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Bicontinuous cubic liquid crystals as sustained delivery systems for peptides and proteins

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Importance of the field: Self-assembling lipid-based liquid crystalline systems are a broad and active area of research. Of these mesophases, the cubic phase with its highly twisted bilayer and two non-intersecting water channels has been investigated extensively for drug delivery. The cubic phase has been shown to accommodate and control the release of drugs with varying physicochemical properties. Also, the lipids used to prepare these delivery systems are generally cheap, safe and biodegradable, making these systems highly attractive. Early research investigating the potential of cubic phases as delivery systems showed that several peptides or proteins entrapped within these gel-based systems showed retarded release. Furthermore, entrapment within the cubic phase protected the selected peptide or protein from chemical and physical degradation with its native confirmation and bioactivity retained.
Areas covered in this review: In this review, the literature pertaining to the delivery of various bioactives from cubic liquid crystalline phases is examined, with a particular focus on peptides and proteins. The scope and limitations of the cubic phases in this respect and the future of cubic liquid crystalline systems as sustained delivery systems are highlighted.
What the reader will gain: The reader will be able to gain an understanding of the properties of the bicontinuous cubic phase and how its structural attributes make these systems desirable for sustained delivery of bioactives, in particular peptides and proteins, but also how these same structural properties have hindered progress towards clinical applications. Current strategies to overcome these issues will also be discussed.
Take home message: The bicontinuous cubic phase offers great potential in the field of peptide and protein delivery, but limited research in this area precludes definite conclusions to its future in this respect.

Keywords: cubic phase, cubosomes, glycerylmonooleate, liquid crystals, peptides, phytantriol, proteins, sustained delivery

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1. Introduction

Advances in biotechnology over the last few decades have led to a large number of candidate protein- and peptide-based pharmaceuticals. With continued growth in the field, especially in subspecialties such as genomics and proteomics, it is to be expected that more macromolecules with therapeutic potential will emerge [1].

Notwithstanding the advances in technology, the challenges associated with formulating and administering peptide/protein-based pharmaceuticals remain significant. These obstacles include, but are not limited to, poor bioavailability owing to their inherently poor absorption properties, susceptibility to chemical and physiological degradation, and short *in vivo* half-lifes, thus limiting their delivery primarily to parenteral routes [2,3]. Various formulation strategies to overcome these issues

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Article highlights.

- Progress in biotechnology has led to an increased repertoire of lead peptide and protein pharmaceuticals, which represents a significant challenge for formulation science.
- Self-assembled liquid crystalline systems, in particular the bicontinuous cubic phase, show great potential as delivery systems for peptides and proteins.
- The bicontinuous cubic phase consists of a highly twisted bilayer with two non-congruent water channels that can accommodate a wide range of actives with varying physicochemical properties.
- A variety of lipids have been investigated as potential cubic phase-forming lipids, however a significant proportion of available literature has focused on the unsaturated fatty acid, glyceryl monooleate (GMO).
- GMO is commonly used in foods and therefore is generally regarded as safe. However, it is vulnerable to hydrolytic degradation owing to the presence of an ester functionality. The potential of phytantriol as an alternative liquid crystal-forming lipid to GMO is discussed.
- Several peptides and proteins have been entrapped within the cubic phase and have shown retarded release. Entrapment within the cubic phase protected the selected peptide or protein from chemical and physical degradation as the native confirmation and bioactivity was retained.
- Phytantriol matrices were able to entrap and release the model protein ovalbumin (Ova) in a sustained manner with only 30% of the protein released over a period of 2 weeks. Furthermore, matrices were able to retain the cubic microstructure on addition of Ova. By contrast, owing to hydrolysis, similar GMO-based matrices had converted from the cubic to the hexagonal phase.
- Although the future of liquid crystalline systems is regarded with enthusiasm, progress towards commercialization as sustained delivery systems for peptides and proteins is slow. Significant development, especially application of these systems in *in vivo* animal models to assess their suitability for human application, needs to occur.

This box summarizes key points contained in the article.

have been proposed and are now under investigation. These include the use of polymeric micro- and nanoparticulate systems, lipid micro- and nanoparticulate systems, *in situ* depot-forming systems, implantable systems and chemical conjugation [2-5]. Lipid-based lyotropic liquid crystalline mesophases have been shown to entrap and sustain the release of a range of bioactives, including peptides and proteins [6-11].

Liquid crystals can be broadly divided into two groups: thermotropic and lyotropic liquid crystals [12]. The structures shown by thermotropic liquid crystals are governed principally by changes in temperature. Lyotropic structures are more complex and are influenced by changes in various factors, such as the molecular shape and concentration of the amphiphile, in addition to environmental conditions such as temperature, pressure and pH [12,13]. This discussion is restricted to lyotropic liquid crystalline systems.

Liquid crystal-forming lipids are water-insoluble but swellable polar lipids with an amphiphilic nature containing a polar head group and a nonpolar hydrophobic tail. When exposed to a polar environment, depending on the temperature and solvent concentration, these molecules are capable of self-assembling into various well-defined structures, with long-range order in one, two or three dimensions (3D). These structures are collectively referred to as liquid crystals or mesophases [12,14,15]. Contact with the polar environment causes the molecules to position themselves in such a way as to minimize the free energy of the system, where the polar solvent penetrates between the amphiphilic molecules, exposing the hydrophilic regions to the aqueous environment and causing the hydrophobic regions to be sequestered [13]. This phenomenon is commonly referred to as the hydrophobic effect [16,17]. In addition to optimization of the hydrophobic effect, some packing constraints also play a critical role in the resulting structures. These are determined by the geometric properties of the amphiphilic molecule, specifically packing and curvature [18].

The rich array of structures displayed on swelling of the lipids range from simple micellar and planar bilayer structures to more intricate and complex 3D bilayer structures such as the inverted hexagonal phase and the inverted or bicontinuous cubic phase. This diversity in lipid polymorphism has been implicated in several biological processes, with membrane lipids forming a wide variety of liquid crystalline phases, and is discussed extensively in several reviews [19,20].

At low lipid concentration, there is molecular disorder within the system and the molecules are present as monomers. Above the critical micellar concentration (CMC), the amphiphilic molecules spontaneously arrange into molecular aggregates of various shapes, such as spheres, rods and disks, and are termed micelles [15].

The lamellar phase (L_α) is probably the best understood and most investigated of all lyotropic mesophases. It is a one-dimensional stacking of amphiphilic bilayers where the amphiphiles arrange themselves in such a manner that the head groups are in contact with water and the tail groups are orientated away from water, forming double layers. The double layers pack parallel to one another, separating planar sheets of water [21]. Hexagonal phases are the most common non-lamellar mesophases with curved interfaces [21]. The hexagonal phase occurs as normal (H_1), or reverse/inverse (H_2) structures, based on the dense packing of cylindrical micelles arranged in a two-dimensional hexagonal lattice [13]. The cubic phase is usually observed between the L_α and H_2 phase in a given temperature versus water phase diagram. There are two types of cubic phase, the discontinuous cubic phase, which is comprised of discrete normal or inverse micelles distributed in an ordered cubic arrangement, and the inverse bicontinuous cubic phase, Q [13,21,22]. This review focuses on the bicontinuous cubic

liquid crystals. The structure, the swelling and release of bioactives, particularly of peptides and proteins, and the potential application of the bicontinuous cubic phase as a sustained delivery system are addressed.

2. Bicontinuous cubic liquid crystals

2.1 Structure of the cubic phase

The bicontinuous cubic phase, referred to hereafter simply as the cubic phase, consists of a single continuous lipid bilayer that is twisted so that it separates space into two non-intersecting water channels. Luzzati and co-workers [23,24] were the first to identify the existence of cubic phases in lipid–water systems in the early 1960s. Independently, their work was supported by Fontell *et al.*'s [25] work on ternary systems of amphiphiles, oils and water, and by Lutton [26], who examined the aqueous phase behavior of monoglycerides. A decade later, Larsson and colleagues [27] systematically elucidated the structure of the cubic phases in monoglyceride–water systems using NMR and X-ray scattering techniques to show that the cubic phases contained continuous hydrophilic and hydrophobic regions. The pioneering work of these early researchers also led to the realization that cubic phase structures could be described using concepts of differential geometry and minimal surfaces.

A minimal surface is described as a surface where the two principal curvatures are equal but opposite in sign, resulting in a mean curvature of zero at all points, consequently leading to saddle-shaped or hyperbolic surfaces [28]. Bicontinuous cubic phases are generally based on three minimal surfaces, the P-surface (primitive), the D-surface (diamond) and the G-surface (gyroid), and are associated with the space groups Im3m, Pn3m and Ia3d, respectively [13]. Space groups are derived from all the possible symmetry operations for the system of interest. Minimal surfaces can be extended to fill space in 3D, forming infinite periodic minimal surfaces, dividing space into two congruent sub-volumes [18]. For cubic phases, this means they consist of two water channels in addition to a highly twisted bilayer.

The structure of the cubic phase has been characterized using various techniques. Amar-Yuli *et al.* [29] recently grouped these techniques into two categories, direct techniques and indirect techniques. Direct techniques such as small angle X-ray scattering (SAXS) and optical and electron microscopy are important in providing information about phase identification. Indirect techniques such as spectroscopy, including NMR, and rheology provide supplementary information.

Macroscopically, the cubic phase is an extremely viscous, almost solid-like, material with a large specific surface area (~ 400 m^2/g) [30]. Using cryogenic field emission electron microscopy (cryo-FESEM), the internal microstructure of the cubic phase can be viewed in 3D, with two distinct regions evident (**Figure 1**) [9,31]. The holes opening to the external surface are postulated to be the two water channels of the cubic phase, whereas the rough nodules are most likely to be the lipidic regions. The highly twisted nature of the cubic phase described using differential geometry is clearly evident in the micrographs, and it is this unique microstructure that has led to considerable attention being given to the cubic phase as a promising delivery system for a range of bioactives [11,32,33].

2.2 Bicontinuous cubic phase-forming lipids

The potential of various classes of polar lipids such as phospholipids [34-36], alkyl glycerates [8,37] and glycolipids [38] to form cubic phases has been reported in the literature. Nonetheless, a significant proportion of cubic phase research is centered around unsaturated mono- and diglycerides, in particular glyceryl monooleate (GMO) and mixtures of GMO with other lipids or structural derivatives based on GMO [7,33,39-41]. These lipids are commonly used as emulsifying agents and food additives and are generally regarded as safe and biocompatible [29,42,43].

GMO is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of monooleate. It can be produced in several ways, such as by direct esterification of fatty acids, mainly oleic acid and glycerol, or by transesterification of refined vegetable oils such as erucic canola or sunflower oil [43]. **Figure 2** shows the chemical structure of GMO, where it can be seen that the acyl chain (oleic acid) is attached to the glycerol backbone by an ester bond. The active hydroxyl groups of the glycerol impart the polar characteristics on the molecule, whereas the acyl chain contributes to the nonpolar portion.

Phytantriol, also shown in **Figure 2**, is another lipid that has gained considerable interest in the past decade as it also forms cubic liquid crystalline phases [44-46]. Structurally it is different from GMO; however, the phase behavior of phytantriol as a function of water concentration and temperature is strikingly similar to that of GMO, as shown in the phase diagrams in **Figure 2** [44,45]. Phytantriol is commonly used as a thickener in cosmetic products [46,47]. Fatty acid-based materials such as GMO are prone to hydrolysis, whereas phytantriol has been shown by SAXS to be structurally more stable in aqueous environments [9,48].

The various phases formed by GMO and phytantriol as a function of water are shown in **Figure 2** [49]. The rate of swelling and the resulting swollen mesophase have been shown to affect the release of some actives from GMO-based mesophases [10,41,50]. Rizwan *et al.* investigated the swelling behavior of phytantriol and GMO-based matrices (**Figure 3**) [9]. The uptake of water by either lipid was found to be rapid until they approached their respective equilibrium water content. For GMO matrices, the maximum swelling achieved within 24 h was 0.39 g/g. By contrast, phytantriol matrices took ~ 48 h before they were fully swollen and the maximum water uptake was 0.37 g/g. The rate of swelling of GMO matrices at 0.12 g/gh was significantly faster when compared with phytantriol matrices, where the rate of swelling was 0.07 g/gh. The authors

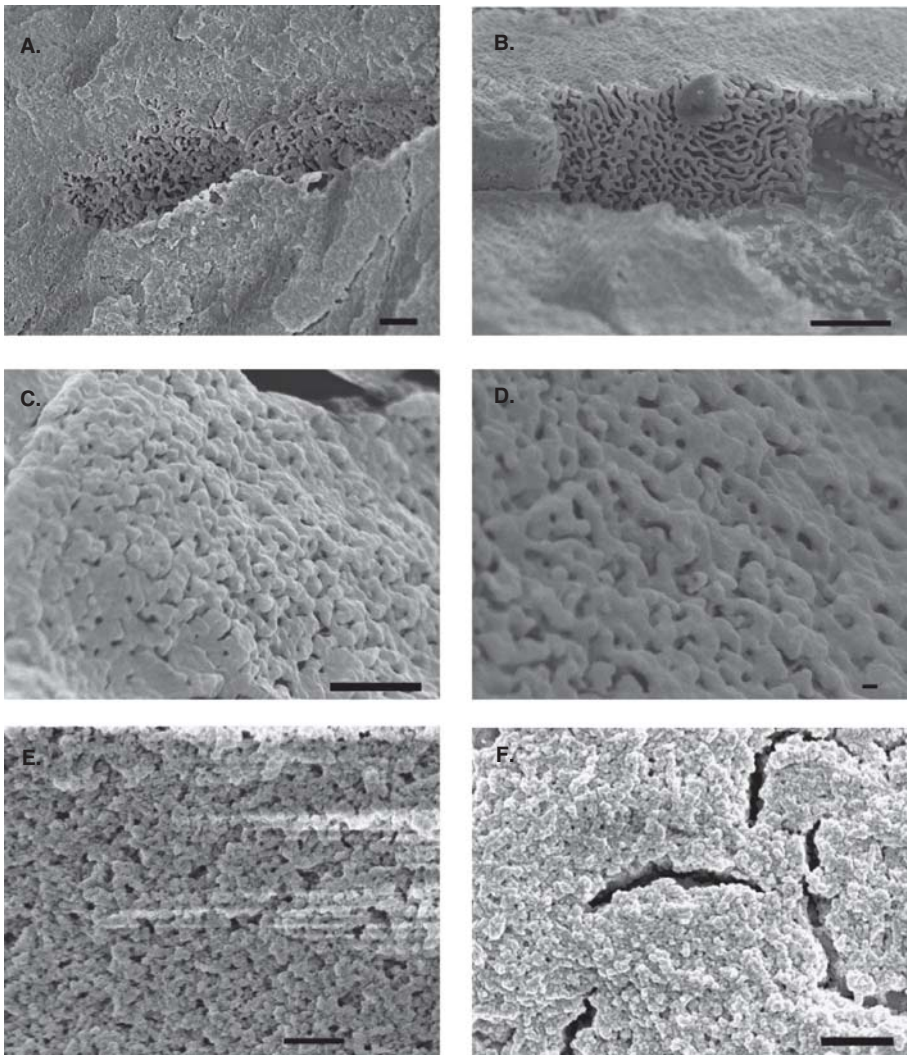

Figure 1. Cryogenic field emission electron microscopy micrographs of the (A–D) cubic liquid crystalline phase of phytantriol in excess water and (E, F) glyceryl monooleate cubic phase containing 30% w/w water. All samples were equilibrated for a minimum of 1 week before analysis. The bar represents 1 μm, except in panel **D,** where the bar = 100 nm.
Adapted with permission from [9].

hypothesized that the greater rate of absorption of water from the external environment by GMO as compared with phytantriol could potentially affect the rate of release of the active out of the cubic microstructure. The more swollen matrices would facilitate more rapid diffusion and faster release than less swollen matrices with reduced channel diameter.

2.3 Release of bioactives

The combination of hydrophilic and hydrophobic domains, as a result of the water channels and a highly twisted bilayer, means that the cubic mesophase is an optimal environment where drugs of various physicochemical properties can be trapped and their release retarded. Sustained release of various bioactives trapped within the cubic phase has been reported [7-10,33,50-53].

A large number of molecules have been incorporated within the cubic phase, ranging from low-molecular-mass drugs to macromolecules (see [11,32] for an extensive list). Incorporation of a hydrophilic active into the cubic phase is generally achieved by mixing an aqueous solution of the active with the cubic phase-forming lipid. The hydrophilic active will reside predominantly in the hydrophilic channels of the cubic phase. By contrast, lipophilic actives are generally solubilized in the lipid before formation of the cubic phase, where they will reside in the bilayer. Amphiphilic actives will tend to reside at the interface between the hydrophobic–hydrophilic domains [11].

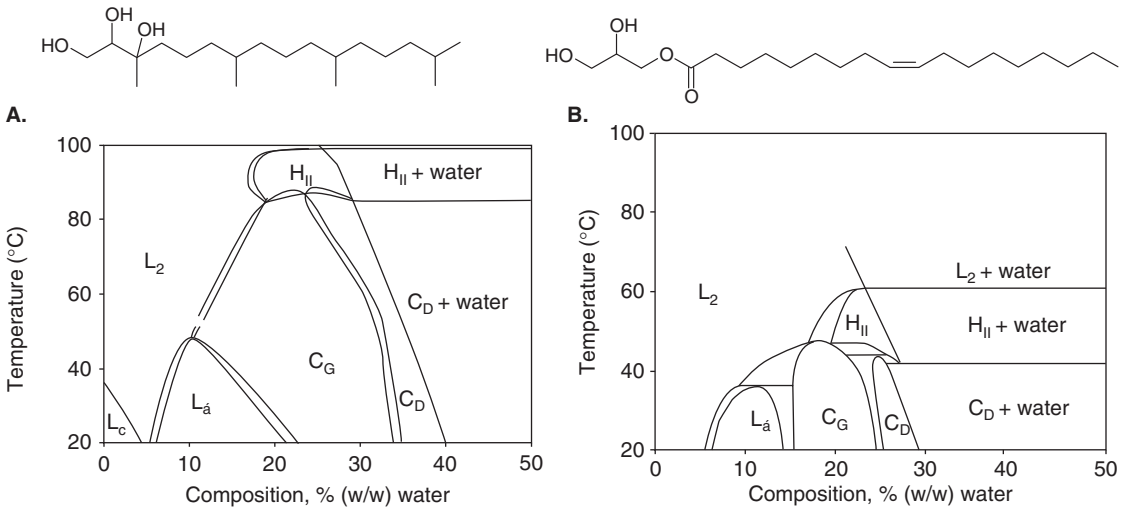

Figure 2. Phase behavior of (A) glyceryl monooleate–water and (B) phytantriol–water systems. Phase notations: L_C, liquid crystal; L_2, fluid isotropic solution; L_α, lamellar phase; H_{II}, reverse hexagonal phase; C_G, gyroid cubic phase (Ia3d); C_D, diamond cubic phase (Pn3m). The chemical structures of the two lipids are on the top of each respective phase diagrams.
Adapted with permission from [49].

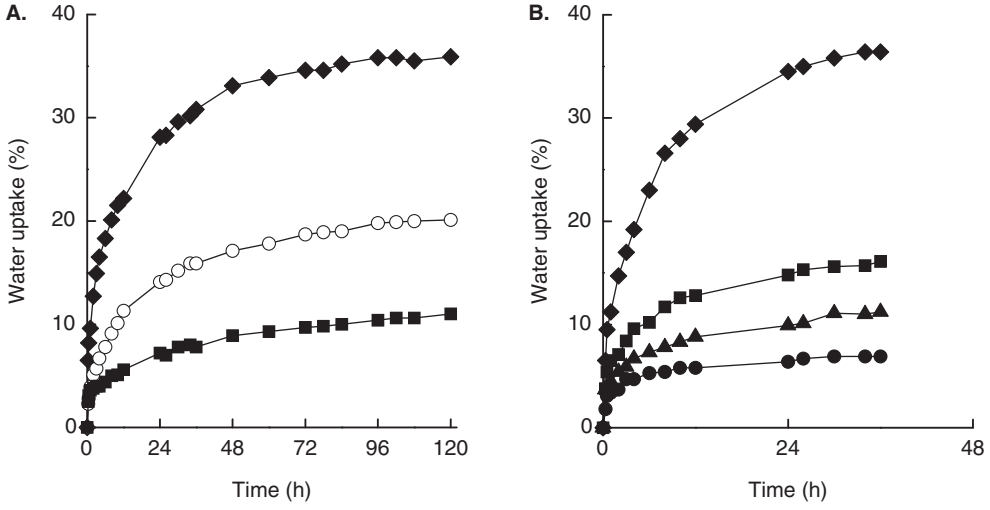

Figure 3. Water uptake as a function of time for selected binary samples of (A) phytantriol and (B) glyceryl monooleate with initial water loading of (◇) 0, (○) 15, (■) 20, (▲) 25 and (•) 30% w/w over the swelling study period. Data presented are the mean of three independent experiments ± s.d.
Adapted with permission from [9].

The mechanism of release of the entrapped active from the cubic phase typically obeys Higuchi's square root of time function [54], shown in the equation, and is thus thought to be under diffusion control.

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2}$$

where Q is the mass of drug released per unit area of matrix, C_o is the initial concentration of drug in the matrix, D is the diffusion coefficient of the drug in the matrix and t is time.

A plot of the mass of drug released as a function of the square root of time allows confirmation of the release kinetics of the incorporated drug from the matrix. A straight line resulting from such a plot is indicative of diffusion-controlled release, where the slope of the straight line is proportional to D, the diffusion coefficient of the drug through the matrix.

Release of the incorporated active is thought to be controlled at least partly by the unique microstructure of the cubic phase, where the active has to diffuse through the structure to access the external solution. The pore size and tortuosity of the water channels and the stiffness and high viscosity of the cubic phase contribute to the sustained release of the entrapped active. Furthermore, factors such as size and polarity of the bioactive, in addition to the features of the mesophase, also affect release kinetics. Size or molecular mass of the active influences the diffusion coefficient and will determine its overall rate of release [7]. The polarity of the active also has implications on the types of interaction (such as hydrogen bonding or electrostatic interactions) that could potentially occur between the active and the liquid crystalline system, and affects the mesophase type and release [7].

Modulating the pore size of the aqueous channels, thereby customizing the mesophase, would allow greater control of the release rate to achieve the desirable release kinetics *in vivo*. This can be achieved through selection of the liquid crystal-forming lipid and/or by adjusting external conditions such as temperature and lipid composition [7].

3. Bicontinuous cubic phase for delivery of peptides and proteins

The large interfacial area of the cubic phase, in theory, should be able to accommodate large macromolecules such as proteins without disrupting the cubic microstructure. There are extensive reports in the literature where the cubic phase, because of its resemblance to biological membranes, has been used successfully to crystallize various proteins for subsequent crystallographic analysis [55-57]. Despite these favorable attributes, there are relatively few studies that describe the entrapment and release of peptides or proteins from the cubic liquid crystalline phase, with most studies limited to GMO-based systems (**Table 1**).

To achieve controlled release, the incorporated active should not affect the cubic microstructure. Structural transformation in the presence of bioactives can affect the solubilization capacity and interfacial reactivity and alter the expected release profiles. There are reports in the literature that show addition of drugs or solvents to the GMO/water system have led to modifications of the liquid crystalline structure and affected the release kinetics of the entrapped drug [58-63].

Early work by Ericsson and co-workers suggested that the cubic phase was not only able to retard the release of peptides but also protected the peptides from degradation and facilitated retention of native conformation and biological activity [6,64]. The authors showed sustained release of the peptide hormone somatostatin for 6 h *in vivo* in rabbits following intramuscular and subcutaneous injections [6]. Furthermore, they went on to load selected oligopeptides (desmopressin, lysine vasopressin, renin inhibitor, H214/03) at high levels into GMO-based cubic gels and demonstrated protection of the peptides against enzymatic degradation *in vivo*.

Leslie *et al.* were able to encapsulate bovine hemoglobin (BHb) in GMO cubic phase with only 45% of the entrapped BHb released after 144 h under *in vitro* release conditions [65]. Sadhale and Shah reported that protection of insulin from agitation induced aggregation when entrapped within the GMO-based cubic phase with retention of biological activity [51,52].

Clogston and Caffery quantified the release characteristics of various amino acids, peptides and proteins varying in their size, shape and charge from the cubic phase [7]. Cubic phases with different sized aqueous pores were prepared using monoacylglycerols with different acyl chains. The authors showed that the rate of diffusion of a particular active was dependent on its molecular size and could be adjusted by manipulating the aqueous channel size of the host cubic phase. Furthermore, the authors were able to show that greater control of the release rate could be achieved by simply adjusting the nature and the degree of the interaction (e.g., electrostatic interactions) between the lipid bilayer of the hosting cubic phase and the aqueous additive.

These studies clearly demonstrate the advantages the cubic phase offer to encapsulated labile bioactives with respect to protection against chemical and physical degradation, presumably owing to the reduced activity of water within the cubic phase [11].

Recently, Lopez *et al.* [66] evaluated the antigenic effect of orally administered GMO-based cubic phase containing Apx toxins found in the bacterium *Actinobacillus pleuropneumoniae* and responsible for porcine pleuropneumonia on mice. Modest changes in T- and B-lymphocyte proliferation were observed with detection of antibodies against the various Apx toxins investigated. The presence of antigen-specific antibodies led the authors to conclude that the cubic phase was able to protect the antigen against degradation and allow generation of an immune response.

Rizwan *et al.* also explored the potential of the cubic phase for the delivery of vaccine antigens. Furthermore, the possibility of using alternative liquid crystal-forming lipids to GMO was examined. The model protein ovalbumin (Ova), a large soluble globular protein with a molecular mass of ~ 45 kDa, was used to demonstrate the potential of phytantriol and GMO cubic phase as sustained delivery systems. The effect of Ova (labeled with fluorescein isothiocyanate [FITC]) on the liquid crystalline behavior of phytantriol and GMO was investigated by polarizing light microscopy (PLM). It was found that, in general, after the addition of the protein to phytantriol matrices at all water loadings the mesophase structure was comparable to that of the respective binary mixtures [9]. However, for GMO samples containing FITC-Ova at 20 and 25% w/w there was a conversion from isotropic (in binary mixtures) to fan textures (in the presence of FITC-Ova), which was not observed for samples at higher water levels. Fan textures indicated the conversion to the H_2 phase, which was confirmed with SAXS.

Table 1. Selected examples of 'proteinaceous' drugs with different molecular masses that have been incorporated within the cubic phase.

Compound	Molecular mass (Da)	Ref.
Tryptophan	204	[33]
Melatonin	232	[10]
Ubiquinone-10	863	[81]
Desmopressin	1069	[82]
Insulin	6000	[51,52,82]
Cytochrome *c*	11,700	[33]
Lysozyme	14,400	[33]
Myoglobin	15,300	[33,64]
Hemoglobin	17,000	[65]
Ovalbumin	45,000	[9,33]
Conalbumin	47,000	[33,64]
Serratiopeptidase	60,000	[83]
Bovine serum albumin	67,000	[64]

Release behavior of FITC-Ova from the various matrices was also quantified. The *in vitro* release of FITC-Ova was faster and the extent of release was greater from phytantriol matrices as compared with GMO matrices, with ~ 30 and 10% released, respectively, over 2 weeks ($p < 0.05$) (**Figure 4A**). The differences in the release kinetics observed between the two different lipid systems was primarily a result of the conversion of GMO-based systems from cubic to hexagonal phases under the release study conditions, as confirmed by SAXS. The authors postulated that the conversion was most probably because of hydrolytic degradation of GMO leading to free oleic acid favors a transition from the cubic to the hexagonal phase. This phenomenon was also reported by Caboi *et al.* [67]. The conversion from the cubic phase, where water channels are open to the external environment, to the hexagonal phase, which is thought to be composed of closed infinitely long rod-like micelles [68], means that hydrophilic actives such as FITC-Ova are more efficiently trapped within these aqueous channels as compared with the cubic phase. Release from closed micellar systems would occur during random destruction/construction of the micelles, explaining why the release of FITC-Ova from the hexagonal phases is significantly retarded as compared with the cubic phase. The plot of release versus square root of time shown in **Figure 4B**, particularly within the first 48 h, shows diffusion as the dominant mechanism of release, consistent with other reports of release of actives from cubic phases [8,10,41,50].

4. Expert opinion

The viscous cubic phase has been utilized successfully for applications such as membrane protein crystallization, but its scope as a potential sustained release system, although extensively investigated, appears to be limited. The unique structural properties of the bicontinuous cubic phase, such as its high loading capacity of bioactives ranging from small molecular mass drugs to large macromolecules in addition to protection against chemical and physiological degradation with conserved biological activity, show why such a system is a desirable delivery platform for a wide range of pharmaceuticals, particularly peptides and proteins. Nonetheless, translation into a usable system for human application is still a long way away, primarily because of the practical limitations associated with using a viscous gel-based system. Administration of the viscous cubic phase by means of traditional delivery routes is impractical.

Alternative strategies for utilizing the cubic phase while circumventing the practical issues associated with handling the viscous gel have been investigated over the last two decades. One strategy to overcome this issue was to formulate the active of interest within the less viscous lamellar phase gel, which is easier to administer and once *in vivo* would theoretically absorb body fluids from the surrounding environment and convert to the viscous cubic phase [59,69]. Limitations with this approach were quickly realized, however, such as the risk of burst release from the lamellar phase with an associated risk of dose dumping [69]. Furthermore, the loading capacity of actives within the simple bilayer structure of the lamellar phase was significantly lower when compared with the complex cubic structure. Moreover, incorporating actives within the hydrated bilayer increases the risk of phase transformation and jeopardizes the physical integrity of the delivery matrix [11].

Another promising strategy recently described in the literature made use of injectable precursors that could be transformed into either the cubic or the hexagonal phase with changes in temperature, rather than relying on the formulations to undergo phase transitions by absorbing fluid [70]. The theory was that the transformation and therefore the rate of release of the entrapped active would be mediated through alterations in the temperature of the external environment. Subcutaneous administration of such a system containing glucose showed changes in the release profile of glucose, with changes in temperature induced by placing a heat or cool pack at the site of administration. The potential of such an 'on-demand' system, which can be delivered easily by subcutaneous injection and triggered to form the desired mesophase, is an interesting avenue of interest for the use of bicontinuous liquid crystals for sustained delivery.

Notwithstanding the success so far, more work is needed to increase the repertoire of peptide or protein pharmaceuticals incorporated within the cubic phase and to characterize their release kinetics in order to determine the suitability of such systems for human applications. Interactions between the drug/protein and the cubic mesophase *in vivo* with respect to the effect of the biological environment on release kinetics and biodegradation is another important area to which little attention has been paid.

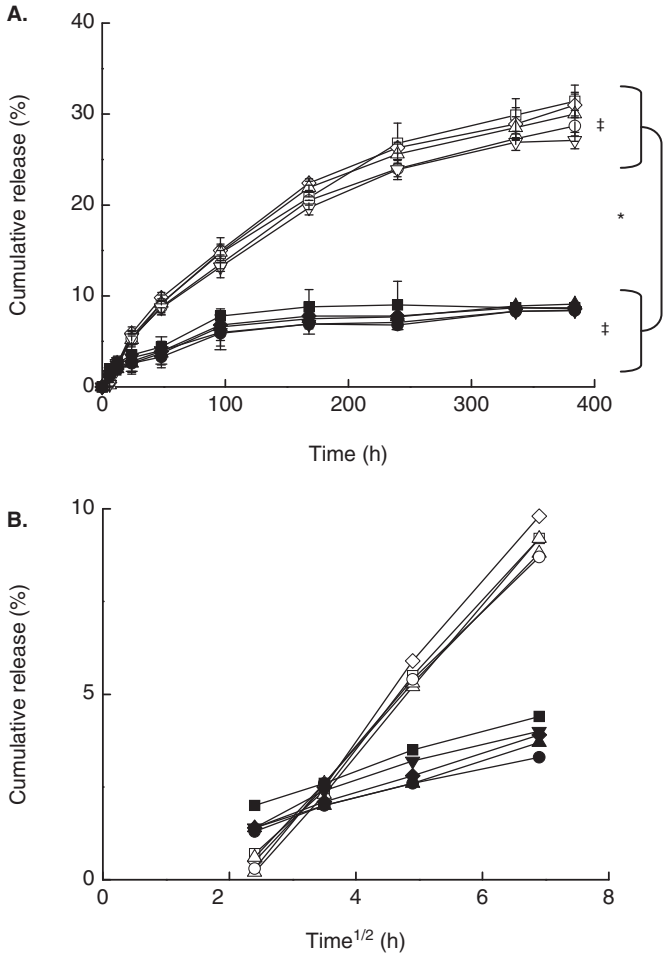

Figure 4. A. Release of fluorescein isothiocyanate-ovalbumin from phytantriol (open symbols) and glyceryl monooleate (filled symbols) matrices with initial water loadings of (■) 10, (◇) 20, (▲) 25, (•) 30 and (▼) 35% w/w at 37°C in PBS, pH 7.4. **B.** Release plotted as a function of the square route of time for the first 48 h of the release study. The correlation coefficients for both curves were at least 0.99. The linear fit is indicative of diffusion-controlled release for at least the first 48 h. Data presented are the mean of three independent experiments ± s.d.

Adapted with permission from [9].

*$p < 0.05$.

‡$p > 0.05$.

Finally, liquid crystalline mesophases in excess solvent can be dispersed into particles, which tend to retain the microstructure of their respective parent phase [31]. Dispersions based on the lamellar liquid crystalline phase are the well-known liposomes, those based on the hexagonal phase are known as hexosomes and dispersions of the cubic phase are referred to as cubosomes [39,71-73]. Cubosome dispersions have a lower viscosity than the viscous cubic phase, but retain the beneficial structural properties. They have been postulated to offer similar advantages to those of the non-dispersed cubic phase, such as solubilization of a wide variety of actives with varying polarity resulting from their bipolar nature, high entrapment of actives and the protection of sensitive actives (peptides and proteins) against rapid degradation [45,74]. These attributes make cubosomes commercially appealing, with potential applications extending from new material synthesis to nanoparticulate delivery systems for bioactives [75]. This has prompted many groups to try and exploit the physicochemical properties of cubosomes for various applications, though none with any commercial success so far [76,77].

Rizwan *et al.* (manuscripts in preparation) formulated phytantriol-based cubosomes and investigated their ability to act as a sustained release antigen delivery system in an *in vivo* mouse model. The cubosomes were well tolerated *in vivo* and cubosomes containing adjuvants resulted in significantly greater expansion of T cells and the production of similar levels of Ova-specific IgG as compared with alum, the most widely used vaccine adjuvant in a prime and boost

paradigm. Furthermore, cubosomes led to greater expansion of T cells *in vivo* as compared with liposomes. These results therefore bring to light the potential of cubosomes for the delivery of subunit vaccines.

Another barrier to the commercialization of cubic liquid crystals as potential sustained delivery systems for peptide and protein-based bioactives is the lack of a suitable, scalable manufacturing method for preparing structurally well-defined and stable dispersions. In addition, the range of lipids available with a suitable phase behavior for the preparation of these systems is limited [74,77]. Current research is focused on addressing these issues [8,78-80]. With the current level of interest in liquid crystalline research for various applications, their future as potential sustained delivery systems, whether it is in the gel or dispersed form, appears promising.

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Declaration of interest

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