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Progress in liquid crystalline dispersions: Cubosomes

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Abstract

Dispersed particles of bicontinuous cubic liquid crystalline phase, cubosomes, are self-assembled nanostructured particles that can be formed in aqueous lipid and surfactant systems. Contributions to cubosome research have come from the fields of biology, material science, medicine, and mathematics and much is known about their formation and properties. At the center of much of the discovery and innovation is the technique of cryo-transmission electron microscopy. Most of the research into cubosomes is motivated by potential applications in drug delivery and material synthesis although no commercialized product based on cubosomes is known. Recent advances in understanding and use of cubosomes are discussed in the context of some of the more promising application areas and the opportunities for microscopy techniques to make unique contributions to these areas.

Keywords: Cubosome; Surfactant; Lipid; Cubic phase; Drug delivery; Material template; Biological interfaces; Liposome; Cryo-TEM

1. Introduction

Of the many liquid crystalline structures self-assembled from aqueous surfactant systems, bicontinuous cubic phases possess a special status. Natural exhibitions of differential geometry, cubic phases are composed of contorted bilayers that partition hydrophobic and hydrophilic regions into continuous but non-intersecting spaces. Their discovery and subsequent structural characterization, it can be argued, sparked the current broad exploration of the complex relationship between surfactant structures from the molecular to nanometer to millimeter scales [1••,2–4]. Of their many unique properties, the ability of cubic phases to exist as discrete dispersed colloidal particles (Fig. 1), or cubosomes [5••,6,7•] is perhaps the most intriguing. Whereas most concentrated surfactants that form cubic liquid crystals lose these phases to micelle formation at high dilutions, a few surfactants have optimal water insolubility. Their cubic phases exist in equilibrium with excess water and can be dispersed to form cubosomes.

Just as small-angle X-ray scattering was crucial to the discovery and structural characterization of bulk cubic phases, cryo-transmission electron microscopy, or cryo-TEM, has been central to studies of cubosome dispersions. The field evolves rapidly and new intermediate and equilibrium structures [8] are still being discovered and direct cryo-TEM observation of new morphologies has no substitute. In addition to providing direct measures of cubosome size and shape, cryo-TEM also serves as a quantitative probe of liquid crystalline structural periodicity. Cubosome researchers are now almost obligated to show cryo-TEM images, lest readers doubt that cubosomes are used. Similarly, the contribution of the book *The Language of Shape* to the field is felt so strongly that it seems equally inadvisable to publish in this area without including a *Mathematica*-rendered image of the cubic unit cells, as in Fig. 2. Having vetted this article, it seems appropriate to try and address some new directions for cubosome research.

Applications often determine the rate and volume of research in a given area, and study of cubosomes has been similarly driven. Despite the breadth of work already performed, no commercial product is known to incorporate cubosomes, although patent art exists [9••,10]. In that light, this review selectively examines recent research to provide a subjective discussion of areas where cryo-TEM and other microscopy techniques can provide insight. Three intertwined application areas are the focus: Active ingredient delivery, Cubic phase–tissue interfaces, and material synthesis. Recent cubosome reviews exist [11,12] and this article builds on these to examine more recent literature and subjectively identify new or promising directions of cubosome research.

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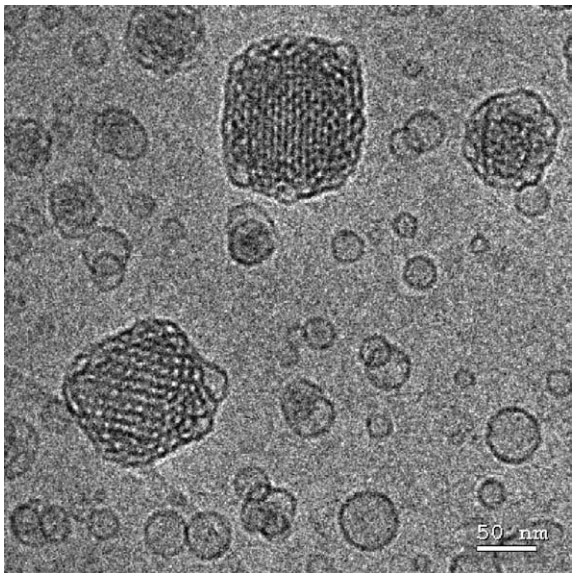

Fig. 1. Cryo-TEM image of cubosomes and vesicles formed by sonicating monoolein in aqueous Poloxamer 407 solution.

A common theme is kinetic aspects of cubosome creation and use.

2. Active ingredient delivery

Drug and other active ingredients require a range of delivery techniques and vehicles because of their variable solubility, availability, and stability and the field is an extremely active one encompassing numerous devices and methods [13]. Starting from their discovery in studies of fat digestion, the potential of cubosomes as encapsulation and delivery vehicles was obvious. Cubic phases with a bicontinuous structure have a high solid-like viscosity so cubosomes reflect the bulk phase's native symmetry in their cubic faceted shapes (Fig. 1). The bicontinuous structure of the cubic phases enables solubilization of diverse molecules ranging from proteins to small-molecule drugs [14••], and the bicontinuous structure's tortuosity leads to diffusion-controlled release of the solubilizates [15]. Not surprisingly the first and still the broadest application pursued for cubosomes has been as controlled release vehicles [12] despite some inherent limitations.

It is common to hear cubosomes cited as excellent controlled-release vehicles for delivery of active materials, especially drugs. But while the diffusivity of solubilized molecules is reduced by about 33% in bulk cubic phases [15], the much smaller length scale of cubosomes makes them difficult to directly use for controlled release. Boyd [16••,17] examines a range of hydrophilic and hydrophobic drugs and finds only burst release from cubosomes in aqueous media. Apparently, earlier claims of controlled release from cubosomes did not accurately characterize the transport behavior in dispersions. Still, altering cubosome charge, viscosity, and structure can improve release kinetics [18–21] but controlling the responsiveness of cubosomes in vivo might do more.

Beyond controlled release is targeted delivery of actives, for example to cancerous tumor vasculature. Here DNA–lipid complexes in the form of liposomes have shown promise during local injections by clustering around tumor sites and exhibiting efficient transfection. No clinical work on DNA–lipid complexes in cubosome form is known, but the extension is an obvious one and recent DNA–lipid cubic phase discoveries [22] offer intriguing promise. Some interactions with biological systems can be undesirable, as when lipase enzyme is present [23]. When injected into plasma, cubosomes are apparently hydrolyzed, but the solubilized actives remain encapsulated to some extent [24]. Through what phases do these particles pass as they degrade and how do solubilized proteins and nucleic acids affect the progression? The actual interactions in blood plasma should be quantified with cryo-TEM and compared to the work of Borné et al. [25] on lipase degradation of cubosomes.

Beyond the effects of the immediate environment on cubosomes, microscopy can also convey the sometimes profound structural influences that solubilized molecules can have on the cubic phase. Such effects are exemplified in the work of Angelova et al. [26••], who have created a new class of particles, "proteocubosomes": cubosomes fragmented from bulk cubic phase by incorporated proteins. Proteocubosomes possess a tertiary structure constructed hierarchically of ordered and disordered subunits, "nanocubosomes". Although the thermodynamic stability of such hybrid structures is an open question, their fascinating formation from cubosome building blocks is one of the first and most successful attempts to alter and customize the structure of cubosomes. The relationship between proteocubosomes and the intermediates observed by Borné [23] is not known, if any exists. A large factor in the success and performance of cubosomes as delivery vehicles rests on their interactions with biological surfaces.

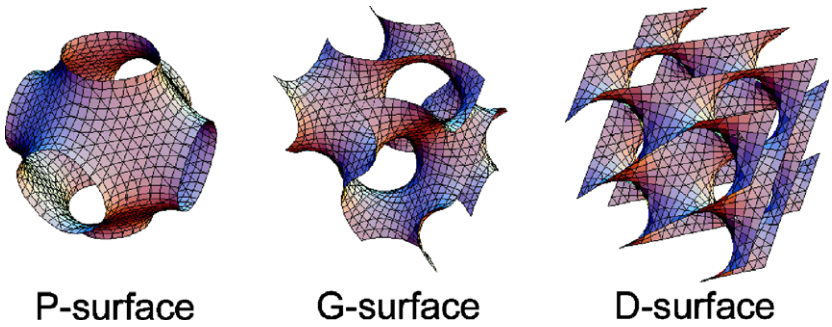

Fig. 2. Calculated approximate representations of the cubic unit cells most frequently encountered in aqueous surfactant systems.

3. Cubic phase–tissue interfaces

Some of the earliest observations of cubosomes and related structures were in biological systems, including plant membranes [27•] and human digestion models [28]. Building on liposome usage as model "cells" the thought came to incorporate proteins into cubic phases. Buchheim and Larsson [29] allow the protein casein to diffuse into a cubic phase and then carry out a beautiful work of freeze fracture electron microscopy by showing the boundary between two different cubic phases at the limit of casein infiltration. The pioneering work established the idea of probing the dynamics of the interface between a biological system and a liquid crystalline phase. A step beyond such work is the now well-established success of in meso crystallization of membrane proteins in cubic phases for structural determination [30]. What has not been approached is the imaging of such systems to test current hypotheses of transport and partitioning as crystals nucleate and grow inside liquid crystals. Here re-use of the techniques of Vekilov's group [31] may be of value, as they have tracked fundamental nucleation behavior by directly imaging proteins as they form critical and sub-critical nuclei visible by AFM. Cryo-TEM imaging of crystal nucleation and growth in cubosomes could provide verification of the proposed need for a local lamellar phase inclusion within the cubic phase to feed monomers to the growing crystals [32].

Other progress in cubic–biological interfaces highlights the unique bioadhesive nature of cubosomes [33]. Although the mechanism is not yet understood, imaging study of the adhesion, deformation, and failure of tissue–cubosome bonds could provide a dynamic window into the adhesion phenomenon and its roots. Recent work on adhesion and failure of vesicle–surface bonds [34] offers an excellent basis to understand the wetting and adhesion physics of such interactions in the context of tissue–cubic liquid crystalline interfaces. For example, what sort of deformation occurs as bioadhesive cubic phases are removed from biological surfaces as a function of interfacial energy? Theoretical work on cubic phase deformation may also be of use here [35].

The work of Lars Norlén offers a tantalizing means of blurring the boundary between cubic self-assembly structures in surfactant and biological systems in his theory of a bicontinuous cubic structure of the stratum corneum in human epidermis [36,37•]. Norlén's use of cryoelectron microscopy of vitreous sections allows direct imaging of the epidermal spaces and finds distinct similarity between cubosomes and the cubic structures of the intermediate filaments in skin [38]. Recent reviews build on these new insights to examine the broad applicability of cubic phases as substrates for bioelectrodes [39–41]. Hoath et al. have a vision of using cubosomes as an adapter that allows continuity between electrical and other medical sensors and the interior of the human body without invasive punctures or other modifications. They show [39,40] that cubic phase on human skin is superior to existing skin treatments because it simultaneously acts as a protective barrier, a moisture sink, and perhaps most importantly a permeable mass. With applied cubosomes the skin is still able to transpire but is protected and moisturized. What would be fascinating is to probe via imaging the adaptation, if any, of the cubic phase at the tissue surface in response to the local dermal structure. Might there be a shifting of local structure over time to better mate with the skin lipids, similar to the change in director twist in cholesteric liquid crystals interacting with organized surface structures? Despite the broad interest in cubosomes and their "soft" nature, commercial applications have also driven the pursuit of these phases in a more permanent form.

4. Material synthesis

The bicontinuous cubic phase structure was recognized early as a fascinating template for more rigid, high surface area nanometer-scale structures. The pioneering work at Mobil of Kresge et al. [42] formed a new aluminosilicate zeolite matrix by initiating sol–gel ceramic formation from precursors that were solubilized and thus shaped by the self-assembly forces at work on the CTAB surfactants themselves. The new material, MCM-48, is the monolithic substrate for catalytic reactions used in petroleum cracking operations. Templating of bulk bicontinuous cubic and other self-assembled phases has broadened into a self-sustaining field of its own and a recent review exists [43•]. A logical extension of bulk cubic phase templating is templating of cubosomes. Lu et al. [44] exploit liquid crystalline phase transition kinetics in evaporation-induced self-assembly processes for the aerosol synthesis of nanostructured particles. More recently Yang et al. [45] polymerized cubosomes and preserved the local microstructure.

It is also possible to extend beyond the well-known cubosome symmetries using both kinetic and equilibrium structures on the colloidal and larger length scales. Lynch et al. [46] followed the formation of unique pyramidal droplets during temperature-induced shifts along the cubic phase boundary in the $C_{12}E_2$-water system. They also observed coupling of the structures formed and their orientation with the surface of the capillary containing the phase, perhaps early evidence that such structures can be tuned by surface forces as suggested above. Staggeringly complex geometries have been formed in lyotropic and thermotropic cubic phase particles by Impéror-Clerc and coworkers [47–49•], again using careful temperature schedules to form all of the crystalline facets. As new triply continuous cubic phases [50] are explored the range of geometric templates will further expand. As mentioned in the above paragraph, proteocubosomes also expand the available structural templates and suggest the use of proteins not as delivery passengers but as structural adjuncts that broaden the symmetries and defects contributing to available cubosome structures and shapes. Here the use of 2D cryo-TEM microscopy images in conjunction with newly developed models to extract the three-dimensional structure [51] could rapidly accelerate the exploration of previously unknown kinetic and thermodynamically stable geometries. Experimental microscopy could truly blend with theoretical models.

Cubic phases possess broad potential as templates both at molecular and colloidal length scales. Recent breakthroughs in

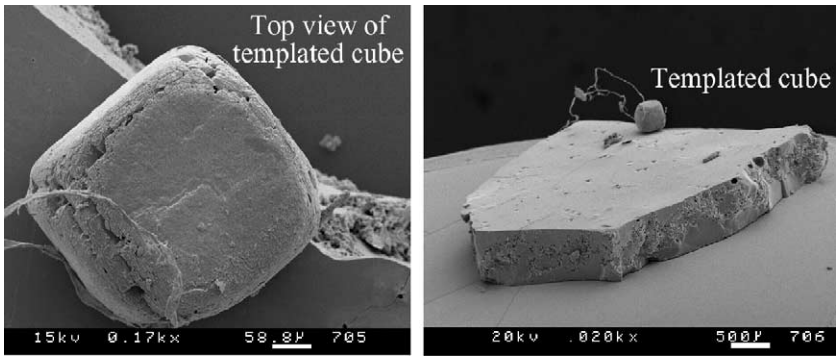

Fig. 3. SEM image of a silica cube templated by coating a cubosome with a silica sol, drying, then dissolving the cubosome.

cubosome yield [52•] are tantalizing in the possibility they offer to apply cubosomes the way monodisperse polymer colloids are now. For example, photonics researchers produce ordered structures by first equilibrating polymer colloids in colloidal crystal phases [53] in a metal oxide solution, then solidifying the structure and burning out the polymer particles. However, some symmetries have a favorable band gap for photonics applications but are difficult to access using colloidal crystals based on spherical particles. It would be interesting to assemble monodisperse cubosomes, explore their colloidal self-assembly structures, and template them by infiltration of silica solutions. Then the cubosomes could simply be dissolved away with organic solvents, leaving behind rigid structures based on cubic building blocks rather than spherical ones. As a crude proof of concept Fig. 3 shows a 100 micron cube formed by coating a large cubosome with silica sol, evaporating the water to consolidate the silica, and dissolving the cubosome. Larger-scale structures would require ordering the cubosomes first and then templating, but this could be done by forming the cubosomes from vesicle or emulsion precursors, ordering these, and then slowly crystallizing them into cubosomes using the techniques of Ref. [52•].

The importance of templating to material science is growing and as researchers become increasingly adept a shift in emphasis from equilibrium phases to kinetic structures is logical. An example is the use of kinetic myelinic structures [54] as templates during lamellar phase formation [55]. Recent work has shown that certain kinetic pathways leading to cubosomes through lamellar intermediates produce structures clearly influenced by myelinic structure formation [56]. What has not been done is to probe the internal structure of such kinetically intermediate forms using cryo-TEM. Even simple systems exhibit the potential for much structural complexity, and future microstructural probes of, for example, poorly resolved regions of the ternary ethanol–monoolein–water [57] phase diagram may yield additional kinetic and possibly equilibrium structures of interest.

5. Concluding remarks

Cubosome research currently progresses through both incremental advances and breakthroughs. Much knowledge exists in proprietary circles given the patent and literature activity but without a well-known commercialized example it is difficult to coalesce the field around some fundamental and central challenges. Nevertheless, the areas discussed above are just a few of the potential applications of cubosomes where microscopy could directly address unknowns. Other areas of potential include a re-examination of the key conclusions of the last 20 years using the new Freeze Fracture Direct Imaging techniques [58] that avoids some of the artifacts associated with cryo-TEM and FF-TEM. Finally, the rheological and flow behavior of cubic phases is at the core of their uniqueness, but few fundamental studies exist that link the flow, deformation, and hydrodynamic behavior of cubosome dispersions with their structure and performance. Here examples from the liquid mixing community offer the most hope for physically based interpretations.

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