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Lyotropic liquid crystal systems in drug delivery

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Lyotropic liquid crystal systems, such as reversed bicontinuous cubic and hexagonal mesophases, are attracting more and more attention because of their unique microstructures and physicochemical properties. Various bioactive molecules such as chemical drugs, peptides and proteins can be solubilized in either aqueous or oil phase and be protected from hydrolysis or oxidation. Furthermore, several studies have demonstrated sustained release of bioactive molecules from reversed cubic and hexagonal mesophases. This article gives an overview of recent advances and current status of reversed cubic and hexagonal mesophases, especially with respect to their preparation methods and applications in the field of drug delivery. In addition, potential problems and possible future research directions are highlighted.

Introduction

Lyotropic liquid crystal (LLC) systems that commonly consist of amphiphilic molecules and solvents can be classified into lamellar (L_α), cubic, hexagonal mesophases, and so on. In recent years, LLC systems have received considerable attention because of their excellent potential as drug vehicles. Among these systems, reversed cubic (Q_2) and hexagonal mesophases (H_2) are the most important and have been extensively investigated for their ability to sustain the release of a wide range of bioactives from low molecular weight drugs to proteins, peptides and nucleic acids [1–5].

Reversed cubic and hexagonal mesophases are often formed by polar lipids in an aqueous environment. The structure-forming lipids can absorb a certain amount of water and then spontaneously form gel-like phases with unique internal structures, into which drugs can be incorporated. Moreover, non-toxic, biodegradable and bioadhesive properties also contribute to their applications for drug delivery [6]. Owing to infinite swelling capability, reversed cubic and hexagonal mesophases can also be dispersed in equilibrium with excess water and form colloidal dispersions with superior thermodynamic stability [7,8]. At present, reversed cubic and hexagonal mesophases are being investigated as candidates for aural, buccal, gastrointestinal, intravenous, lung, nasal, oral, rectal and vaginal administration of drug with considerable progress [1].

In the following sections, we briefly introduce the cubic and hexagonal mesophases based on recent literature, including their textures, preparation methods, phase behaviors and applications in drug delivery. This article is not meant to provide an exhaustive review but rather to present some highlights. In particular, we discuss the current status of investigations with respect to the applications of cubic and hexagonal mesophases as drug vehicles and then propose new or promising directions of research.

Structures of reversed cubic and hexagonal mesophases

For reversed bicontinuous cubic and hexagonal mesophases, three macroscopic forms are typically encountered: precursor, bulk gel and particulate dispersions.

Structure of cubic mesophase

The structure of cubic mesophases is unique and comprises a curved bicontinuous lipid bilayer (with an estimated thickness of 3.5 nm) extending in three dimensions and two interpenetrating, but non-contacting, aqueous nano-channels (with a fully

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swollen diameter of approximately 5 nm), with a high interfacial area of 400 m^2/g [1,7,8]. At present, the cubic mesophases prepared by unsaturated monoglycerides or phytantriol (PT) are the most frequently investigated liquid crystal structures for drug delivery [9–11]. The compartmentalization in cubic mesophases can be used to introduce guest drugs of hydrophilic, lipophilic or amphiphilic nature (Fig. 1a). Hydrophilic drugs will be located close to the emulsifier polar head or in the water channels, whereas lipophilic drugs will be localized within the lipid bilayer and amphiphilic drugs in the interface [12].

The bulk phase is commonly a clear, viscous, semi-solid gel that is similar in appearance and rheology to cross-linked polymer hydrogels [13]. Its high viscosity makes it difficult to handle and limits its application and, furthermore, the bulk phase can cause the irritation reaction when in contact with the biological epithelia [14]. To overcome these issues, an innovative strategy has been formulated: to disperse the bulk phase into water in the form of small particles. The dispersed cubic particles are denoted as 'cubosomes', which can stably exist in equilibrium with aqueous solution with the internal bicontinuous structure unchanged [15,16].

Based on X-ray crystallographic studies, three distinct reversed bicontinuous cubic phases can be identified: the double-diamond lattice (Pn3m, Q_{224}), the body-centered cubic phase (Im3m, Q_{229}) and the gyroid lattice (Ia3d, Q_{230}) [6,17].

Structure of hexagonal mesophase

Hexagonal mesophases are closed and extended micellar columnar structures [18], and the long-range order is two-dimensional. It has been reported that there is no direct contact between water inside and outside the hexagonal phases [19]. Likewise, the dispersed reversed hexagonal particles denoted as 'hexosomes' can also be obtained by dispersing the hexagonal gel into aqueous solution [15,16]. To date, the hexagonal mesophases composed of glycerate-based surfactants such as oleyl glycerate (OG) and phytanyl glycerate (PG) have shown great potential in drug delivery [20,21]. As can be seen in Fig. 1b, hydrophilic drugs will be entrapped in the internal water domain, whereas lipophilic drugs will be located within the lipid domain and amphiphilic drugs in the interface.

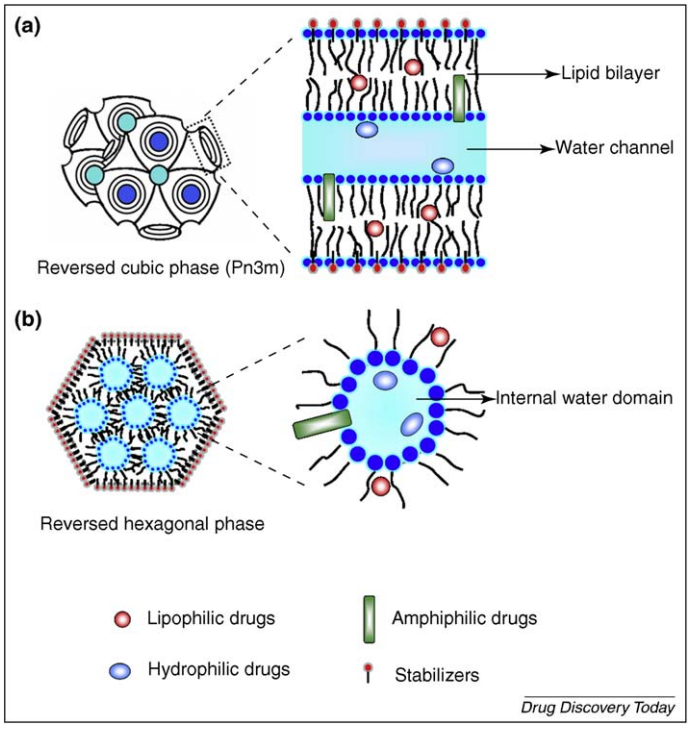

FIGURE 1

Structures of **(a)** reversed bicontinuous cubic and **(b)** hexagonal mesophases, inspired by Sagalowicz *et al.* [12]. Possible localizations of drugs in the mesophases are also pointed out. Note that for simplicity, only partial lattice is represented.

Preparation methods for reversed cubic and hexagonal mesophases

As a rule, cubic and hexagonal gels can be prepared more easily than their dispersions. For example, liquid crystal gels could be prepared by simply blending aqueous phase with lipid phase using vortex or ultrasonication [21]. The manufacture of cubosomes or hexosomes is more complicated, however; therefore, we mainly concentrate on the preparation methods of LLC nanoparticles. The schematic diagrams are represented in Fig. 2.

Top-down approach

This approach was primarily reported by Ljusberg-Wahren in 1996 [22]. The extreme viscous bulk phase is prepared by mixing structure-forming lipids with stabilizers, then the resultant is dispersed into aqueous solution through the input of high energy (such as high-pressure homogenization [HPH], sonication or shearing) to form LLC nanoparticles. At present, HPH is the most extensively used technique in the preparation of LLC nanoparticles [23].

Wörle *et al.* [24] investigated the parameters influencing the properties of glyceryl monooleate (GMO)-based cubosomes. Based on the results observed, the concentration of F127 and temperature during HPH were regarded as crucially important parameters. Recently, a novel approach of shearing was proposed to fabricate LLC nanoparticles using a laboratory-built shearing apparatus [25]. Compared with the well-established ultrasonication approach, the shearing treatment could effectively prepare more stable and homogeneous cubosomes or hexosomes with high content of the hydrophobic phase (oil + lipophilic additives) within a short time (less than one minute). It seems that the preparation procedure is simple enough to be realized conveniently. In fact, the operation units in this procedure require several cycles to achieve the desired nanoparticles with appropriate characteristics, and the high-energy input is also regarded as a barrier to the temperature-sensitive ingredients [23]. In addition, the cubosomes prepared through top-down approach are always observed to coexist with vesicles (dispersed nanoparticles of lamellar liquid crystalline phase) or vesicle-like structures, which will hamper the investigations on plain cubic mesophases.

Bottom-up approach

The key factor in the bottom-up approach is hydrotrope, which can dissolve water-insoluble lipids to create liquid precursors and prevent the formation of liquid crystals at high concentration [26]. Compared with the top-down approach, this dilution-based approach can produce cubosomes without laborious fragmentation. In other words, it needs less energy input. Moreover, this approach is far more efficient at generating small particles. The

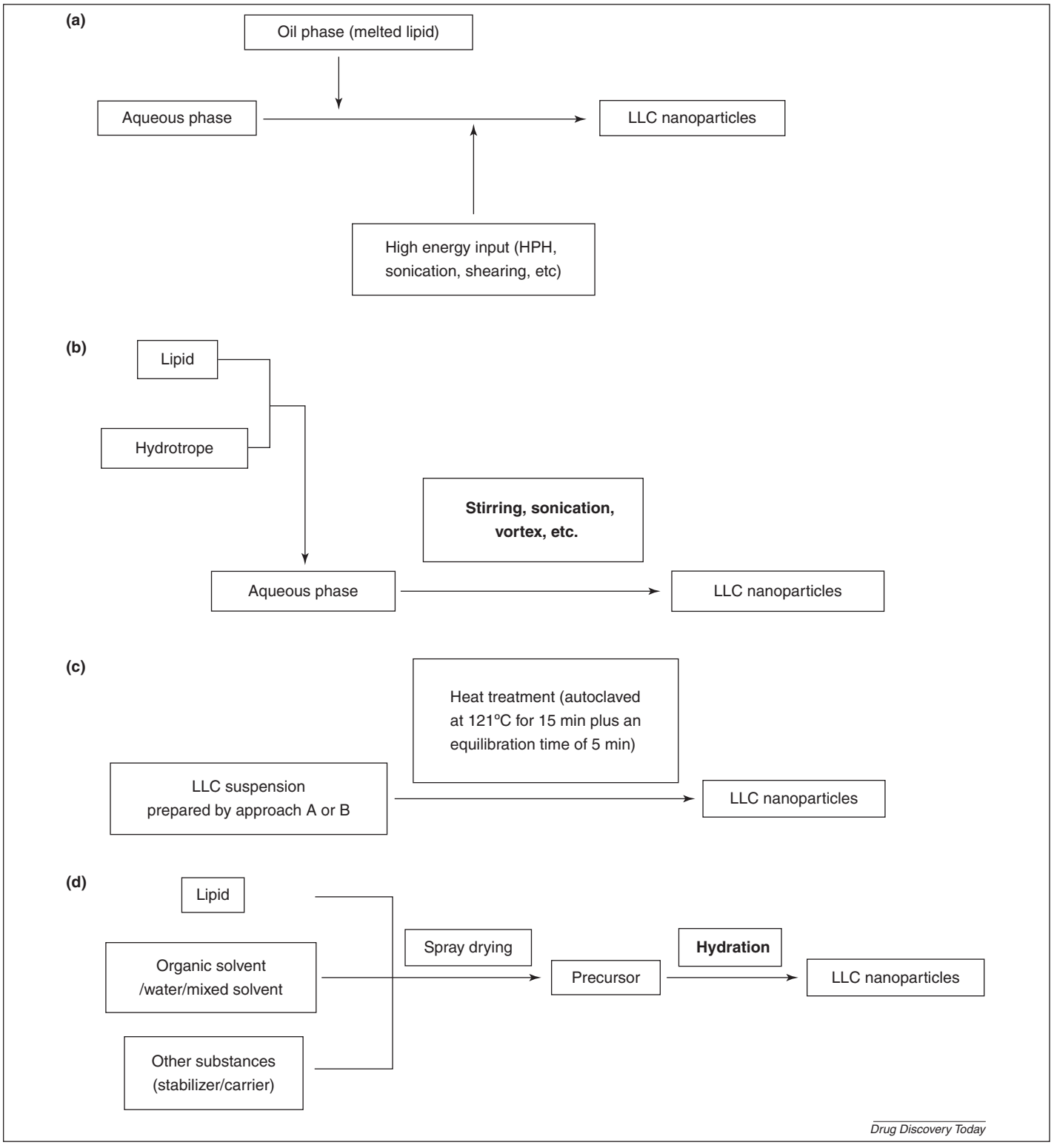

FIGURE 2

Schematic diagrams of preparation methods for cubosomes or hexosomes according to the literature [13,24,27–31]. **(a)** Top-down approach. **(b)** Bottom-up approach. **(c)** Heat treatment. **(d)** Spray drying.

reason for this might relate to the forming mechanism of cubosomes. The dilution-based approach can be regarded as a process of small particles forming big particles through aggregation, which is analogous to the use of precipitation processes to produce nanoparticles, whereas the top-down approach is more analogous to the attrition of big particles. In addition, cubosomes prepared through dilution show long-term stability, which might be attributed to the homodisperse stabilizers onto the surface of cubosomes [23].

Indeed, the use of hydrotrope can simplify the preparation process and produce cubosomes possessing similar or even better properties than those fabricated by the top-down approach. It should be noted, however, that this process via dilution is a

pathway by charting trajectories on the ternary phase diagram (lipid–water–hydrotrope), which requires knowledge of the full phase behavior; hence, the extent of dilution is difficult to control precisely. Owing to the addition of hydrotrope, many issues arise, such as the effects exerted by varying concentrations of hydrotrope on the physicochemical properties of LLC nanoparticles and the possible occurrence of irritation and allergic response when the mesophase formulations are administered. Finally, this bottom-up approach cannot effectively avoid forming vesicles. Through cryo-TEM, many vesicles and vesicle-like structures were also observed to coexist with cubosomes [14].

Heat treatment

The coexistence of cubosomes with vesicles is speculated to provide multiphasic manipulation of the sustained release of drugs [1]; hence, to better investigate the release behavior of plain mesophases, vesicles should be eliminated as much as possible. In this case, heat treatment can be regarded as a good approach. Note that in the strictest sense, heat treatment is not an integrated process for the manufacture of cubosomes because it only promotes the transformation from non-cubic vesicles to well-ordered cubic particles. The dispersed particles, therefore, can be produced by a simple processing scheme comprising a homogenization and heat-treatment step. From the reported studies, heat treatment could cause a decrease in the small particle size fraction that corresponded to vesicles and form more cubic phases with narrow particle distribution and good colloidal stability [27–29].

Taking the whole process of preparation into account, it is obvious that the transition takes place during the procedure of heat treatment. The reason for transition could be speculated as an elevated temperature giving rise to a reduction in solubility and stability. When the temperature was below cloud point, the surfactant had a high solubility and thus the particles could exist stably and the phenomenon of fusion was hardly observed. Once reaching cloud point, the solubility of surfactant decreased notably and a notable fast fusion among vesicles would occur [27]. This hypothesis was also verified by Wörle *et al.* [28]. Although masses of vesicles can transform to cubic nanoparticles through heat treatment, it does not mean that all the LLC systems are suitable for this procedure – in particular, the systems loading drugs that cannot provide sufficient stability under the condition of high temperature (usually above 120°C), such as some proteins and temperature-sensitive drugs, are not suitable.

Spray drying

To widen the applications of cubosomes in pharmaceutical field, dry powder precursors can be fabricated by spray drying and used for the preparation of oral solid formulations and inhalants. This approach was originally proposed and investigated by Spicer *et al.* [30]. In his research, the powder precursor could be prepared through drying a pre-dispersed aqueous solution that consisted of GMO, hydrophobically modified starch and water or contained GMO, dextran, ethanol and water, and then the colloidally stable dispersions of nano-structured cubosomes could be created by hydration of the precursors. Afterward, Shah *et al.* [31] prepared GMO-based cubosome precursor containing diclofenac sodium through spray drying. The precursor was proven to have more effective and prolonged anti-inflammatory and analgesic activity than pure drug when administered perorally; it is noteworthy, however, that residual solvent content is still a problem that cannot be ignored.

Phase behaviors of reversed cubic and hexagonal mesophases

Generally, molecular geometry has an important role in determining mesophase behavior, thus crucial packing parameter (CPP) can be introduced to predict molecular geometry in a surfactant–water system. CPP = $v/a_0 l$, where v is the hydrophobic chain volume, a_0 is the cross-sectional area of the surfactant headgroup and l is the hydrophobic chain length [32]. Depending on CPP, different self-assembly structures can be formed (Fig. 3). When CPP = 1, lamellar liquid crystalline structure forms. When CPP is smaller than 1, oil in water self-assembly structures form, such as normal micelles (L_1) and normal hexagonal (H_1) phases. When CPP > 1, reversed self-assembly structures form, such as reversed cubic structure, reversed hexagonal structure and reversed micelles (L_2) [12].

Based on the published literature, many factors can influence the phase behaviors of cubic and hexagonal mesophases. Addition

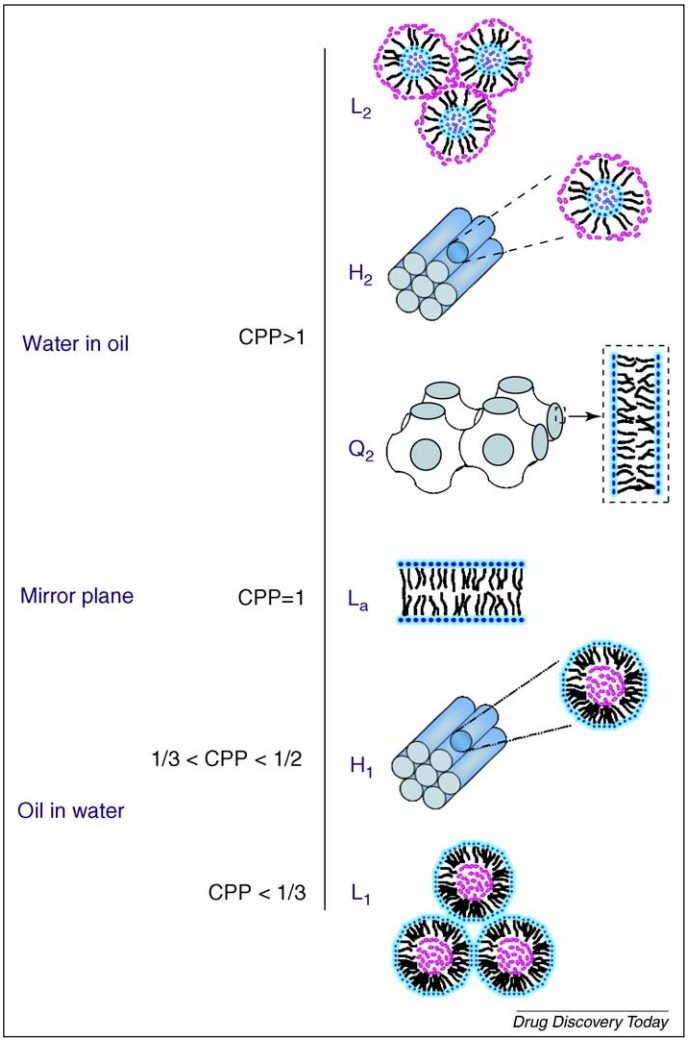

FIGURE 3

Schematic diagrams of different existing surfactant self-assembly structures and their corresponding CPP, inspired by Yaghmur and Glatter [8]. Going from the top down corresponds to a decrease in the CPP.

of a third substance – such as oleic acid, triolein, diglycerol monooleate, soybean phosphatidylcholine, retinyl palmitate, and tetradecane – could modulate the textures of mesophases and even result in phase transition [25,33–35]. It was reported that increase or decrease of temperature or pressure could also induce the phase transition of mesophases and, moreover, the pressure-dependent structural transition displayed opposite trends in lipid systems as compared to the influence of temperature [36,37]. In addition, salt concentration and pH value had been proven to have an effect on the phase behavior of mesophases to a certain extent [38,39]. An intimate knowledge of phase behavior will provide original ideas for using LLC systems for drug delivery.

In vitro release behavior of drugs from reversed cubic and hexagonal mesophases

Bulk cubic and hexagonal mesophases have been investigated as sustained drug delivery systems for more than 18 years [40]. It is widely accepted that release of drugs from these mesophases in most cases has been shown to follow Higuchi diffusion-controlled kinetics [41], where the cumulative amount of drug diffusion through matrix presents a linear dependence with the square root of time. The release behavior is related to many aspects, such as the properties of drugs, initial water content, type of LLC phases, swelling capacity, drug loading, electrostatic interaction between drugs, lipid bilayers and so on [2,42–48]. For dispersed mesophases, in-depth investigations on drug release were conducted by Boyd [49]. Lipophilic compounds containing diazepam, griseofulvi, propofol and rifampicin were employed as model drugs, and the pressure ultrafiltration method was used to determine the release behavior of these drugs. The results showed that cubosomes should be classified as a burst release delivery system, in which drug was released by diffusion from the cubic phase matrix. In the subsequent study, the release of irinotecan from hexosomes was also measured using ultrafiltration and an analogous phenomenon of burst release was again found [20]. The reason can be elucidated as follows: because of dividing the bulk mesophases into lots of small particles, the surface area greatly increases, and thus drugs can be transported into aqueous phase much faster from cubosomes or hexosomes.

Applications of reversed cubic and hexagonal mesophases as drug delivery carriers

Cubic and hexagonal mesophases as injectable vehicles

Reversed bicontinuous cubic and hexagonal phases are highly viscous, and this mechanical stiffness makes them clumsy to handle and difficult to inject [1,6,50]. To overcome this defect, some corresponding approaches have been proposed, such as application of flowable precursor forms [21,51] and use of LLC nanoparticles [20,52,53].

According to the phase diagram of structure-forming lipid, the transition from lamellar phases to cubic phases can be completed upon heating from room temperature to body temperature or swelling with water. Therefore, lamellar phases with inherently fluid properties can act as precursors of viscous cubic phases. Once injected into the body via subcutaneous or muscular approach, flowable lamellar phases will gradually absorb water from body fluid or surrounding tissues and, subsequently, convert to cubic phases, which can form the sustained release depot *in situ* [6]. Hexagonal phases also cannot be directly injected because of the limitation of high viscosity. One way to circumvent this problem is using L_2 phase with low viscosity as precursors. For example, OG and PG phases underwent a transition of L_2 to H_2 at 37°C with increasing water at approximately $7 \pm 1\%$, and it had been demonstrated that a drug-containing L_2 phase precursor prepared by OG or PG could transform to reverse hexagonal phase, which showed sustained release behavior when injected into excess aqueous solutions [21]. It should be noted, however, that losing a considerable volume of water from the topical environment can cause irritation to the body; this issue should be considered carefully when lamellar and L_2 precursor systems are administered *in vivo*. Another limitation is that the precursor systems containing lamellar and L_2 phases all show relatively rapid release [21,54], which is not conducive to the sustained release of drugs from cubic and hexagonal mesophases. Therefore, how to reduce the amount of drugs released from precursor systems and shorten the duration of transformation should be deliberated.

Recently, Fong *et al.* [51] designed a delicate protocol to prepare an 'on demand' drug release delivery system, which could vary the release rate of model drugs (glucose) through the transition of Q_2 to H_2 induced by tuning temperature. They employed the PT-based mesophases loading 3% of vitamin E, which was proved to have a transition temperature of Q_2 to H_2 at approximately 37°C. In a drug release experiment under dynamic temperature (30°C to 40°C to 30°C), when the temperature was switched to 40°C, the release rate was suppressed for the transition from Q_2 to H_2. Once the temperature was back to 30°C, the release rate was immediately returned to close to the original release rate because of the structure reverting to cubic mesophase. The result obtained from a similar release study under dynamic temperature (40°C to 30°C to 40°C) also verified that the switching of temperature stimulated the variation of release rate. In *in vivo* absorption studies, when injected subcutaneously at 40°C, the drug released from hexagonal mesophase slowly. After switching the temperature to 30°C, a phenomenon of statistically significant increase in plasma concentration was observed; furthermore, this system displayed a more sustained manner than other control formulations.

Because of their small particle size, low viscosity, biocompatibility and thermodynamic stability in excess water, cubosomes and hexosomes are particularly suitable for intravenous injection. Leesajakul *et al.* [52] investigated the interplay between GMO-based cubosomes and plasma *in vitro* and *in vivo*. *In vitro* study revealed that GMO would be adsorbed out of the particles by albumin that had binding sites for GMO. From *in vivo* study, when injected intravenously, cubosomes were disintegrated in a short period of time. However, Chol-py (a fluorescence probe) incorporated still showed the property of long-term circulation, which might be attributed to the sustained behavior of cubosome remnant particles. This study shows probable ways in which cubosomes are degraded in blood circulation, but whether the texture of the remnant particles changed and which specific forms the drugs solubilize in after disruption of cubosomes are still not clear.

Oral administration of drug-loaded cubic and hexagonal mesophases

Lipid formulations such as lipid suspensions, solutions, emulsions and self-emulsifying lipid-based formulations can all increase the oral bioavailability of poorly water-soluble drugs [55,56]. To realize

the expected aim that the formulations can exhibit sustained release of oral drugs *in vivo*, there are still three principal factors that need to be noticed. First, the formulations must possess the inherent property of sustained release, which is a major precondition. Second, they should stably exist in the gastrointestinal fluids to provide a persistent matrix from which drugs can be slowly released. Note that this requires the formulations to resist the digestive process to a certain extent. Third, the property of bioadhesive can extend the formulations' retention time in the gastrointestinal tract, providing more time for drug absorption [57].

According to current literature, although GMO-based mesophase formulation has been shown previously to enhance the bioavailability of co-administered poorly water-soluble drugs [58,59] and exhibits the first and third features described above, it cannot provide sustained release owing to its sensitivity to the digestive process [48]. With in-depth investigations of some novel materials (including PT and OG) that can resist the effect of digestive enzymes, some progressions have been made, especially in the aspect of bioavailability enhancement and sustained release of oral drugs, showing promising perspectives of applications.

Boyd *et al.* [57] investigated the oral bioavailability of a poorly water-soluble drug, cinnarizine, incorporated in different types of LLC phases. Through animal experiments, the OG-based hexagonal formulation showed a considerably higher relative bioavailability that was almost 3.5 times greater than that of the control suspension of cinnarizine and 3 times greater than the GMO-based cubic formulation. Furthermore, it was intriguing that the OG matrix provided extended absorption of drug for over 120 h, which was several times longer than the other two formulations, indicating long residence of the formulation in the gastrointestinal tract and a poor sink condition *in vivo* inhibiting drug release. It also should be noted that OG was more resistant to the digestive process than GMO, which was proven in *in vitro* digestion studies, and this might also be responsible for sustained drug absorption. Recently, Lee *et al.* [48] employed glucose-loaded $Q_{2\ GMO}$, $Q_{2\ PT}$ and $H_{2\ PT+Vit\ EA}$ phases as researching objects to investigate *in vivo-in vitro* correlation and realized the control over absorption of hydrophilic drug *in vivo* through manipulation of matrix nanostructure for the first time.

The oral administration of drugs incorporated into LLC nanoparticles has also been reported [60–62]. Chung *et al.* [60] prepared GMO-based cubosomes containing insulin and investigated the hypoglycemic effect generated by oral administration of this formulation. The blood glucose concentration–time profile showed that the insulin formulation could provide a hypoglycemic effect comparable to intravenous administration of insulin over six hours. Simvastatin incorporated in GMO-based cubosomes was administered orally and the relative bioavailability to the control drug crystal powder was 241%. Moreover, the cubosomes showed sustained release of simvastatin over 12 h in beagle dogs. The author presumed that the mechanism of enhancing bioavailability might be related to the hydrophilic surface of cubosomes, which stimulated the permeation through the stagnant aqueous layer of the intestinal mucosa [62].

Topical application of cubic and hexagonal mesophase formulations

Topical drug delivery is an attractive alternative to oral administration. Its main drawback is the limited absorption of drugs through the skin barrier, and investigations on topical drug uptake are necessary to facilitate the design of efficient topical drug delivery systems. At present, stratum corneum (SC) is considered to be the rate-limiting barrier in transdermal drug delivery [63]. Many studies have shown that cubic and hexagonal mesophase formulations are capable of penetrating through SC and becoming candidates for topical drug delivery systems [64–72].

At present, GMO-based and PT-based mesophases are the most widely investigated LLC systems for topical drug delivery. It has been proven that these mesophases can statistically significant enhance permeation of drugs such as acyclovir [64], δ-aminolevulinic acid [65], indomethacin [66], cyclosporine (Cys A) [67–69], vitamin K [70] and diclofenac salts [71,72]. There are several natural characteristics that the reversed cubic and hexagonal phases present to make them suitable for topical drug delivery: (i) sustained release of drugs incorporated, (ii) bioadhesive properties, (iii) solubilization of hydrophilic and lipophilic drugs and protecting them from physical and enzymatic degradation, and (iv) the nontoxic permeation enhancers GMO and PT as structure-forming materials [10,65,68].

Bender *et al.* [73] used two-photon microscopy to elucidate the penetration pathway of sulphorhodamine B, a fluorescent hydrophilic model drug that was incorporated in GMO-based and PT-based cubic mesophases and then applied in human skin *in vitro*. The results revealed different penetration approaches for the control formulations and cubic mesophase formulations: the intercellular pathway seems to be predominant when using the water solution and the ointment, whereas the intercluster pathway seems to dominate the skin absorption for the cubic mesophases. In addition, drugs could penetrate into the deeper layer of skin by using cubic mesophases and, moreover, GMO-based cubic mesophase – compared with PT-based cubic mesophase – seemed to permeate the lipid matrix more readily.

Topical applications of δ-aminolevulinic acid and its methyl ester that were incorporated into GMO–water and GMO/PT–propylene glycol–water systems, respectively, showed fast penetration in comparison to the standard ointment during the studies of one hour short-term and 24 h continuous applications. The difference between GMO and PT in terms of enhancing drug permeation mainly relied on the discrimination of the swelling extent and the rheological property [65]. Lopes *et al.* [68] reported that Cys A incorporated in GMO-based cubic and hexagonal phases could statistically significant elevate the penetration of Cys A. Moreover, the cubic phase formulation favored retention of Cys A in the skin, whereas the hexagonal one favored its penetration into deeper skin layers and its transdermal delivery.

Cubosomes and hexosomes have also been used for topical drug delivery. Compared with the cubic and hexagonal gels, the dispersions show some unique advantages. First, the good fluidity and large surface area of the dispersions provide tighter contact with the skin. Second, the dispersions can be embedded by the other formulations. Last, but not least, they does not cause skin irritation after topical application [10,66,70]. Topical applications of carbomer-indomethacin loaded cubosomes, carbomer-blank cubosomes and carbomer with an indomethacin water suspension had been reported to show different drug release behavior and effects on UVB-induced erythema through human test [66]. The first formulation statistically significant prolonged

anti-inflammatory activity when exposed under UVB irradiation for six hours after removing it, whereas the other two formulations exhibited decreased activities to a certain extent. Release studies also verified the persistently higher concentration of indomethacin in SC after application of the first formulation. Cys A incorporated in hexosomes comprising GMO, oleic acid and water was reported to be capable of enhancing drug permeation when applied topically [69]. In an *in vitro* permeation study using the drug-loaded hexosomes, the concentration of Cys A in epidermis and dermis ([E + D]) was two times higher than that after application of the control formulation (olive oil solution of Cys A). Similarly, statistically significant enhancement of drug concentration in [E + D] (2.8 times) was derived from *in vivo* study. Moreover, a skin irritation test demonstrated that the daily application of this formulation did not cause skin irritation. In addition, Lopes *et al.* [67] found that a high concentration of GMO (20–70%) could suppress the transdermal delivery of Cys A. Similar results were also obtained that the increase of GMO concentration might prevent the penetration of Vit K into the deep layer [70]. These phenomena might be caused by an intense interaction between GMO and lipophilic drugs.

Mucosal drug delivery using cubic and hexagonal mesophases

The structure-forming materials (such as GMO, PT, OG and PG) all possess not less than two hydroxyl groups, which make them available for hydrogen bonding to mucus membranes, and therefore the cubic and hexagonal mesophases are good candidates for mucosal drug delivery [74–77].

To facilitate operation, the flowable precursor systems are employed, which can form the viscous cubic or hexagonal gels by absorption of body fluid *in vivo*. It was reported that GMO-based gel was used for vaginal delivery of propantheline bromide and oxybutynin hydrochloride, and sustained release behaviors of both drugs were observed for over a period of 18 h *in vitro* [74]. However, no data involving *in vivo* experiment were exhibited. Lee *et al.* [75] reported that GMO in the cubic and lamellar mesophases could be eroded without the action of an enzyme and then penetrate across excised porcine buccal mucosa. Moreover, the flux of a [D-Ala^2, D-Leu^5] enkephalin incorporated from the cubic and lamellar mesophases was enhanced statistically significant compared with PBS solution during the initial three hours. Likewise, there were no *in vivo* results presented.

Not only the bulk mesophases but also their dispersions could be utilized for mucosal drug delivery. Swarnakar *et al.* [77] reported that after application of progesterone loaded hexosomes on the albino rabbit mucosa for 12 h, an obviously enhanced transmucosal flux was observed and that it was fivefold higher than that of progesterone loaded gel and nearly fourfold higher than plain progesterone suspension. In addition, lipid extraction phenomena and evident pores were exhibited in the epithelium of mucosa through FT-IR and confocal laser scanning microscopy, indicating a probable intercellular 'virtual channel' for hexosomes permeating.

Concluding remarks and further perspectives

This review mainly discusses the current applications of reversed cubic and hexagonal mesophases as drug vehicles, with a major focus on their applications *in vivo*. Table 1 presents some major investigations with respect to the cubic and hexagonal mesophases as drug vehicles in recent years (2004–2009). Note that animal experiments were also conducted in these studies to validate these mesophase formulations. Based on the current literature, cubic and hexagonal mesophase formulations maintain a good momentum of growth and show broad prospects for development.

Although the cubic and hexagonal mesophases possess advantageous characteristics, there is still a long way to go before their

TABLE 1

Cubic and hexagonal mesophases as drug vehicles reported in recent years[a]

Type of LLC phases	Lipid system	Bioactive molecule	Administration route	Refs
Cubic bulk phase	PT/water; PT/VitEA/water	Glucose	Subcutaneous injection	[51]
Cubosomes	GMO/F127/water	Chol-py	Intravenous injection	[52]
Lipid-based liquid crystalline nanoparticle	Phosphatidylcholine/glycerol dioleate/Tween 80/water	Somatostatin	Intravenous injection	[53]
Cubic bulk phase	GMO/water; OG/water	Cinnarizine	Oral administration	[57]
Cubic bulk phaseHexagonal bulk phase	GMO/water; PT/water; PT/VitEA/water	GlucoseAllura RedFITC-dextran	Oral administration	[48]
Hexagonal bulk phase	OG/water	Sodium pamidronate	Oral administration	[78]
Cubosomes	GMO/water	Omapatrilat	Oral administration	[61]
Cubosomes	GMO/F127/water	Simvastatin	Oral administration	[62]
Cubic bulk phase	GMO/water; PT/water; GMO/propylene glycol/water; PT/propylene glycol/water	δ-Aminolevulinic acid	Topical application	[65]
Cubosomes	GMO/F127/water	Indomethacin	Topical application	[66]
Cubic bulk phaseHexagonal bulk phase	GMO/water; GMO/oleic acid/water	Cys A	Topical application	[68]
Hexosomes	GMO/oleic acid/F127/water	Cys A	Topical application	[69]
Hexagonal bulk phaseHexosomes	GMO/water; GMO/F127/water	Vit K	Topical application	[70]
Hexosomes	GMO/oleic acid/F68/water	Progesterone	Mucosal application	[77]

[a] *In vivo* experiments were conducted in these investigations.

clinical application. For injectable cubosomes and hexosomes, more approaches should be exploited to increase the effective drug loading and control sustained release actions – for instance, multi-component cubic and hexagonal mesophases are good candidates that might meet these requirements. For oral formulations, the current investigations are mainly concentrated on the cubic and hexagonal gels loading lipophilic drugs, whereas the investigations involving the transport of hydrophilic drugs and the use of LLC nanoparticles are still very limited. In addition, some new structure-forming materials such as PT, OG and PG have exhibited superior properties to conventional materials. At present, however, understanding of them is still insufficient, especially in safety, biological stability and appearance *in vivo*. For topical applications, especially for mucosal drug delivery, there are still few studies with respect to *in vivo* experiments; thus, more work should be performed to validate the mesophase formulations *in vivo*. In the future, all these aspects should be brought to the forefront and investigated further.

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