid aggregates which may be preferentially formed for a given lipid is best predicted by the principle of packing parameter [18]. The critical packing parameter (P) was described by Israelachvili [21] and recently it has been well discussed by H Abdelkader [18] is given by (equation 1). It relates the molecular shape and properties of certain lipid to its favored curvature at the lipid water interface.

$$P = \frac{v}{al} \quad \text{(Equation 1)}$$

Where (v) and (l) represent the volume and length of hydrophobic chain respectively, while (a) is the optimal surface area of the polar head group. (Table 1) shows the shape and topology of the formed mesophases which can be illustrated as following [17, 22]:

- a) The normal micelle, when the critical packing parameter (P) < 1/2, in which oil is domain in water (oil in water) with the resulting curvature occurs towards the chain region. According to this value of critical packing, parameter the amphiphiles occupy a cone shape space and hence this conical geometry leads to formation of spheres [23].
- b) Closed lipid bilayer, when the critical packing parameter $1/2 < (P) < 1$, this property derives the lipid molecules to assemble forming closed lipid bilayer structure [18].
- c) Open lipid bilayer (lamellar curvature), when the critical packing parameter (P) = 1 and characterized by zero curvature with identical cross-sections of the polar heads and the lipophilic tails. This phase with no interfacial curvature as the amphiphilic lipids under the critical packing parameter occupy an apparently cylindrical space [23].
- d) Inverted micelles, when the critical packing parameter (P) > 1, it is water in oil version and the resultant curvature occurs towards the water region. According to the (P) value, the amphiphiles occupy an inverse cone shape space.

This geometry leads to formation of inversed spheres [17, 21].

Lipid assembling into a closed lipid bilayer system results in formation of different lipid-based nanovesicles which are widely used in pharmaceutical applications this assembly depends mainly on the (P) value of the assembling lipid this including lipid assembling into liposomes which are composed of one or two concentric phospholipid bilayers that are separated by water compartments [24], niosomes which are formulated from non-ionic surfactant consisting of one or more surfactant bilayers enclosing aqueous spaces in presence of membrane stabilizer as cholesterol[25] and also, lipid could be assembled into cubosomes which restricted to certain amphiphilic lipids as glyceryl monooleate and phytantriol that are able to self-assemble into bulk cubic crystallographic structures which are farther dispersed in water in the presence of suitable stabilizer resulting in formation of our interesting structure "cubosomes" [26].

3. Component of cubosomes

Cubosome was firstly mentioned by Kåre Larsson in 1980s in his review on cubic lipid/ water phases [27], Larsson followed by Patton and Carey when they described their observation in fat digestion studies where dispersed particles of the bicontinuous cubic structures were formed as a result of combining of simulated stomach contents with lipase and bile salts [28]. However, the work on cubic structures was established by Larsson, he discovered that cubosomes can be formed from the bulk cubic structure when dispersed in water into submicron particles with identical internal structures to the parent cubic structure [29]. Cubosomes are organized in three dimensions as "honeycomb" structure and they are mainly composed of amphiphilic lipids which dispersed in water in the presence of suitable stabilizers [1].

3.1. Amphiphilic lipids

3.1.1. Glycerol monooleate (GMO)

Glycerol monooleate (GMO) is the most commonly used amphiphilic lipids in preparation of cubosomes and it is generally referred to as monoolein [30, 31]. GMO is a polar unsaturated monoglyceride, with a melting point: 35-37 °c, storage temperature -20°c, having HLB value 3 [32] and it is clear and colorless in appearance. It is a synthetic mixture of glycerides ester of oleic acid and other fatty acids and mainly consists of monooleate which able to self-assemble in water into bicontinuous cubic structures [11]. (Figure 1 A) represents the chemical structure of GMO, it shows that GMO with hydrophilic and hydrophobic characters at the same time (amphiphilic molecule) this is attribute to the presence of hydroxyl groups in the head portion which responsible for formation of H- bonds with water and the presence of hydrocarbon chains in the tail [1]. It is commonly used as an emulsifier in food industry, characterized by being safe, non-toxic, biodegradable and biocompatible material and it was first

recommended as a biocompatible encapsulating material in 1984 [33].

3.1.2. Phytantriol (PHYT)

Phytantriol which is a common constituent in cosmetic products, is considered as a brilliant alternative to GMO in cubosomes preparation [34]. Phytantriol (**Figure 1 B**) (3, 7, 11, 15-tetramethyl-1, 2, 3-hexadecantriol) has the ability to form a bicontinuous cubic structure in aqueous media under physiological condition and temperature. Recently it gained more interest, compared to monoglycerides, in biomedical field due to its high chemical stability compared to monoglycerides as a result of absence of ester group [35], its enhanced skin penetration properties [36], improved moisture retention [37] and it is commercially available with high purity (95 %), while monoglycerides are with different purities as they are produced from various sources [38]. PHYT-based liquid crystalline matrices were found to be able to sustain the release of different drug molecules especially those having hydrophilic properties and thus it is considered as a remarkable sustained drug delivery system [39]. Rizwan et al, approved its higher stability during the incorporation of hydrophilic additives [40].

3.2. Stabilizers

Despite the bulk cubic aggregates are thermodynamically stable ,however, when they are dispersed in aqueous media, the dispersed particles aren't kinetically stable as they tend to aggregate as a result of exposure of hydrophobic portions to the external hydrophilic aqueous media [41], thus using stabilizing agents become a crucial step in cubosomes preparation to prevent re-coalescence of the dispersed particles into the parent bulk cubic structure when dispersed in water [42]. The main function of the stabilizer is to provide an electrostatic barrier between particles to prevent close particle contact and thus keeping the dispersed particles in a stable form. This effect is produced through the participation of the used stabilizer in the lipid water assembly without disrupting the cubic liquid crystallinity, thus the choice of an appropriate stabilizer is a critical step. The most commonly used stabilizing agents are Pluronics, especially F127 (Poloxamer 407) which considered to be the "gold standard" (**Figure 1 c**) [15]. Pluronics are water-soluble self-assembled triblock copolymers which composed of polyethylene oxide (PEO) and polypropylene (PPO) arranged in PEO-PPO-PEO configuration where PPO and PEO portions are responsible for hydrophobic and hydrophilic properties respectively [42]. In case of cubosomes, the stabilizing action of F127 is thought to be a result of adsorption of the hydrophobic (PPO) portion onto the surface of the particles, while the hydrophilic (PEO) portion extends out into the aqueous media providing steric shielding [17]. Stabilizer, depending on the dispersed particles, is usually used with a concentration up to 20 % w/w while GMO- polymer mixture is usually used with a concentration between 2.5 % (w/w) and 10 % depending on the total weight of the dispersion [1]. Recently, Chong et al studied the ability to use a verity of non-ionic

molecules as stabilizers for cubosomes preparation with GMO and PHYT lipids and compared their effect with F127. Interestingly, they revealed that poly stearate (ethylene oxide) stabilizers were found to be more effective in stabilization of PHYT- cubosomes than the gold standard F127. However the reason for this improved stability still unclear [43].

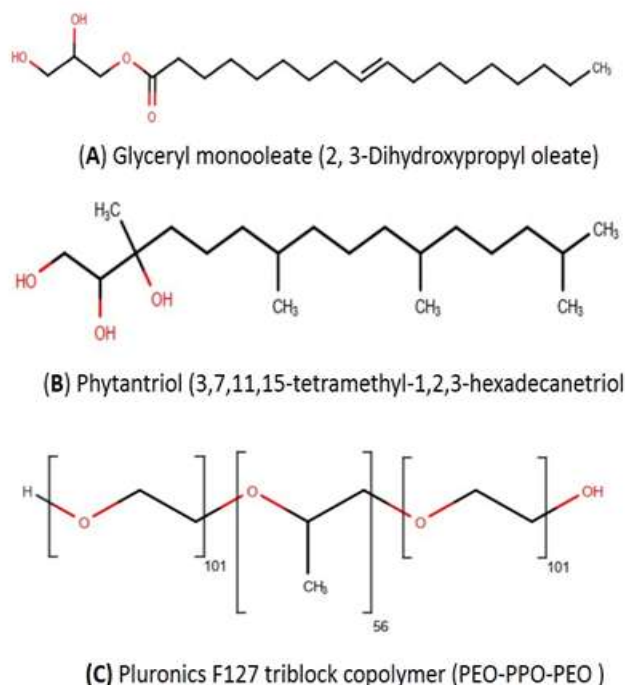


Figure 1: Molecular structure of cubosomes forming lipid (A) glycerol monooleate, (B) phytantriol, and (C) stabilizer (poloxamer 407).

4. Preparation of cubosomes

Generally, there are two main approaches for cubosomes preparation, the top-down and bottom-up approaches, both of them require the utilization of suitable stabilizer such as F127 to prevent cubosomes dispersion aggregation, as described before. However, stability, biocompatibility and optimal drug release remain the main target in the choice of optimum preparation method [17].

4.1. Top-down approach

The top down-method is the most widely used technique for cubosomes preparation (**Figure 2**) [44], it involves two main steps. Firstly, mixing the cubosomes forming lipid with a suitable stabilizer to form the bulk viscous cubic aggregates. Secondly, dispersion of the produced viscous cubic aggregates in aqueous media by the application of high energy as high-pressure homogenizer or sonication finally resulting in the formation of cubosomes[17]. Fortunately, cubosomes prepared by the top-down method are found to be stable against aggregation up to a year. However, this method with drawbacks in large scale production as the formation of viscous cubic aggregates require high energy input to be dispersed into cubosomes, unfortunately, these may be a problem when incorporation of temperature-sensitive bioactive agents, especially peptides and protein are required [1].

4.2. Bottom-up approach

This approach is commonly referred to as solvent dilution method, it involves dispersion of mixture containing cubosomes forming lipid, the stabilizer and a hydrotrope in excess of water with the application of minimal energy input (**Figure 2**) [41]. Hydrotrope is the key factor in the bottom-up approach as it is added to dissolve water-insoluble lipids to form lipid precursors and prevent the formation of liquid crystals at high concentration [45, 46]. Hydrotrope is a molecule able to solubilize molecule that able to solubilize poorly soluble agents in aqueous media by hydrotropic solubilization which means enhancement of solubility of one solute by addition of another solute. Urea, sodium alginate and sodium benzoate are among the most commonly used hydrotropes. The solubilizing mechanism of hydrotrope involves complex formation between the hydrotrope and the hydrophobic agent [47, 48]. The bottom-up technique provides more advantages over the top-down approach as it requires less energy input thus it can be safely used for the preparation of cubosomes loaded with temperature-sensitive agents also the yielded cubosomes show long term stability due to the homogenous dispersion of stabilizers onto the surface of the produced nanovesicles [1].

release [9]. They are found to improve ocular bioavailability of the loaded drugs because they have long residence time at the corneal surface and characterized by mucoadhesive properties due to the presence of GMO leading to improve corneal permeability and consequently improve ocular bioavailability of the incorporated drugs [49]. Interesting results were obtained when cubosomes were studied as a topical ocular drug delivery systems. In vitro permeation study of cubosomes loaded with dexamethasone through excised rabbit corneas, results showed that cubosomes formulation was found to increase the apparent permeability coefficient. Additionally, the precorneal residence time test and pharmacokinetic study of aqueous humor samples results revealed that cubosomes formulations cause a significant increase in precorneal retention time compared to Dex-Na phosphate eye drop and consequently result in an overall increase in dexamethasone concentration in the aqueous humor [10].

Also when cubosomes were used for ocular delivery of tropicamide, a mydriatic agent, comparative evaluation studies of tropicamide-loaded cubosomes with commercial conventional ophthalmic solution, the results revealed no significant difference in in-vitro corneal permeation characteristics but significantly faster onset and higher intensity of mydriatic action resulted through in vivo study for the cubosomes formulation [50]. (**Table 2**) summarizes few examples of cubosomes loaded drugs for ocular application, all results showed great benefits of cubosomes for ocular drug delivery in prolonging the precorneal residence time, improving ocular bioavailability of loaded drug also histopathology studies proved that cubosomes preparation are safe and nonirritant for ocular uses.

5.2. Dermatological applications

In transdermal drug delivery, the stratum corneum which is highly organized outer most layer of skin, represents a strong barrier for skin penetration of topically applied drugs [55]. However, cubosomes with their unique structure and properties provide a promising vehicle for transdermal drug delivery. Because of the bioadhesive properties of cubosomes to the stratum corneum as a function of GMO, they can be effectively used in topical and mucosal drug delivery [6]. Recently there are several dermatological applications of cubosomes. An important dermatological application is vaccination through transcutaneous (TCI) immunization. However, microneedles (MNs) and cubosomes have been effectively used as a synergistic approach for the delivery of vaccines through the skin. Results showed that the use of MNs enhances the permeation of the aqueous peptide mixture through the skin layers and cubosomes formulated peptide showed longer retention within the skin. Consequently, the use of combined approaches of both MNs and cubosomes were found to be an efficient system for local delivery of antigen to the targeted cells in the skin [56]. (**Table 3**) summarizes examples of the dermatological applications of cubosomes for topical delivery of various drugs.

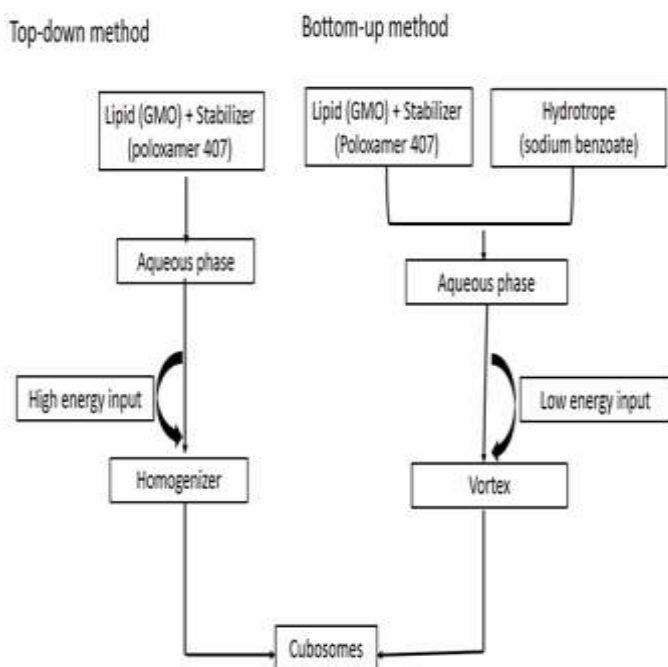


Figure 2: Diagrammatic illustration of cubosomes preparation approaches

5. Applications of cubosomes in drug delivery

5.1. Ocular applications

Many recent studies have concerned with the application of cubosomes in ocular drug delivery. Utilizing their benefits of being biodegradable, able to encapsulate all 3 types of drug molecules as hydrophilic, hydrophobic and amphiphilic, and they render bioactive agents with targeted release and controlled

5.3. Oral applications

Cubosomes are also attained special interest in their use in oral drug delivery for different compounds including poorly-water soluble, poorly absorbed drugs and drugs with large molecular size. They facilitate the absorption of orally administered drugs possibly due to their bioadhesive properties, interaction with intestinal cell membrane or induced secretion of physiological surfactants during lipid digestion in the gastrointestinal tract [63-65]. Chung *et al.* [66] showed more interesting results for oral treatment of fasted streptozotocin-induced diabetic rats by oral administration of cubosomes loaded with insulin. They prepared insulin loaded cubosomes with special care to keep insulin active. A mixture of GMO, emulsifier and water were microfluidized at 80 C° and then cooled to room temperature.

However, as insulin cannot be stable under severe conditions, cubosomes were prepared at room temperature by stirring and were used in the form of large aggregates (10 µl-1 ml). Their results showed that cubosomes could provide stable biocompatible oral delivery of insulin with reproducible hypoglycemic effect and enhanced adsorption on the intestinal epithelia due to the mucoadhesive properties of GMO. In addition, cubosomes provide a promising vehicle for oral delivery of poorly-water soluble. They incorporate the poorly-water soluble drug, in the solubilized form, within the lipid bilayer portion of their structure consequently, prevent drug precipitation in the GIT tract and additionally improve the intestinal adsorption due do the mucoadhesive properties of GMO [1] (**Table 4**) showing examples of applications of cubosomes in oral drug delivery .

5.4. Anticancer applications

Important applications of cubosomes are the oral and topical delivery of anticancer agents. Cubosomes as a novel drug delivery system were effectively used for targeted delivery of chemotherapeutic agents with improved bioavailability, pharmacokinetics and safety profiles of the loaded drugs [51]. Utilizing the unique structure and properties of cubosomes for improvement of oral bioavailability of administered drugs. Studies showed that cubosomes improve oral bioavailability of 20(S) protopanaxadiol (PPD), an anticancer drug, which has low oral absorption this could be attributed to the effect of cubosomes on the enhancement of absorption due to its bioadhesive properties [71]. Also, It is reported that selective and sustained delivery to the tumor site of 5-Fluorouracil not only improves the antitumor activity but also reduces its side effects when compared with the clinically available 5-FU formulations [72, 73]. Cubosomes were effectively used for targeted delivery of 5-Fluorouracil (5-FU), a water-soluble drug, selectively into liver tissue. The *in vitro* release profile showed that cubosomes formulation had biphasic release profile, with an initial burst release of drug during the first hour, followed by a relatively slow release of the remaining drug after 4.5 h. Also results showed higher liver concentration of 5-FU from cubosomal formulation compared do solution form, this could be attributed to the higher systemic absorption of 5-FU loaded cubosomal form due to the higher permeability of cubosomes through the epithelial membrane as a result of structural similarity between the lipid bilayer of cubosomes and the microstructure of cell membrane [29, 74]. (**Table 5**) showing examples applications of cubosomes for anticancer drug delivery.

Table 1: Shape and topology resulting from self-assembling of amphiphilic lipid in aqueous media.


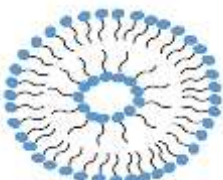


	Type of curvature	Critical packing parameter (P) value	Shape and topology
(a)	Micelles (curvature occurs toward the chain)	$\frac{v}{al} < 1$	
(b)	Closed lipid bilayer structure	$1/2 < \frac{v}{al} > 1$	
(b)	Open lamellar structure (zero curvature)	$\frac{v}{al} = 1$	
(c)	Inverted micelles (curvature occur towards the water region)	$\frac{v}{al} > 1$	

Table 2: Examples of applications of cubosomes as ocular drug delivery system.

	Loaded drug	Oil used	Stabilizer used	Pharmacological uses	Conclusion	References
1	Dexamethasone (DEX.)	GMO	Pol. 407	Treatment of anterior ocular inflammation.	Cubosomes improve the precorneal retention time and ocular bioavailability of DEX.	[10]
2	Flurbiprofen (FB)	GMO	Pol. 407	NSAID used for treatment of ocular inflammation.	Cubosomes showed low ocular irritation and improved transcorneal permeation of FB.	[49]
3	Ketorolac	GMO	Pol. 407	NSAID used to relieve itching eyes caused by seasonal allergies.	Cubosomes improve the transcorneal permeation and prolong the precorneal retention time of ketorolac and also, the histopathology studies showed that ketorolac loaded cubosomes was safe for ocular uses.	[47]
4	Timolol (TM)	GMO	Pol. 407	Non-selective beta-blocker drug used for treatment of glaucoma.	Timolol loaded cubosomes showed increased corneal penetration, prolonged precorneal retention time and enhanced intraocular pressure-lowering effect more than the commercially available eye drops.	[50]
5	Cyclosporine A	GMO	Pol. 407	Immunosuppressive agent used in the treatment of inflammatory and immune related ocular diseases.	Cubosomes showed low ocular irritation, improved ocular bioavailability and increased precorneal retention time of cyclosporine A.	[51]
6	Pilocarpine nitrate(PN)	GMO	Pol.407	Treatment of open-angle glaucoma and acute angle-closure glaucoma,	Cubosomes led to increment in the apparent permeability coefficient compared to the commercial eye drops with an enhanced effect on decreasing the intraocular pressure of rabbits and lower ocular irritation effect.	[52]

Table 3: Examples of dermatological applications of cubosomes.

	Loaded drug	Oil used	Stabilizer used	Pharmacological uses	Conclusion	References
1	Capsaicin	- (GMO) - (PYT)	Pol. 407	Used in the treatment of psoriasis, pruritus, and contact allergy.	Cubosomes provided sustained release of capsaicin, prolonged skin retention with no irritation to skin and render capsaicin stable under strong light and high temperature.	[55]
2	Silver sulfadiazine (SSD).	GMO	Pol. 407	Used for the treatment of infected burns.	Cubosomes in gel formulations were effectively used in treatment of second degree of burns with better patient compliance, excellent healing results and with fewer side effects compared to commercially available products.	[56]
3	Indomethacin	GMO	Pol. 407	Anti-inflammatory drug.	Cubosomes prolong the anti-inflammatory activity of the loaded drug as a result of cubosome depot effect on the epidermis.	[2]
4	Hydroxypropyl β cyclodextrin / minoxidil complex	GMO	Pol. 407	Minoxidil used for hair growth.	In vitro skin permeation of minoxidil loaded cubosomes were higher than that of minoxidil dissolved in propylene glycol/water/ethanol.	[57]
5	Antimicrobial peptide (AMP) LL-37	GMO	Pol. 407	Used for treatment of skin infection caused by staphylococcus aureus.	Cubosomes provide protection for the loaded antimicrobial peptide from proteolysis enzymes compared to the pure LL-37 and effectively killed the infectious bacteria with no skin irritation induced.	[16]
6	Erythromycin	GMO	Pol. 407	Treatment and prevention of several types of acne as a result of its bacteriostatic activity against <i>Propionibacterium acnes</i> .	Cubosomes were effective in the topical delivery of erythromycin in a non-invasive and sustained manner for treatment and prevention of acne.	[58]
7	Dapsone	GMO	Pol. 407	An antibiotic and anti-inflammatory agent used in the treatment of acne, leprosy and systemic lupus erythematosus.	Cubosomes enhance permeation of dapsone across the epidermal layers at the local site, reducing systemic side effects with higher transdermal flux value compared to marketed formulation.	[59]
8	Palmitoyl peptides (palmitoyl- GHK and palmitoyl- GQPR).	PYT	Pol. 407	Pal-GHK and pal-GQPR have anti-wrinkle properties when applied topically to the skin (Skincare product).	Phytantriol cubosomes effectively incorporate pal-GHK and pal-GQPR, released in a sustained manner up to a few days and significantly increase the stability at room temperature.	[60]

Table 4: Examples of oral drug delivery utilizing cubosomes formulation

	Loaded drug	Oil used	Stabilizer used	Pharmacological uses	Conclusion	References
1	Insulin	GMO	Pol. 407	Used in the treatment of type 1 diabetic induced rats (insulin-dependent diabetes)	Cubosomes protected the loaded insulin from proteolysis enzymes and the loaded insulin was stable upon storage and has a superior effect on controlling hyperglycemia in a predictable and reproducible manner.	[64]
2	Ibuprofen	PYT	Pol. 407	Non-steroidal anti-inflammatory drug with analgesic property.	Cubosomes provide sustained release of ibuprofen leading to sustained plasma level of ibuprofen with enhanced systemic bioavailability.	[65]
3	Simvastatin	GMO	Pol. 407	Used to lower bad cholesterol and fats and raise good cholesterol in the blood.	Cubosomes enhanced the bioavailability of the water-insoluble simvastatin when administered orally.	[66]
4	Piperine	GMO	-Pol. 407 -Tween 80 -Cremophor RH 40	It is a natural alkaloid with memory enhancing potentials used in the treatment of Alzheimer's disease (AD)	Cubosomes were found safe on brain cells, with superior effect over free drug and were effectively able to restore the cognitive functions of the treated animals. Tween 80 successfully used as a stabilizer for cubosomes preparation with a suitable size for brain delivery.	[67]
5	Amphotericin B	PYT	Pol. 407	An antifungal drug used for the treatment of several types of fungal infections as histoplasmosis and Leishmaniasis.	Cubosomes loaded with amphotericin B were characterized by improved relative bioavailability and didn't show nephrotoxicity compared to marketed product Fungizone®	[68]

Table 5: Applications of cubosomes for anticancer drug delivery.

	Loaded drug	Oil used	Stabilizer used	Pharmacological uses	Conclusion	References
1	Dacarbazine	GMO	Pol. 407	First-line chemotherapy medication against melanoma.	Cubosome delivery of dacarbazine reduces the serious side effect of intravenous administration. The loaded drug within cubosomes was in the amorphous or molecular state these physicochemical characters improve the shelf life, efficacy and safety.	[73]
2	5-fluorouracil (5-FU)	GMO	Pol. 407	Antineoplastic agent widely used in the treatment of advanced gastrointestinal cancers including hepatocellular carcinoma	Cubosomes were effective in targeted drug delivery of orally administered 5-FU. Cubosomes significantly increase the liver concentration of 5-FU about 5-fold greater than that observed with 5-FU solution.	[74]
3	20(S) protopanaxadiol (PPD)	GMO	Pol. 407	Anticancer drug	Cubosomes improved the oral bioavailability of PPD as a result of enhanced absorption of poorly-water soluble drug.	[69]

Conclusion

Cubosomes are among a special class of lipid-based nanovesicles which characterized by liquid crystalline nature of their nanostructure, prepared from amphiphilic lipid which self-assembled in water and in presence of stabilizer into cubosomes. Recently numerous published reports proved their potential uses as a novel drug delivery system. Cubosomes have been approved as an effective ocular drug delivery with enhanced ocular residence time, bioavailability and no irritation to the eye. Oral application illustrated that cubosomes can be used effectively to increase absorption of poorly water soluble drugs, protect the liable drug from enzymatic degradation and in targeted drug delivery. They provide a promising vehicle for effective transdermal drug delivery with enhanced skin permeation and low irritation potential. Interestingly, cubosomes were applied for delivery of anticancer drugs with reduced serious side effects of the chemotherapeutic agents and targeted drug delivery.

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