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AN OVERVIEW ON CUBOSOMES AS REMARKABLE NANOCARRIER FOR DRUG DELIVERY

VANDANA GUPTA
Department of Pharmaceutical Sciences, Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Uttar Pradesh, India

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ABSTRACT

Lipids have been extensively used as main ingredients in various drug delivery systems, such as liposomes, solid lipid nanoparticles, nanostructured lipid carriers and lipid-based lyotropic liquid crystals. Over the past few years, lipid-based lyotropic, bicontinuous cubic phase liquid crystals have been investigated for their applicability to controlled delivery of active ingredients. Lipid-based lyotropic liquid crystals have highly ordered, thermodynamically stable internal nanostructure, thereby offering the potential as a sustained drug release matrix. The emulsification of cubic lipid phases in water results in the production of cubosomes that can be defined as nanoparticulate disperse systems characterised by high biocompatibility and bioadhesivity. The unique microstructure of cubosomes have the potentials to control the release of active ingredients, improve drug bioavailability and reduce toxicity, enhance the stability of drugs and to increase the penetrability of drug after topical application. This reflection will provide an overview of the lipids used to prepare cubic phase at physiological temperature, as well as the influencing factors on the phase transition of liquid crystals. In particular, the most current research progresses on cubic phase as drug delivery systems and its applications will be discussed. It might act as smart lipid nanoparticles for drug delivery.

Keywords: Cubosome, Cubic phase, Pluronic F127, Liquid crystalline, Bicontinuous

INTRODUCTION

Bicontinuous cubic phases are optically isotropic, achingly viscous and solid like liquid crystals with cubic crystallographic conformity. Prior to their structural evaluation, these phases were coined ‘viscous isotropic phases’. Bicontinuous cubic phases consist of two asunder, continuous but nonintersecting hydrophilic regions divided by a lipid bilayer that is contorted into a periodic minimal surface with zero average curvature.

*Corresponding author: vandana.gupta@shiats.edu.in, vandanargpv@gmail.com

Cubosomes are liquid crystalline nano-construct particles with the same distinctive properties of the bulk cubic phase, however cubosome dispersions have much lower viscosity (Spicer 2004).

In cubic phases, the minimal surface is structured by the self-assembled bilayer that occurs as the hydrophobic or hydrophilic portions of the surfactant molecules line up to minimise their interaction with the opposites. Monoglycerides as monoolein (MO) form infinite periodic minimal surfaces (IPMS) by self-assembly in excess water. Minimal surfaces are best described by analogy with their most readily observed natural form: soap films. The surface formed by a soap film between two rings is a catenoid, a simple form of minimal surface whose two principle curvatures are equal but opposite in sign at every point, resulting in an average curvature of zero and a negative Gaussian curvature (Spicer 2005). Depending on the conditions, a wide array of structures with differing complexity emerges. These aggregates can have two-dimensional order, such as the classical monolayer and bilayer structures (lamellar phase) or higher complexity with three-dimensional architectural features (Fontell 1992; Seddon 1992; Yang, Armitage and Marder 2004), including the micellar (L), hexagonal (H) and cubic phases (Q) (Figure 1).

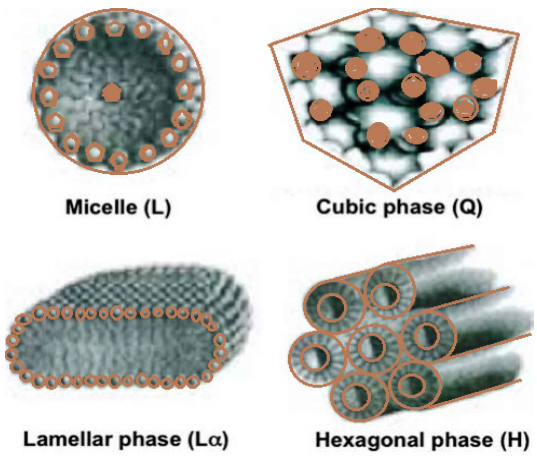

Figure 1: Various phases of amphiphilic self-assembled systems. Representative two-and three-dimensional structures of amphiphile self-assembled systems (Yang, Armitage and Marder 2004).

Cubosomes at a Glance

Luzzati and Husson (1962) first acknowledge the presence of cubic phases in lipid-water system using X-ray scattering evaluation. Fontell, Mandell and Ekwall (1968) drew similar outcomes regarding cubic phase in ternary systems of ampiphiles, oils and water in aligned. Around the same time, another researcher published extensive investigation of the aqueous phase behaviour of monoglycerides. Monoglycerides are polar lipids with destitute water solubility that exhibit aqueous phase behaviour echoing their structural analogy to non-ionic surfactants (Lutton 1965). Landh and Larsson (1996) have patented the preparation of colloidal dispersions of non lamellar lyotropic crystalline phases and have designated the particles 'cubosomes'. Cubosomes usually have been constructed by means of time-consuming methods requiring high-energy aid. Similarly Gustafsson *et al.* (1997) have explored the production and structure of aqueous dispersions of lipid based lyotropic liquid crystalline phases. The dispersions were comprised either on glycerylmonooleate/

sunflower oil or glycerylmonooleate/retynilpalmitate mixtures plus a nonionic triblock polymer (Poloxamer 407) in water. Dispersions were produced by drop wise addition of a melt of lipids and Pluronic in water, followed by attrition of size by homogenisation under high pressures at 80°C. Further, Siekmann *et al.* (2002) have reported the preparation and characterisation of dispersions composed of MO-rich monoglycerides with or without purified soya phospholipids. Dispersions were produced by equilibration of the monoglycerides/ phospholipid/water cubic phase, consequent fragmentation by a solution of Pluronic F127, predispersing by probe sonication and finally high pressure homogenisation. Moreover some authors have investigated experimental protocols for cubosomes preparation based on the use of organic solvents. In particular Spicer *et al.* (2001) have recommended a method based on a dilution process of an ethanolic solution of MO with an aqueous solution of Pluronic. Ethanol was used as a hydrotrope to create a liquid precursor, instinctively forming cubosomes after dilution. Moreover, Nakano *et al.* (2001) have suggested a method for the production of cubosomes based on hydration of a dry film of MO/poloxamer with an aqueous buffer.

Cubosomes have been already substantiated to be exceptional drug delivery carrier applied also for anticancer drugs and for the diffusion of drugs across the blood–brain barrier. When complexed with specific ligands, e.g. folic acid they target the tumour tissues that over express folate receptors and enhance the drug delivery efficiency. When impregnated with imaging and therapeutic moieties, they can be advantageous for theranostic nanomedicine (Godlewska *et al*. 2019; Zhang *et al*. 2020). Combined delivery of two drugs contained into cubosomes has been recently reported (Li *et al*. 2019). Dual-manner cubosomes were applied for fluorescence-magnetic resonance (MR) imaging. When loaded with an anticancer moiety and near infrared fluorescent (NIRF) probe, they showed enhanced targeting experties toward HeLa cancer cells (Barriga *et al*. 2019). MO is the widely used lipid to prepare cubosomal drug delivery systems (Murgia, Biffi and Mezzenga 2020; Mertins, Mathews and Angelova 2020). Cubosomes are arranged into bilayers within the surfactant and wrapped into a three-dimensional, periodic and minimum surface, generating a densely packed design. The substance is an optically transparent, very clammy bicontinuous cubic liquid-crystalline phase with a remarkable structure in the nanoscale range. They are quite easy to make, and the enhanced permeation ability and emulsification tracts of lipids enable them to accommodate hydrophobic, hydrophilic and amphiphilic compounds while ensuring the targeted and controlled release of bioactive compounds (Sivadasan *et al*. 2023).

Structure of Cubosome

Cubic phase comprised of a curved bi-continuous lipid bilayer elongated in three dimensions and separating two congruent networks of aqua channels, so it can accommodate hydrophilic, amphiphilic and hydrophobic components. As a deserving delivery system for various pharmaceuticals ranging from low molecular weight drugs to proteins, peptides, amino acids and nucleic acids, the cubic phase has received considerable attentions. Further studies of amphiphilic lipid-water systems have provided evidence for the existence of three cubic structures, with the lipid bilayer following the gyroid (Ia3d), diamond (Pn3m) and schwarz primitive (Im3m) surface (with the minimal surface equivalent to the bilayer mid-surface). Cubosomes comprise curved bicontinuous lipid bilayers that are organised in three dimensions as honeycombed structures and divided into two internal aqueous channels that can be exploited by various bioactive ingredients, such as chemical drugs, peptides and proteins. Owing to promising properties such as thermodynamic stability, bioadhesion, the ability of incorporating hydrophilic, hydrophobic and amphiphilic substances, and the

potential for controlled release through functionalisation, cubosomes are regarded as promising vehicles for different routes of administration.

Cubosomes are discrete and nanostructured particles formed from disintegration and steric stabilisation of inverse bi-continuous cubic phases of lipids. Therefore, cubosomes have much larger specific surface area and still remain the inner structures and the sustained release drug delivery system.

The self-assembly structure formed not only on the type of surface active agent used but also on many other factors, such as temperature and water content. In the MO/water, a reversed microemulsion (also called a fluid isotropic or L_2 phase), a lamellar (L_α), a reversed hexagonal (H_{ii}) and a reversed bicontinuous cubic liquid crystalline phase can form. The self-assembly structure is generally influenced by addition of an external molecule. For example, the addition of oil, such as tetradecane (TC), induces a structural transition from cubic to H_{ii} or L_2 in the MLO/water system. The concept of the self-assembly of amphiphilic moieties is linked with two principles: opposing strength and packing parameter. In accordance with the principle of opposing strength, molecular positioning of amphiphilic moieties in a polar solvent decreases free energy. The vehicle may move through them and expose the hydrophilic parts to the aqueous domain, while securing the hydrophobic domain from the vehicle. At this moment, opposing strength begin to emerge, as hydrophobic proximity arises at the interface linking hydrophobic hydrocarbon tails and the hydrophilic head groups on the amphiphilic moieties. The hydrophobic effect phenomenon happens due to this approach. The packing parameter concept describes the lipid agglomerates that form favouredly with any lipids (Almoshari 2022).

Factors Influencing the Phase Transition of Cubosomes

Many factors influence the phase behaviours of cubic liquid crystals, such as the molecular structures of lipids, pressure, temperature, salt concentration, pH, addition of a third substance and steric factors (Abourehab *et al.* 2022). A thorough understanding of these influencing factors on the phase transition will contribute better quality control as well as expanding the lipid applications in drug development and delivery.

The molecular structures of lipids play an important role in the assurance of phase behaviour. The critical packing parameter (P) is used to anticipate the nanostructure of formed liquid crystal with the formula P = V/al, where P is critical packing parameter, V is the hydrophobic chain volume, a is the cross-sectional area of the polar head group and l is the hydrophobic chain length (Israelachvili 1994). It is essential to note that P-value (and therefore the self-assembled nanostructure) will change along with other parameters such as temperature and solvent conditions. Depending on P, different self-assembled liquid crystalline structures will be formed (Sagalowicz *et al.* 2006). When P = 1, lamellar liquid crystalline structure forms. When P < 1, oil-in-water self-assembled structures form, such as normal micelles (L1), normal cubic structure (V1), and normal hexagonal phases (H1). When P > 1, water-in-oil self-assembled structures form, such as reversed micelles (L2), reversed cubic structure (V2) and reversed hexagonal structure (H2). Salt concentration (Awad *et al.* 2005; Yaghmur *et al.* 2008; Yaghmur, Sartori and Rappolt 2011) and pH value (Okamoto *et al.* 2008; Alam *et al.* 2011; Muller, Salonen and Glatter 2010) also have an influence on the phase behaviour of liquid crystals to a certain extent. For example, Awad *et al.* (2005) found out that low concentrations of Ca^{2+} induced the Lα phase to the cubic phase transition in the suspensions of MO and dioleoylphosphatidylglycerol mixtures. In addition, Negrini and Mezzenga (2011) studied about a system composed of monolinolein (MLO) and linoleic acids (97/3, w/w) which reversibly change from a reverse bicontinuous cubic phase to a reverse hexagonal phase, when the pH was dropped from 7 to 2 (Yaghmur

et al. 2011); Yaghmur *et al.* (2011) investigated the impact of pH on the phase structures of 6% bupivacaine-loaded glyceryl monooleate (GMO)-based liquid crystals and it was found out that the different self-assembled structures were produced by increasing the pH from 6.0 to 7.4, which induced the structural transition of Pn3m + H2→H2. In addition, additives will modify the textures of liquid crystals which result in phase transition. Yaghmur *et al.* (2005) introduced TC to the MLO water-Pluronic-F127 system and observed that, when mass of TC/mass of MLO = 19, the system transformed from cubosomes to hexosomes and, when mass of TC/mass of MLO = 75, the system transformed from hexosomes to a concentrated microemulsion. Later on they (Yaghmur *et al.* 2006) found out that diglycerol monooleate or soybean phosphatidylcholine had a counter effect on that of TC which turned back the self-assembled nanostructures in the TC-loaded dispersions from hexosomes to cubosomes. Amar-Yuli and Garti (2005) investigated the impact of incorporation of triglycerides (TAGs) with various chain lengths into the binary GMO/water system, and the results suggested that the solubilisation of TAGs in the system facilitates transition from lamellar or cubic phases to hexagonal structure at room temperature. Dong *et al.* (2006) found out that the presence of 5% w/w vitamin E acetate in the phytantriol (PT)-water system abolished the transition temperature of the cubic phase to hexagonal phase from 60ºC to below 25ºC. This revealed that lipophilic components in liquid crystalline formulations may have a significant impact on the phase behaviour.

Constitution of Cubosomes

Bicontinuous cubic phases are formed by natural lipids, cationic (Stroem and Anderson 1992) and nonionic surfactants, (Lynch *et al.* 2000) and polymer systems, although the lipid most widely used to produce bicontinuous cubic phases is the monoglycerides MO. Monoglycerides spontaneously form bicontinuous cubic phases upon the addition of water, are relatively insoluble (allowing the formation of colloidal dispersions of cubosomes) and are resistant to changes in temperature. When lipid molecule is heated, instead of melting directly convert into an isotropic liquid. The ability to exist in several different phases is an important property of pure lipids and lipid mixtures; it depends on temperature, hydration and lipid class. In general monoglycerides exhibit different phase behaviours when they introduced to water. Surfactants, which are used in the production of cubosomes, are Pluronic F127 in a concentration range between 0% w/w and 20% w/w with respect to the disperse phase. The concentration of the monoglycerides/surfactant mixture generally takes between 2.5% w/w and 10% w/w with respect to the total weight of the dispersion. Polyvinyl alcohol (PVA) used in addition to Pluronic as a stabilising agent of the dispersion. The investigators observed that the stabilising effect of the stearate category of poly (ethylene oxide) is more efficient than the Pluronic F127 when *accustomed* in the preparation of PT cubosomes. Especially, product Myrj59®, which is 100 poly (ethylene oxide) units, was more appreciable in stabilising PT cubosomes in comparison to the gold standard Pluronic F127 (Umar *et al*. 2022).

Cubic phase-forming lipids

Various types of lipids have been extensively studied as the carriers to prepare lipid-based lyotropic liquid crystals because of their low toxic and biodegradable properties. In general, lipid-based lyotropic liquid crystals are formed by swelling of certain amphiphilic lipids due to their amphiphilic nature comprising a polar head group and a hydrophobic tail. Amphiphilic lipids spontaneously form thermodynamically stable self-assembled structures in aqueous

environment and eventually develop into cubic and/or hexagonal liquid crystals depending on temperature and water content. Till date, there have been few materials known to exhibit this kind of phase behaviour. In this part, we mainly introduce the lipids which are commonly used to form cubic and/or hexagonal liquid crystals at physiological temperature.

GMO, 2,3-dihydroxypropyl oleate (Shah, Sadhale and Chilukuri 2001; Peng *et al.* 2010); PT, 3,7,11,15 tetramethyl-1,2,3-hexadecanetriol (Barauskas and Landh 2003; Han *et al.* 2010) and other lipids such as MLO (Negrini and Mezzenga 2011; Yaghmur *et al.* 2005; Tang *et al.* 2012), monoelaidin (Chung and Caffrey 1995; Czeslik *et al.* 1995; Kulkarni 2011), phosphatidylethanolamine (PE) (Boni and Hui 1983; Tenchov *et al.* 1996; Tenchov, Koynova and Rapp 1998), oleoylethanolamide (Mohammady, Pouzot and Mezzenga 2009), phospholipids (Driever *et al.* 2013; Meikle *et al.* 2017), PEGylated phospholipids (Tenchov and Koynova 2012), alkyl glycerates (Tiberg and Johnsson 2011; Zabara and Mezzenga 2014) and glycolipids (Kitamoto *et al.* 2009) have been observed to form cubic phase. Among them GMO and PT are the most commonly studied to form cubic phase liquid crystals as drug delivery systems. GMO or MO, is a polar unsaturated monoglycerides. The chemical structure of GMO is shown in Figure 2(a). GMO is a nontoxic, biodegradable, and biocompatible material and is listed in the FDA's *Inactive Ingredients Guide.* Although GMO is a well-known emulsifying agent and food additive since the 1950s, its potential application in drug delivery has not been discovered until in 1984 that GMO was first proposed as a biocompatible material (Rubino *et al.* 2015). It is clearly indicated that GMO forms cubic phase with water in a broad area at physiological temperature. Instead, PT also has been widely used as a cubic phase-forming material in the past decade (Akbar *et al.* 2017; Wang *et al.* 2019). PT is a well-known active ingredient used in cosmetic industry, such as hair and skin care products (Chen *et al.* 2015). Recently, PT has been receiving more attention since it has a phytanyl backbone but does not possess an ester bond, which provides an improved chemical stability compared to other fatty acid-based materials, such as GMO, in aqueous and model gastrointestinal conditions (Jain *et al.* 2018; Rajabalaya *et al.* 2017). A recent research reported the synthesis of four novel aminolipids and developed pH-responsive nanostructured lipid nanoparticles (LNP), which exhibited a slow-releasing hexagonal structure (H2) at physiological pH and quick release bicontinuous cubic phase (Q2) at the acidic tumour pH. Out of the four studied aminolipid-based cubic systems, the cubosomes containing N-(pyridin-4-ylmethyl) oleamide (OAPy-4) or N-(2(piperidin-1yl) ethyl) oleamide (OAPi-1) exhibited best response means a pH-induced H2 to Q2 phase transition in a tumour-relevant pH range 5.5–7.0 (Rajesh *et al*. 2022).

Glycerol monooleate

Glycerol monooleate is one of the most widely investigated amphipathic lipid used in the production of various liquid crystalline drug formulations (Esposito *et al*. 2018) Glycerol monooleate (Figure 2) is a glycerol fatty acid ester. It has a cis double bond at C_9. From the molecular point of view, glycerol monooleate has the acyl chain which is by an ester bond attached to the glycerol backbone (Esposito *et al*. 2018; Freag *et al.* 2016). The two remaining carbons of the glycerol moiety are free, giving polar characteristics to this part of the molecule. This hydrophilic part can form hydrogen bonds with water in an aqueous environment (the head group). The hydrocarbon chain (the tail) gives hydrophobic properties to glycerol monooleate.

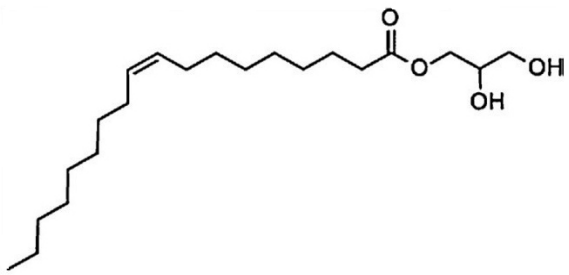

Figure 2: Chemical structure of glycerol monooleate.

Furthermore, glycerol monooleate is a nontoxic, biodegradable and biocompatible material, classified as GRAS (generally recognised as safe) and it is FDA approved inactive ingredients (Esposito *et al*. 2018; Bode *et al.* 2013; Priddy *et al.* 2017). One important manner of the use of glycerol monooleate as a safe parenteral material is the necessity to confirm its biological tolerance. Although glycerol monooleate abandon after *in vivo* subcutaneous and intramuscular injection, principally by lipase activity, its non-irritant effect on the tissues has not been absolutely confirmed. Glycerol monooleate has haemolytic properties and therefore it is not suitable for intravenous administration. Glycerol monooleate was first used in 1930 in the margarine manufacture (Esposito *et al*. 2018). Today, it is used as a processing aid in the preparation and stabilisation of emulsions and foams in bread, cakes, margarine, ice creams and chewing gums. Its major activities are absorption at interface or on solids, promotion of wetting phenomena, co-crystallisation, complex formation (with proteins or starch components) and self-association. In the pharmaceutical field, glycerol monooleate was first used as an emulsifier and an absorption enhancer in presence of bile salts (Esposito *et al*. 2018; Xu *et al.* 2017; Herai *et al.* 2007). As an absorption enhancer, glycerol monooleate apparently acts by causing a temporary and reversible interruption of the lamellar structure of the lipid bilayer in the stratum corneum and, in turns, increasing intercellular lipid fluidity. Glycerol monooleate was first considered as a biocompatible encapsulating material in 1984. Since then, many researchers have investigated and reported its new applications and drug release properties.

Phytantriol (PT)

PT also has been wide spectrum used as a cubic phase-forming material in the past decade (Malheiros *et al.* 2022; Vallooran *et al.* 2020). PT is a well-observed active ingredient applicable in cosmetic industry, like hair and skin care products. Now days, PT has been acquiring more considerations since it has a phytanyl foundation but does not contain an ester linkage, which favours an enhanced chemical stability over to other fatty acid-based substances, such as GMO, in aqueous and model gastrointestinal situations (Sun *et al.* 2021; Jabłonowska *et al.* 2021)**.**

Phosphatidylethanolamine (PE)

It has been investigated that PE system is capable to form immensely stable Im3m and Pn3m cubic phases as a consequent of a temperature cycling through their L_α - H_{II} transition, as observed by X-ray diffraction (Hong *et al.* 2019; Shah *et al.* 2022). PE acquire a conical

shape due to the relative volume difference between its small ethanolamine head group and long, unsaturated acyl chains at the *sn*-1 and *sn*-2 positions. Because of its shape and lack of a charged head group, PE emerges negative curvature when integrated into membranes, which is recorded to increase the membrane fusion and can be applicable in drug delivery (Lou and Best 2020).

Oleoylethanolamide

Oleoylethanolamide is a natural cognate of the endogenous cannabinoid anandamide. In a reported literature, the phase diagrams and morphologies of oleoylethanolamide-water-arginine liquid crystalline structures were determined by combining cross-polarised optical microscopy (CPOM) and small angle X-ray scattering (SAXS). The results showed that the phase behaviour and stability of the liquid crystalline phases are highly dependent on arginine concentration. Upon enhancing the arginine concentration, transitions from Ia3d cubic phase to Pn3m cubic phase were observed, together with remarkable shrinking of the whole phase diagram region in which bicontinuous cubic phases are stable. By comparing the impact of arginine with that of glucose as a low molecular weight hydrophilic substance, it was observed that arginine has an ability to dehydrate the oleoylethanolamide polar head group, associating in the destabilization of Ia3d reversed cubic phases in assistance of Pn3m cubic phases (Mohammady, Pouzot and Mezzenga 2009; Chen, Ma and Gui 2014).

Due to a prolonged coexistence area of Pn3m cubic phase and water-arginine solutions, oleoylethanolamide-water-arginine liquid crystals were investigated as feasible delivery systems for amino acids in aqueous environment. A report indicated that oleoylethanolamide-water liquid crystals can effectively accommodate amino acids and are encouraging systems for a controlled release of these and other hydrophilic moieties in aqueous surrounding (Marson *et al*. 2022; Urandur *et al*. 2018).

Phospholipids

Generally lipidic mesophases with a Pn3m, Im3m or Ia3d consistency are characterised by water channels with a diameter of 3 nm–5 nm. The geometric constraint limits large hydrophilic molecules, such as high molecular weight proteins, hormones and antibodies from being admitted in the mesophase. But that crucial constitutional limitation can be overcome by excipients that enhance the water-channel proportions, including hydration-modifying agents such as phospholipids and cholesterol. Phospholipids with their two hydrophobic tails actually form bilayers. However, this is not usually emphasised, such bilayers can endure in various topological phases. These may be lamellar, like the L_{α} phase, which dispersed in solution forms spherical shapes or with saddle-shaped angles such as the L_3 sponge phase or cubic phases (Q_{II}) such as the primitive (P, Im3m), diamond (D, Pn3m) and gyroid (G, Ia3d) cubic phases. Ultimately, very apolar lipids may reveal large adverse interfacial angles and form an inverted hexagonal phase (H_{II}) or even inverted micelles (De Lange *et al*. 2021a; De Lange *et al*. 2021b).

Glycolipids

Glycolipids are carbohydrate-mediated component that appertain to the glycoconjugate group, consist of other biopharmaceuticals, such as glycoproteins, glycopeptides, lipopolysaccharides and peptidoglycans. Glycolipids self-organise into various liquid crystalline phases, such as hexagonal or columnar phases and lamellar or smectic

phases. They are usually birefringent and form characteristic structure under the polarising microscope (Zahid *et al*. 2022). Amongst them, cubic phases are not birefringent and hence are recorded as a dark band owing to their isotropic property. Although, they exhibit elevated viscosity than the usual isotropic structures. Glycolipids reveal productive surface properties that can be varied by changing the hydrophobic string length and the number of carbohydrate entities. In aspect of their notable lyotropic characteristics, glycolipids have been specially outlined and altered to give alluring self-assemblies that conduct to the emergence of nanostructure products or micro-emulsions, which have implicit to be used for drug delivery prospects (Patrick *et al*. 2018; Iskandar *et al*. 2021). An exceptional research investigation has demonstrated that glycolipids based micro-formulation not only indicated better drug solubilisation but also begot less pain upon injection in comparison with the other marketed formulations (Goh 2019).

Structure of bicontinuous liquid crystal

A number of cubic phase liquid crystals have been proposed, however, three bicontinuous liquid crystal structures (D-surface), (G-surface) and (P-surface) are common and can be described in terms of nodal surfaces (Behroozi 2009; Fraser, Separovic and Polyzos 2009; Squires *et al.* 2005). Figure 3 shows the surfaces of bicontinuous liquid crystal that are particularly fascinating because their discovery was purely mathematical, prior to knowledge of the structures existence in cubic phases. Since the structures formed by the monoglycerides bilayer in these cubic mesophases correspond to specific infinite minimal periodic surfaces (IMPS), they are also referred to as G-type (gyroid surface) and D-type (diamond surface). The MO-water system forms the D-surface at high water levels and the G-surface at lower water levels (Oka 2015). For more complex systems, e.g. in the MO-poloxamer 407 (Pluronic F127)-water system, another mesomorphic cubic phase or P-type (primitive surface) has been described (Wörle *et al.* 2006).

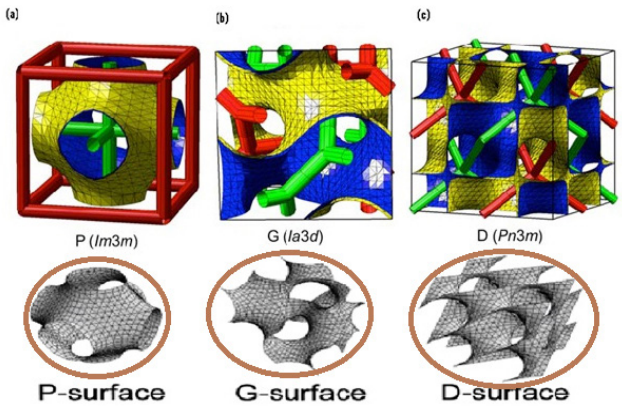

Figure 3: Three bicontinuous liquid crystal structures (P-surface), (G-surface) and (D-surface) in terms of nodal surfaces. Approximate mathematical models of the common inverse cubic-phase unit cells: Ia3d [gyroid (G)], Pn3m [diamond (D)] and Im3m [primitive (P)] (Spicer *et al.* 2001).

Phospholipids can be incorporated into cubic mesophases. The lipid bilayer units in simple MO/water systems form a three-dimensional network which separates two identical water-channel systems that have a water pore diameter of about 5 nm in the fully hydrated cubic phase (Landh 1991; Patton and Carey 1979). Due to its lipid and water domains the cubic phase may in principle solubilise both water and lipid-soluble substances, and molecules with amphiphilic character may partition at the lipid/water-interface. Biodegradability, the ability to incorporate and slowly release a variety of drugs with different physicochemical properties and the possibility to enhance the chemical, physical and/or enzymatic stability of incorporated drugs and proteins have made the cubic phase an interesting candidate for use in drug delivery (Engström *et al*. 1992; Ericsson *et al.* 1991; Shah, Sadhale and Chilukuri 2001; Wyatt and Dorschel 1992). Xiang and co-investigators (2022) have reported the synthesis of bicontinuous porous polymer based cubosome structure. They reported polymer cubosomes as the template, for the controllable synthesis of bicontinuous porous polymers with an ordered single primitive (SP) cubic structure, including polypyrrole (SP-PPy), poly-*m*-phenylenediamine (SP-PmPD) and polydopamine (SP-PDA). Such type of systems which possess 3D interconnected pore channels facilitating a smooth mass transfer and have more attracted features for drug delivery.

Production of Cubosomes Nanoparticles

Dispersed particles of bicontinuous cubic phases were first observed during studies of fat digestion that simulated stomach contents by combining oil with lipase and bile salts (Patton and Carey 1979). Olive oil droplets, mainly triolein, in contact with lipase formed small particles of cubic phases as the enzyme broke the TAGs down into MO that then hydrated, further sodium cholate bile salts provided dispersion and colloidal stability of the cubic phase particles formed by MO and water (Lindström *et al.* 1981). Later research on cubosomes focused on the ternary phase behaviour of the Pluronic F127 (a PEO_{99}-PPO_{67}-PEO_{99} block copolymer)-MO water system because of the polymer's utility at providing colloidal stability to cubosomes against recoalescence to bulk cubic phase. The PPO region of the block copolymer exists either at the surface of the cubic phase particles, or within the bilayer structure, whereas the PEO chains remain in the bulk water phase. Several techniques have been described for the preparation of aqueous dispersions of cubic MO-water phases (Spicer *et al.* 2001; Landh and Larsson 1993; Gustafsson *et al.* 1996; Gustafsson *et al.* 1997; Nakano *et al.* 2001). At its most basic, the production of cubosomes entails the formation of nanoparticles. Two distinct nanoparticles production routes exist: top-down and bottom-up techniques.

Top-down technique

Top-down approach begins with a suitable starting material and then sculpts the functionality from the material. Most cubosomes research over the last two decades has focused on top-down techniques, whereby bulk cubic phase is first produced and then dispersed by high energy processing into cubosome nanoparticles. Bulk cubic phase resembles a clear rigid gel formed by water-swollen cross-linked polymer chains, but cubic phases differ in that they are a single thermodynamic phase and display periodic liquid crystalline structure. Rupture of these cubic phases occurs in a direction parallel to the shear direction and the energy required is proportional to the number of tubular network branches that rupture. Researcher found that rupture of the cubic phase occurs as the bilayer breaks under applied shear stresses and flows along slip planes. The cubic phases exhibit a yield stress

that increases with increasing amounts of bilayer-forming surfactants and oils, and that is inversely proportional to the crystalline unit cell dimension. At high oscillatory frequencies, cubic phases become highly elastic (Radiman, Toprakcioglu and Mcleish 1994).

Bottom-up technique

The bottom-up approach first forms the nanostructure building blocks and then assembles them into the final material. It is more recently developed technique of cubosome formation, allowing cubosomes to form and crystallise from precursors on the molecular length scale. Almgren, Edwards and Gustafsson (1996) discuss the formation of cubosomes by dispersion of L2 or inverse micellar phase droplets in water at 80°C, then by slow cooling to allow the droplets to gradually crystallise into cubosomes.

Most cubosome research over the last two decades has focused on top-down techniques, whereby bulk cubic phase was first produced and then dispersed by high energy processing into cubosomes nanoparticles. It was suggested to use the common conventional emulsification methods (such as ultrasonication, microfluidisation and homogenisation) for the fragmentation and the stabilisation of these monoglycerides self-assembled systems. Ultrasonication of bulk cubic phase produces mostly vesicles that over the course of several weeks transform into cubosomes, likely via membrane fusion. Such metastability is characteristic of cubosome systems because of the slow transport processes involved in forming the high viscosity crystalline structure and the high energy input required to fragment bulk cubic phase.

Another process of development at room temperature to produce cubosomes by simply diluting MO-ethanol (or other hydrotrope) solutions with aqueous Pluronic F127 solutions. The cubosomes have been shown to form by spontaneous emulsification, producing nanoparticle dispersions in the near absence of energy input beyond that required for simple blending of the two liquids (Spicer *et al.* 2002).

Smaller and more stable cubosomes are produced than those by high-energy processes but some vesicles are also produced. A second process was also developed to allow cubosomes production from a powdered precursor. Spray dried powders comprising MO coated with starch or dextran form cubosomes on simple hydration. Colloidal stabilisation of the cubosomes is good when Pluronic F127 is used to provide steric stabilisation against aggregation and coalescence but other suitable polymers can be used as well. The large unfolded bilayers that form in such cases may be stabilised by polymer-induced osmotic, steric or viscous forces, identical to the higher fraction of surface vesicles observed in the Pluronic F127-MO-water system. Dispersion of the nanoparticles produced in the cubosomes formation by several techniques, such as sonication, high pressure homogenisation, spontaneous emulsification and spray drying.

Sonication and high-pressure homogenisation recommend the production of complex dispersions containing vesicles and cubosomes with time-dependent ratios of each particle type. Coarse cubosomes on the micron scale possess the same D-surface cubic structure as their source bulk cubic phase (Siekmann *et al.* 2002) but after homogenisation, the P-surface dominates, either because of the added polymer or other factors. Large scale production of cubosomes and products requires more robust processes. Spicer *et al.* (2001) optimised a room temperature process to produce cubosomes, diluting MO-ethanol (or other hydrotrope) solutions with aqueous Poloxamer 407 solutions by spontaneous emulsification, producing nanoparticle dispersions in the near absence of energy input beyond that required for simple blending of the two liquids. Smaller and more stable cubosomes are developed than those by high-energy processes, but some vesicles are also produced. A second process was also optimised to allow cubosomes production from a

powdered precursor (Spicer *et al.* 2002). Spray-dried powders composed of MO coated with starch or dextran form cubosomes on simple hydration. The polymers immediately provide colloidal stabilisation of the cubosomes.

Spray-drying technique

The spray-drying technique is applied in order to prepare dried form precursors of cubosomes and utilises the organic solvents that evaporate upon the application of air. The lipid-surfactant solvent mixture is atomised with hot air wave, resulting in quick solvent evaporation and the formation of the dry powder of the cubosomes precursor. It begins with combining the lipid with the stabiliser and then solubilising them into ethanol (binary solutions can also be used). A distinct mixture of aqueous phase, consisting of a hydrophilic solid carrier, i.e. sorbitol or dextran, is prepared, followed by combining with the previously prepared lipid mixture. Both mixtures are mingled with continuous stirring, which give rise to the formulation of a low viscosity emulsion. This method is simple, cost effective and usually easy to scale-up (Umar *et al*. 2022)

Solvent evaporation technique

Solvent evaporation is an alternative technique of producing powder cubosomes by using a homogeniser or ultra-sonicator. The method is actually analogous to the spray-drying method, except for the application of a high-energy sonicator. In this technique, firstly, an organic solvent such as ethanol or chloroform is used to dissolve the lipids, followed by adding drop-wise to the other mixture containing stabiliser, such as Pluronics in aqueous phase. The mixture is controlled at an elevated temperature under magnetic stirring. The drug can be dispersed in the lipid or the aqueous surfactant solution. Stirring under elevated temperatures removes the volatile organic solvent, and the mixture is homogenised by ultra-sonication or homogeniser, leading to the formation of cubosomes.

Characterisation of Nanostructured Cubosomes System

To date, cubosomes have been characterised primarily by cryo-transmission electron microscopy (cryo TEM) in combination with SAXS to confirm the internal nanostructure of the particles (Figure 4). Other techniques that have been used to characterize liquid crystalline systems, including freeze-fracture transmission electron microscopy (Gulik-Krzywicki and Delacroix 1994; Angelova *et al.* 2005; Müller-Goymann 2004), scanning electron microscopy and NMR. Cubosomes are immediately identifiable as square particles with a well-defined cubic lattice of dots. Each dot corresponds to the presence of a pore containing aqueous phase. Differential scanning calorimetry is a powerful method to study phase transitions and to analyse them in terms of their position on the temperature scale and of the associated energy (Raemy *et al.* 2005). Further LUMIfuse technique, a microprocessor-controlled analytical centrifuge has been used to determine the physical stability (creaming, aggregation and coalescence) (Amar-Yuli *et al.* 2007; Efrat *et al.* 2007). Other techniques that have been used to evaluate possible variations of particles, dimensional distribution (Esposito *et al.* 2003).

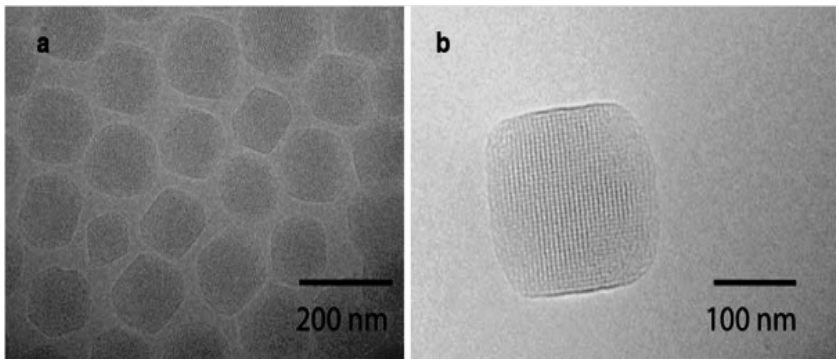

Figure 4: Cryo-transmission electron microphotograph. Visualisation of monoolein-Poloxamer 407-water cubosomes formed by high-pressure homogenisation (Spicer *et al.* 2001).

Stabilisation of nanostructured cubosomes system

Three decades before, in the 1990s, Larsson and co-workers investigated the formation and characterisation of Pluronic F127-stabilised lipid based nanoformulations (cubosomes and hexosomes) that were manufactured using a high-energy emulsification technique. Besides to Pluronic F127, numerous stabilising agents as well as other Pluronics (such as F128), PEGylated lipids, *β*-casein and citrem have been found for the stabilisation of cubosomes, hexosomes and other cubic phase lipid based nanostructured formulations (Prajapati, Salentinig and Yaghmur 2018; Prajapati, Larsen and Yaghmur 2019). By considering the reported major drawbacks (including poor haemocompatibility and cytotoxicity) of most explored Pluronic F127-stabilised cubosomes and hexosomes, it is valued using 'citrem' as a desirable alternative stabiliser, especially for nanoformulations deliberated for intravenous drug delivery applications (Azmi *et al.* 2016; Khaliqi *et al.* 2017). Recent investigation on utilising mPEG-lipid conjugates as stabilising agents of *lipid based nanostructured formulations*; it was found that their lipid moieties may have modulatory impact on nanoparticle-mediated complement activation. Among distinct mPEG-lipid conjugates, it was observed that TPGS-mPEG$_{2000}$ is the most desirable lipopolymer for adequately overcoming complement activation (Helvig *et al*. 2021). Recently, Murgia and co-investigators (2020) recommended polyphosphoester congenator of Pluronic F127 as another choice of stabiliser for fabrication of cubosomes with reduced cytotoxicity. Stabiliser-free cubosomes at definite compositions were also recently manufactured and characterised (Zabara *et al.* 2019).

Applications of Cubic Phase and Cubosomes

Controlled release of solubilised actives is the most popular application pursued by cubosome researchers and excellent reviews exist of attempted delivery applications as well as pharmaceuticals actives that have been solubilised in bulk cubic phase and cubosomes (Drummond and Fong 1999).

Cubic phase is attractive for controlled release because of its small pore size (5 nm–10 nm), its ability to solubilise hydrophobic, hydrophilic and amphiphilic molecules, and its biodegradability by simple enzyme action (Borné, Nylander and Khan 2002). Cubic phase is strongly bioadhesive (Geraghty *et al.* 1997) and is thought to be a skin penetration

enhancer (Lee and Kellaway 2000) suggesting excellent compatibility with topical and mucosal deposition and delivery of active ingredients. Recent studies have emphasised similarities between the bicontinuous structures formed in human skin layers and those comprising cubic phases, offering the promise of better skin transport understanding and treatment (Norlén 2001a; Norlén 2001b). The tortuous structure of cubic phase lends itself well to slowing diffusive release of solubilised actives. Theory predicts the minimum reduction of a solute's free solution diffusivity by 33% (Anderson and Wennerstrom 1990). Experimental measurements of small molecule diffusivity in cubic phases give values on the order of 10^{-10} m^2/sec (Mattisson *et al.* 1996). No commercial applications of cubic phase delivery vehicles are known other than a treatment developed for periodontal disease that is based on triglyceride-MO mixtures combined with the drug metronidazole (Jain *et al.* 2008). The lipid–drug mixture forms a low viscosity liquid that, when applied to the gums and placed in contact with saliva, hydrates to form a bulk cubic phase that then delivers the drug to the gum. Despite the potential of bulk cubic phase as a delivery vehicle, some applications are not compatible with the extremely high viscosity of the bulk cubic phase and require the use of cubosomes.

Compared to liposomes or vesicles, cubosomes posses much higher bilayer area-to-particle volume ratios as well as higher viscous resistance to rupture. Although bulk cubic phase has sufficient length scale to allow controlled release of solutes, cubosomes are too small and have high a surface area for such performance, exhibiting instead burst release (Boyd 2003). Turning this concept around cubosomes should be quite useful for uptake instead of release because they can rapidly absorb pollutants (e.g. for water treatment or cosmetic skin protection) and retain an amount determined by the solute partition coefficient.

From material sciences prospective, the creation of ordered structures with nanoscale pore geometries is of great interest to numerous fields including electronics, photonics, catalysis and medicine. The creation of solid structures using cubic phases as a template usually entails either polymerisation or reaction to form solids from precursors that are solubilised in or comprise the cubic phase matrix. One of the earliest and most successful materials formed in a cubic phase template is the aluminosilicate zeolite MCM-48 (Kresge *et al.* 1992), used for catalytic processing for petroleum. Polymerisation inside the cubosome is successfully carried out, yielding a solid nanostructured particle with cubic symmetry (Yang, Obrien and Marder 2002). Such particles hold promise for use in photonic and semiconductor applications. A novel aerosol processes have been developed that create particles with nanometer scale structure by evaporation of solvent from isotropic phase liquid droplets, simultaneously driving them into cubic phase structure and solidifying the particles (Lu *et al*. 1999). Beautiful structures have been formed using careful growth of faceted cubosome in the $C_{12}E_2$ water system offering future promise of multiple decade length scale control over the morphology of particles formed from such templates (Pieranski *et al.* 2001). Moreover, cubosomes posses various other applications as a drug delivery system, which includes the following. Table 1 reports the updated translational research, patents and applications of cubosomes.

Table 1: Various types of cubosomes with recent update on translational research, patents and applications

Type of cubic system	Active ingredient	Application	Route of administration/ Mechanism	Patent/Article	Reference
An emulsion of a liquid crystal-forming substance	One or more antimicrobial substances, anti-biofilm substances, or combination thereof, alone or optionally in further combination with a mucoadhesive substance	Treatment of mastitis in dairy cattle	Intramammary infusion	Patent	Barrows and Curemast Inc., 2023
A pharmaceutical long acting depot composition comprised of biodegradable carrier is in the form of microspheres, implants, cubosomes, hexosomes, solutions, suspensions, microemulsions, in-situ gelling system or a combination thereof	Varenicline	As an aid to smoking cessation treatment	Parenteral	Patent	Malhotra and Singh, 2023
Liquid crystal phases	Statin	Relates to methods of lowering blood cholesterol	*Via* the oral mucosa	Patent	Szto, Madmon, and Kannar 2021
Cubosomes	Lutein	Enhanced penetration into the skin	Topical	Patent	Lim *et al.* 2021

(continued on next page)

Table 1: (*continued*)

Type of cubic system	Active ingredient	Application	Route of administration/ Mechanism	Patent/Article	Reference
Cubosomes/Water with up to 30% protein and about 5% lipid to about 30% lipid.	Vernix caseosa (Vernix)	The composition may be used to cleanse newborn skin, compromised skin Surfaces, as well as normal skin, to provide hydration/ barrier function, and other applications	Composition contains water-in-oil emulsified particles providing water vapor transport and evaporative water loss properties simulating native Vernix.	Patent	Hoath *et al*. 2011
Solubilising composition comprises cubosome particles	Novel solubilising composition comprising of monoglycerides, emulsifiers and organic solvents.	Stable solubilisation of materials as well as stable long-term storage, and the manufacturing method	Lyophilised liquid composition and the powder of the present invention are physicochemically stable since they neither contain water that causes oxidation or hydrolysis upon storage nor undergo phase separation.	Patent	Jeong *et al.* 2006
Polymer cubosomes	Block co-polymer as main structural component	Used for chemical reactors or bioreactors, carriers capable of cargo loading and release, and scaffolds for nanotemplating	Block co-polymer with nonlinear architectures (dendritic–linear, branched–linear, and branched–branched BCPs) preferentially self-assembled to inverse mesophases in solution	Article	Ha, La and Kim 2020

(continued on next page)

Table 1: (*continued*)

Type of cubic system	Active ingredient	Application	Route of administration/ Mechanism	Patent/Article	Reference
Hyaluronic-Acid-Tagged cubosomes	Copper acetylacetonate	As cancer therapeutics	CD44 receptor is overexpressed in various cancer and act as potential target for nanoparticle delivery, by attaching its natural ligand hyaluronic acid (HA) to the surface of particles	Article	Pramanik *et al.* 2022a
Hybrid theranostic cubosomes	Anticancer drug daunorubicin with a folate-chitosan conjugate and a single strand DNA as model genetic materials	Photodynamic treatment of tumor lesions	NIR-induced photodynamic therapy	Article	Bazylińska *et al.* 2022
Functionalised cubosomes	Lissamine rhodamine	To deliver drug across the BBB	Cubosomes were formulated with Pluronic F127 or Pluronic F68 or Tween 80 and their potential to facilitate the brain uptake of lissamine rhodamine (RhoB), a P-gp substrate and a molecule with poor BBB permeability, which makes tracking its distribution under the confocal microscope	Article	Azhari *et al*. 2021
Engineered cubosomes functionalised with Affimer proteins via copper-free click chemistry	Copper acetylacetonate	Targeting to colorectal cancer cells	Target overexpressed carcinoembryonic antigens on LS174T colorectal cancer cells	Article	Pramanik *et al.*2022b

Potentiality to sustain or control drug release as drug carriers

Cubic phase liquid crystals have the ability to provide sustained drug release. Drugs with a wide range of molecular weights and water solubilities have demonstrated sustained release in a cubic phase, such as aspirin and vitamin E (Wyatt and Dorschel 1992), propantheline bromide and oxybutynin hydrochloride (Geraghty *et al.* 1996), metronidazole (Norling *et al.* 1992), tetracycline (Esposito *et al.* 1996), timolol maleate (Lindell *et al.* 1998), chlorpheniramine maleate (Chang and Bodmeier 1997), propranolol hydrochloride (Costa-Balogh *et al.* 2010), melatonin, pindolol, propranolol and pyrimethamine (Burrows, Collett and Attwood 1994), haemoglobin (Leslie *et al.* 1996), cefazolin (Sadhale and Shah 1998), insulin (Sadhale and Shah 1999a; Sadhale and Shah 1999b), capsaicin (Peng *et al.* 2010), cinnarizine (Rajabalaya *et al.* 2017; Sadhale and Shah 1999b; Nguyen *et al.* 2010) and diclofenac salts (Yariv *et al.* 2010). Lee *et al*. (2009) investigated the *in vitro* sustained release behaviour of a number of model hydrophilic drugs with various molecular weights (14C-glucose, Allura red and fluorescein isothiocyanate dextrans FD-4, FD-20 and FD-70) in two types of liquid crystalline matrixes, namely, V2GMO (a cubic phase prepared from GMO) and V2PT (a cubic phase prepared from PT). The release samples were constrained in micro beakers with a fixed surface area to ensure a constant release area between the liquid crystals and the release media. The results showed that in all cases the cumulative amount of drug release through the matrix followed a linear relationship with the square root of time, which represented a Higuchi diffusion controlled release profile. The impact of phase structure and molecular weight on drug release was also investigated. It was discovered that the release rate of each drug decreased as the matrix was changed from V2GMO to V2PT and the diffusion coefficient of the model drugs was reduced as the molecular weight increased. The results indicated that phase type and molecular weight of drugs had an influence on their release behaviour. In order to confirm whether the *in vitro* release data were able to translate into *in vivo* oral absorption dates, the oral absorption kinetics of 14C-glucose formulations were further studied in rats. In this study, 14C-glucose was chosen as the model drug due to its fast absorption rate; therefore, the kinetics of 14C-glucose in plasma would only be determined by its release rate from the matrix. The pharmacokinetic results showed that the mean t_{max} (time to peak) of 14C-glucose absorption in solution formulations (1 h) was the shortest, followed by V2GMO (1.13 h) and V2PT (1.88 h). It was found out that the oral absorption rate of 14C-glucose followed the order observed *in vitro*, which indicated that the nanostructure of these lipid based liquid crystalline systems had a significant impact on oral absorption of hydrophilic drugs.

Potentiality to improve drug bioavailability and reduce drug toxicity

Cubic phases are utilised to improve the drug bioavailability and reduce drug toxicity (Yang *et al.* 2012) prepared PT-based cubosomes containing amphotericin B (AmB) to improve its bioavailability and reduce nephrotoxicity. After oral administration of an AmB-loaded cubosomal formulation in rats, nephrotoxicity was not observed and the relative bioavailability of AmB was approximately 285% compared to the control group. Chen *et al*. (2012) developed a cyclosporine A-loaded GMO/F127 cubosome system to reduce ocular irritancy. The results showed that cubosomes almost had no irritation, and only transient corneal hyperaemia was observed in one rabbit but recovered within 1h. In addition, no ocular damage or clinically abnormal signs were observed in cornea, conjunctiva or iris. Anticancer agents like paclitaxel (Zhang *et al*. 2020) and docetaxel (Dawoud, Abourehab and Abdou 2020), antiviral (efavirenz) and anti-inflammatory (flurbiprofen) therapeutic

agents, along with antioxidants like curcumin, have significantly increased bioavailability that is attributable to the preservation and delivery of the hosting cubic liquid crystalline phases (Alavi and Nokhodchi 2022).

Potentiality to enhance the stability of drugs

The distinguished structure of the cubic phase is used to accommodate unstable drug substances and protect them from physical and chemical degradation. Sadhale and Shah (1999a, 1999b) reported the ability of cubic phase gel to protect insulin from agitation induced aggregation. The results showed that the native conformation of agitated insulin in cubic phase gels was almost unaffected for 2 months at 37°C, while the majority of insulin in solution appeared to aggregate and precipitate only after 8 days. Therefore, the cubic phase gel was able to protect insulin from agitation-induced aggregation and subsequent precipitation. Furthermore, they (Sadhale and Shah 1999b) investigated the effect of agitation on biological activity of insulin in cubic phase gel by subcutaneous injections of the agitated cubic phase gel, nonagitated cubic phase gel, agitated insulin solution, and normal saline to fasted rats and their blood glucose levels were observed. The blood glucose levels given the non-agitated cubic phase gel and the agitated cubic phase gel were significantly lower ($P < 0.05$) than those in the agitated insulin solution or saline from 40 min to 4 h. The results suggested that insulin was biologically active in both agitated and non-agitated cubic phase gels. However, upon agitation, insulin in solution totally lost its hypoglycaemic activity. In summary, GMO-based cubic phase gel can protect insulin from agitation-induced aggregation. Sadhale and Shah (1998) also evaluated the stability of two model drugs, cefazolin and cefuroxime, in a GMO-based cubic phase gel. The stability of cefazolin was assessed at two different concentrations (200 μg/g and 50 μg/g) at 22°C and 37°C. The results revealed that the degradation of cefazolin at lower concentration was 3-fold and 18-fold slower in cubic phase gel than that in solutions at 22°C and 37°C, respectively. At 22°C and 37°C, the kinetics of degradation at higher concentration of cefazolin was not first order but a lag phase followed by an exponential loss of cefazolin, which may be due to its oxidation. Later on, the oxidation of cefazolin was confirmed by its 18-fold higher stability in the presence of EDTA and nitrogen in solution. In addition, the degradation rate of cefuroxime was two times slower in cubic phase gel than that in solution. In summary, this study clearly demonstrated that cubic phase gel enhanced the chemical stability of cefazolin and cefuroxime.

Potentiality to increase the penetration of drugs

Cubic phases and cubosomes also have the capability to improve the transdermal/topical delivery of small molecules such as acyclovir, used to treat infections caused by certain types of viruses (Nielsen, Helledi and Schubert 2001), paeonol, used for the treatment of atherosclerotic cardiovascular disease (Luo, Shen and Chen 2011), δ-aminolevulinic acid, used to treat actinic keratosis (Bender *et al.* 2005), sulphorhodamine B, a fluorescent dye widely used to investigate cytotoxicity in cell based studies (Bender *et al.* 2008), calcein, also known as fluorexon, fluorescein complex, a fluorescent dye (Yamada *et al.* 2011), and diclofenac salts, indicated for *use* in the treatment of pain and inflammation (Yariv *et al.* 2010), as well as macromolecules such as cyclosporin A, used *t*o prevent organ rejection (Chen *et al.* 2012; Lopes *et al.* 2006). Nielsen, Helledi and Schubert (2001) studied that the cubic phase augmented the penetration of acyclovir by 6 times compared to its commercial product. Luo, Shen and Chen (2011) prepared a cubic gel containing 3% paeonol, 30%

water and 67% GMO, and the *in vitro* skin permeability test indicated that the permeability coefficient of cubic gel was 8.34 ± 0.49 × 10^{-3} cm/h, which was significantly higher ($P < 0.05$) compared to the microemulsion gel (5.88 ± 0.28 × 10^{-3} cm/h) and the control solution (3.06 ± 0.10 × 10^{-3} cm/h). The results indicated that paeonol in cubic gel had higher permeability in comparison to that of paeonol in microemulsion gel and the control solution; therefore, cubic gel would be a better drug delivery system for transdermal delivery of paeonol. Lopes *et al*. (2006) observed that the cubic phase increased the penetration of cyclosporin A in stratum corneum and epidermis plus dermis at 12 h post application *in vitro*. The *in vivo* permeation study showed that the cubic phase increased the concentration of cyclosporin A in the non-viable skin (stratum corneum) when compared to the control formulation. Chen *et al*. (2012) prepared a cyclosporine A loaded cubosome system for ocular drug delivery to improve corneal penetration. The *in vitro* corneal permeation study showed that the steady state permeation rate of cyclosporine A was 1.52-fold compared to that of the oil solution. In addition, cyclosporine A loaded cubosomes demonstrated a significantly enhanced initial penetration rate, where the cumulative amount of cyclosporine A in the first 0.5 h was about 2-fold compared to that of the oil solution. The authors concluded that the main mechanism of the enhanced corneal permeation might be the absorption and/or surface lipid exchange between the liquid crystalline nanoparticles and corneal epithelial cells. Further, the close adhesion of the small lipid colloidal carriers with a large surface area to the lipophilic epithelium surface could promote a quick initial permeation rate. It was also reported that the periodically curved lipid bilayer of liquid crystalline nanoparticles was very similar to the microstructure of the cell membrane (Larsson 1989; Giorgione, Huang and Epand 1998) and the cubic architecture in stratum corneum was almost similar to the structure of cubic phases (Norlén and Al-Amoudi 2004). Furthermore, the interaction between biological tissues and cubic phases may contribute fast permeation rate as well, and this characteristic makes the development of cubosome based products more promising.

Nowadays, some advanced optical technologies have been used to investigate the penetration mechanism of fluorescent materials. The method of fluorescent quantification at the skin surface was used to quantify the penetration of δ- aminolevulinic acid (ALA) and its methyl ester into the tissue (Bender *et al.* 2005). ALA is a precursor of heme which can induce the production of the photosensitizer protoporphyrin IX (PpIX) in living tissues. A fibre-optic probe coupled to a spectrophotometer was used to measure the PpIX fluorescence at the skin surface followed by topical administration of cubic phases. The results showed that, 1 h post application, GMO-based cubic systems and PT/propylene glycol/water cubic systems showed significantly higher fluorescence in comparison to standard ointment over 10 h ($P < 0.05$), and this was probably due to the enhanced drug permeation. The authors also analysed the difference between GMO and PT cubic systems in terms of enhanced drug permeation and concluded that the difference mainly relied on the swelling extent and the rheological parameter of the systems. Noteworthy, the maximum amount of water in the PT-based cubic phase (about 28%) was less than that in GMO-based cubic phase (about 40%), which implied that PT-based cubic phase had more narrow water channels thus resulting in a slower release. In terms of rheological property, PT-based cubic phase had higher viscosity which made it quite difficult to handle and contact with skin thereby led to a lower PpIX level. In addition, propylene glycol was introduced into PT-based cubic phase to make the system softer, and this may be a partial explanation for the higher PpIX level in the PT-based cubic phase with propylene glycol. In another study, two-photon microscopy (Bender *et al.* 2008) was used to visualise the uptake of sulphorhodamine B (incorporated into GMO-/PT-based cubic phases) in full thickness human skin. This technology is able to take images of fluorophores of sulphorhodamine B much deeper into highly light-scattering and light-absorbing tissues compared to confocal laser scanning microscopy but

with minimal photobleaching and phototoxic effects. The results recommended that the dominating delivery route of the cubic phases is *via* micro-fissures caused by microscopic clustering of the keratinocytes in the skin. Therefore, sulphorhodamine B delivered by cubic phases diffused into the surrounding intercellular lipid matrix through these micro-fissures and acted like a source for its sustained release.

ISAsomes as functional lipid based nano-vehicles for drug delivery

The rearmost decade has observed a extended interest in the application of *ISAsomes*, mainly cubosomes and hexosomes, as nano-vehicles for loading various active constituents, diagnostic probes, and antimicrobial peptides (Mertins, Mathews and Angelova 2020; Gontsarik, Yaghmur and Salenti 2021). Especially, a major attention has been conducted towards their application for augmenting the solubilisation of poorly water-soluble active constituents, including curcumin, thymoquinone and cinnarizine (Rakotoarisoa *et al.* 2019). Nonetheless, most observation focused on the entrapment efficiency and the influence of entrapped drug type and concentration, lipid composition, and stabiliser type and concentration on the structural and morphological characteristics, and size of these nano-self-assemblies. Various studies on their drug release properties, cellular responses, and other in vitro and *in vivo* evaluations are still relatively narrow. In addition, further investigations should be needed to gain insight into the impacts of lipid composition and type, and stabiliser type and concentration on the cellular uptake mechanisms of these nano-self-assemblies. Other applications of cubosomes and related nanoparticles, including oral, intravenous, and subcutaneous, topical, trans- and intra-nasal, ophthalmic, and skin drug delivery, and their uses in the development of theranostic nanocarriers, the readers are directed to relevant reports and recent review articles (Bakr, Shukr and Elmeshad 2020; Silvestrini *et al.* 2020).

Oral drug delivery

In the formulation development of nano-structured lipid based systems intended for oral drug delivery, previous studies reported through in vitro and in vivo evaluations on an improved bioavailability and sustaining release of various drugs [including 20(S)-protvopanaxadiol, doxorubicin and cinnarizine] loaded to cubosomes or hexosomes (Von Halling Laier *et al.* 2019). The evaluated nano-structured lipid based systems were comprised of either PT or MO and stabilised with Pluronic F127. For example, it was reported after an oral administration of doxorubicin-loaded PHYT cubosomes to rats on an improved bioavailability, an improved antitumour efficacy and a lower level of cardiotoxicity in comparison to the FDA-approved formulation Adriamycin®, which was intravenously administered (Swarnakar, Thanki and Jain 2014). This improved oral doxorubicin delivery was associated to a longer circulation half-life and an enhanced tumour accumulation of nanoparticles *via* an enhanced permeation and retention (EPR) effect. In another example, Yang *et al.* (2014) reported on an improved oral delivery of amphotericin B loaded to MO cubosomes for anti-fungal infection treatment. As compared to the clinical formulation Fungizone®, which was intravenously administered, a more significant efficacy was reported for the orally administered cubosomal formulation.

Intravenous drug delivery

Recent investigations reported that combination of MR and NIRF imaging modalities for development of F127-stabilised cubosomes and hexosomes have shown as agents with dual MR-NIRF imaging properties. It was found that the administered nano-structured system accumulated up to 20 h of post-administration in the liver and spleen of mice. However, the accumulation level seems to be dependent on the lipid composition and/ or structural features as hexosomes showed a greater level of accumulation in the spleen than the liver in comparison to cubosomes. The MO cubosomes were accumulated in the liver after intravenous administration in mice which was analysed by NIRF *in-vivo* imaging technique (Tran *et al.* 2017; Biffi *et al.* 2017). Moreover, the investigated biodistribution of cubosomes and hexosomes, *in vivo* MR imaging indicated an incvreased contrast in the liver and spleen. An enhancement of MR contrast for *in vivo* imaging was also reported for nitroxide-loaded MO hexosomes. Further investigation by Jain *et al*. (2012) reported on radiolabeling of PEGylated non-lamellar liquid crystalline nanoparticles loaded with paclitaxel [^{99m}Tc-(Technetium radionuclide)-labeled nanoparticulate formulation] and biodistribution after intravenous administration to Ehrlich Ascites tumour (EAT)-bearing mice. They found that PEGylation of these nanoparticles is not only associated with an enhanced safety, but also contributes to an improved circulation time and enhanced tumour accumulation by EPR as compared to corresponding non-PEGylated cubosomes and plain paclitaxel. The observed tumour growth inhibition with the non-PEGylated nanoparticles was attributed to their internalization into the tumours through EPR and other non-specific effects. In another study, the in vivo biodistribution of MO cubosomes loaded with paclitaxel was also investigated. However, the investigations were done after intraperitoneal (i.p.) administration of the nanoparticles to mice (Bye *et al.* 2014; Jain *et al.* 2012; Zhai *et al.* 2020). It was reported on an enhanced tumour accumulation and a reduction of tumour average size following i.p. administration of paclitaxel-containing cubosomes as compared to corresponding control (paclitaxel-free) cubosomes. In addition to paclitaxel-loaded nanocarriers, F127-stabilised MO cubosomes were observed as suitable candidates for loading etoposide, which is a topoisomerase II inhibitor showing anti-proliferative activity. In vivo investigations on i.v. administered etoposide-containing folate-modified and unmodified cubosomes to mice bearing human breast carcinoma MCF-7 indicated that nanoparticle modification with folate was related with an improved anti-proliferative activity as compared with unmodified cubosomes and free etoposide.

Subcutaneous drug delivery

In a reported research, radiolabeled 99mTc-SpmTrien-hexosomes were evaluated for in vivo imaging after subcutaneous (s.c.) administration to right flanks of healthy mice (Tian *et al.* 2017) and footpads of healthy rats by using single photon emission computed tomography in combination with computed tomography (SPECT/CT). It was observed that the radiolabeling method did not influence the mean nanoparticle sizes, and structural and morphological features of hexosomes. It was observed that Pluronic F127, covering the outer surfaces of hexosomes, may play a modulatory role in the detected rapid drainage from the footpad interstitium and the simultaneous recognition by the lymph node macrophages. It was expected that the surface anchored ethylene oxide (PEO) blocks of F127 may form a ‘mushroom-like’ configuration in hexosomes, leading to reduction of interactions within the footpad interstitium, without interfering with lymph node macrophage identification. Further, a liver targeted drug delivery system was reported, Pluronic F127 MO cubosomes loaded with 5-fluorouracil was evaluated after s.c. injection into rats. After 3 h of administration,

it was observed that enhanced accumulation of 5-fluorouracil (about 5-fold increase) in the liver as compared to an aqueous solution of this drug. However, the increase in drug concentration was associated with hepatocellular damage. An elevated permeability of cubosomes to the epithelial membrane may play role in the observed liver uptake. In recent studies, cubosome was also observed in the design of vaccine delivery applications (Nasr, Ghorab and Abdelazem 2015; Liu *et al.* 2016; Rizwan *et al.* 2013).

CONCLUSION

Cubosomes are lipid based nano-spheres that are an exclusive class of liquid crystalline phase which characterised by liquid crystalline property of their nano-architecture, prepared from amphipathic lipids like glycerol monooleate, PT, PE, oleoylethanolamide, phospholipids, glycolipids which self-assembled in water and in presence of stabiliser into cubosomes. Cubosomes nanoparticles formed from monoglycerides-Pluronic F127-water liquid crystalline stages are a unique and intriguing self-assembled material with enormous potential in areas as diverse as health science, chemical science, materials science and consumer products. These systems and the method by which they were prepared may be applicable for manufacturing of various delivery systems for pharmaceutical applications. Two validated methods such as top-down and bottom-up techniques could be conveniently employed to bring out cubosomes either by high pressure homogenisation or ultrasonication techniques. Cubosomes are pertinent to broad spectrum of active medicaments, high molecular weight components like proteins, immunogenic substances and also to cosmetics. They may furnish a promising carrier for effective dermal drug delivery with enhanced dermal penetration and low sensitisation potential, impart the stability of drugs, enhanced drug bioavailability and reduce drug toxicity and further, prolong or controlled the drug release. Cubosomes would have broad spectrum scope of research in developing new formulations with commercial and industrial value due to its high through output.

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