Cubosomes: Bicontinuous Liquid Crystalline Nanoparticles

Patrick T. Spicer

The Procter & Gamble Company, West Chester, Ohio, U.S.A.

INTRODUCTION

Surfactants, lipids, and polymer molecules that have both polar and nonpolar components are termed amphiphilic. The hydrophobic effect drives amphiphilic molecules in polar solvents to spontaneously self-assemble into a rich array of thermodynamically stable lyotropic liquid crystalline phases with characteristic lengths on the nanometer scale. Liquid crystalline phases possess a sufficient average degree of molecular orientational order to be characterized by their structural symmetry, despite their liquid state, and often form in aqueous surfactant systems at relatively high amphiphile concentrations. An interesting example of contemporary interest is the bicontinuous cubic liquid crystalline phase.

Bicontinuous cubic phases are optically isotropic, very viscous, solidlike liquid crystals with cubic crystallographic symmetry. Prior to their structural characterization, these phases were termed "viscous isotropic phases" and considered quite a nuisance in industrial processes. Bicontinuous cubic phases consist of two separate, continuous but nonintersecting hydrophilic regions divided by a lipid bilayer that is contorted into a periodic minimal surface with zero average curvature. The phase's regular structural connectivity results in a very high viscosity, whereas its tortuosity is useful for slowing diffusion in controlled transport applications. The bicontinuous nature of such cubic phases distinguishes them from the so-called micellar or discontinuous cubic phases containing micelles packed in cubic symmetry. In this work, all references to cubic phases refer to bicontinuous cubic phases, unless otherwise noted.

The last several decades have brought about a great understanding of the properties of cubic phases and a realization of their relevance in areas such as medicine, biology, and chemistry. An intriguing property of the cubic phases formed by certain classes of amphiphiles is their ability to be dispersed into particles, termed cubosomes. Cubosomes are liquid crystalline nanostructured particles with the same unique properties of the bulk cubic phase, although cubosome dispersions have much lower viscosity. Although fundamental research has been focused sharply on bulk cubic phases, it is commercial applications that drive much of the existing and still very active research into cubosomes. This chapter reviews historical research into cubic phases and cubosomes, describing the initial discovery and the realization that differential geometry allows the mathematical characterization of cubic phase structures. Cubosome properties are reviewed in the context of particle formation and applications. Where appropriate, interesting open problems are cited and discussed.

CUBIC PHASE STRUCTURE

Luzzati and Husson^[1] and Luzzati et al.^[2] first recognized the existence of cubic phases in lipid-water systems using x-ray scattering measurements. Fontell et al.^[3] drew similar conclusions regarding cubic phases in ternary systems of amphiphiles, oils, and water. In parallel, although without apparent awareness of the lipid work, biologists began documenting structures with cubic symmetry in plant leaf plastid membranes,^[4] identical to what is now accepted as cubic liquid crystalline phase structures. Around the same time, Lutton^[5] published a comprehensive study of the aqueous phase behavior of monoglycerides. Monoglycerides are polar lipids with poor water solubility that exhibit aqueous phase behavior, reflecting their structural similarity to nonionic surfactants. Fig. 1 shows a schematic of Lutton's results for monoglycerides with hydrocarbon chain lengths between C₁₂ and C₂₂. Of particular interest is the large region of cubic phase exhibited by each of the aqueous monoglyceride systems, with the largest being that of monoolein, the unsaturated C₁₈ monoglyceride (Fig. 2). Cubic phases are often found sandwiched between lamellar and hexagonal liquid crystalline phases, especially in nonionic surfactant systems.^[6] However, the monoolein-water system uniquely possesses a cubic phase region spanning large compositional and temperature ranges. It is not entirely clear why monoolein forms cubic phases in such broad compositional and temperature ranges, but surfactant packing concepts offer some conceptual approaches. Monoolein has a slightly larger cross-sectional area of its hydrophobic tail region, leading to a ratio of the hydrophobic tail group area to the hydrophilic head group



Fig. 1 Schematic of the aqueous phase behavior of the monoglycerides showing the regions of cubic phase formation. (From Ref. [5]. (C) American Oil Chemists' Society, 1965.)

area slightly larger than one (~ 1.03 ,^[7]). As a result, the cubic phases are often referred to as reversed or inverse cubic phases, indicating the curvature of the constituent bilayers toward the polar medium. Shortly after Lutton's study, Larsson began to systematically examine the structure of aqueous monoglyceride cubic phases using x-ray diffraction. He found, using nuclear magnetic res-

onance (NMR) and x-ray, that the cubic phases have continuous regions of both hydrophobic and hydrophilic nature^[8] leading to the conclusion that the cubic phase structures can be described using the concepts of differential geometry and periodic minimal surfaces.^[9]

Minimal surfaces are best described by analogy with their most readily observed natural form: soap films. The



Fig. 2 Molecular structure of monoolein, the lipid most commonly used to form cubosomes.



Cubosomes: Bicontinuous Liquid Crystalline Nanoparticles

surface formed by a soap film between two rings is a catenoid, a simple form of minimal surface whose two principal curvatures are equal but opposite in sign at every point, resulting in an average curvature of zero and a negative Gaussian curvature.^[10] Fig. 3 shows approximate plots of the three most commonly studied minimal surfaces in cubic phases. The surfaces in Fig. 3 are particularly fascinating because their discovery was purely mathematical, prior to knowledge of the structures' existence in cubic phases. The P-surface (or primitive surface) and the D-surface (or diamond surface) were both discovered mathematically by Schwarz in 1890, whereas the G-surface (or gyroid surface) was only discovered by Schoen^[11] in the 1960s. In cubic phases, the minimal surface is formed by the self-assembled bilayer that occurs as the hydrophobic or hydrophilic portions of the surfactant molecules line up to minimize their interaction with their opposites. The three structures are all bicontinuous (i.e., they divide space into two continuous but nonintersecting regions); in the case of cubic phases, two separate regions of hydrophilic material (water channels) form. Following suggestions by Scriven^[12] that minimal surfaces could explain liquid crystal structure, Larsson et al.^[9] applied the minimal surface description to the cubic phase observed in the monoolein-water system and noted the connection to the structures formed in plastid systems. However, Longley and Mcintosh^[13] found evidence of an alternative symmetry in the monooleinwater cubic phase, leading Larsson^[14] to realize that two cubic phases are formed, both with minimal surface structures. The monoolein-water system forms the D-surface at high water levels and the G-surface at lower levels, as shown in the phase diagram in Fig. 4.^[15] Qiu and Caffrey^[16] later updated elements of the monoolein-water phase diagram, but the version put forth by Larsson (Fig. 4) remains the benchmark. The P-surface is formed in the monoolein-water system, but only when a third component, such as caseins^[17] or amphiphilic block copolymers,^[18] is added. The strength of the minimal surface approach to cubic phase characterization is its



Fig. 3 Unit cubes of the P-surface, G-surface, and D-surface formed in bicontinuous amphiphilic systems. (*View this art in color at www.dekker.com.*)



Fig. 4 Aqueous phase behavior of the monoolein–water system, updated to reflect the existence of two cubic phases. (From Ref. [25]. (C) American Chemical Society, 1989.)

quantitative mathematical basis. Unfortunately, the analytical representation of minimal surfaces in Fig. 3 is still mathematically very difficult. A significant advance came in the discovery that nodal surfaces are closely related to minimal surfaces and offer a simple equation that can be used to plot and study cubic phase structures.^[19] The structures plotted in Fig. 3 are generated using nodal surfaces and the equations used are also shown. Extensive reviews of the history and mathematical description of bicontinuous phases are given in Refs. [20] and [21], whereas a more recent review^[22] generally discusses bicontinuous geometry in amphiphilic systems. Contemporary with the discovery of minimal surface applicability to cubic phases was the realization that, in certain cases, the cubic phases can exist in a particulate form, possessing the same bicontinuous structure as the bulk cubic phase.

CUBOSOMES—DISPERSED CUBIC PHASE PARTICLES

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.^[23] Olive oil droplets, mainly triolein, in contact with lipase formed small particles of cubic phases as the enzyme broke the triglyceride down into monoolein that then hydrated. Lindstrom et al.^[24] studied the phenomenon further, observing that sodium cholate bile salts provided dispersion and colloidal stability of the cubic phase particles formed by monoolein and water, and that such particles formed in other ternary systems as well. One of the first published instances of the word "cubosome" appears in a review by Larsson^[25] of the

Marcel Dekker, Inc.

270 Madison Avenue, New York, New York 10016



implications of bicontinuous cubic phases to biological membranes. Later research on cubosomes focused on the ternary phase behavior of the Poloxamer 407 (a PEO₉₉–PPO₆₇–PEO₉₉ block copolymer)–monoolein–water system because of the polymer's utility at providing colloidal stability to cubosomes against recoalescence to bulk cubic phase.^[18] 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. Landh^[18] observed cubosome formation in a three-phase region including disordered bicontinuous (L₃ or sponge) phase, cubic phases, are critical elements of some biological processes and occur naturally in bacterial cells, for example.^[26]

Just as bulk cubic phases can be mathematically characterized using nodal surface approximations, so, too, can cubosomes. Andersson et al.^[27] model different</sup>cubosome structures by extending nodal surface descriptions^[19] to describe closed-off surfaces such as those formed by cubosomes. Fig. 5 shows two examples of such simulated cubosomes, in this case a D-surface that has been closed off on its surface by a sphere and by a cube. A comprehensive overview of the structures that can be simulated by such techniques is given by Jacob and Andersson.^[28] Many experimental studies of cubosomes utilize cryo-transmission electron microscopy (TEM) micrographs that allow the visualization of cubosome particles and the verification of their lattice symmetry, although x-ray measurements are required to confirm phase structures. Cryo-TEM allows the visualization of hydrated phase structures by vitrifying, instead of freezing, the sample in a thin film suspended between polymercoated grid struts.^[29,30] Insight into bulk cubic phase structure has also been gained in some cases by freeze-fracture electron microscopy.^[31,32] More recent work has



Fig. 5 Calculated nodal surface representations of a D-surface cubosome closed off on its surface with a sphere and a cube. (*View this art in color at www.dekker.com.*)



Fig. 6 Cryo-TEM micrograph of cubosomes and vesicles formed by ultrasonic dispersion of bulk cubic phase in aqueous Poloxamer 407 solution. (From Ref. [36]. © American Chemical Society, 2001.)

shown the possibility of in situ study of cubosomes using atomic force microscopy.^[33]

Although small amounts of cubosomes are apparently produced by simple addition of dispersing agents such as sodium cholate to bulk cubic phase,^[24] mechanical dispersion of bulk cubic phase is the most studied and most direct cubosome formation technique. Gustafsson et al.^[34,35] formed cubosomes by high shear microfluidization and ultrasonication of bulk cubic phase in contact with aqueous Poloxamer 407. The cubosomes produced exhibit the P-surface structure based on x-ray diffraction and, interestingly, resemble cubes despite their nonsolid nature. The molecular-scale cubic symmetry of the lipid bilayer comprising cubosomes is thus manifested in the nanometer-scale cubosome particles, although their appearance is often orientation-dependent.^[34,36] An example of the different types of particles formed by ultrasonication of bulk cubic phase in aqueous Poloxamer 407 solution is seen in Fig. 6, a cryo-TEM micrograph of the dispersion. The particles range in size from about 10 to 500 nm in diameter. Cubosomes are immediately identifiable as square particles with a well-defined cubic lattice of dots. Each dot corresponds to the presence of a porecontaining aqueous phase. Along with well-formed cubosomes, more disordered cubosomes that have a more spherical shape are also visible. Gustafsson et al.^[34] point out that the absence of grain boundaries in the cubosomes indicates they are single-crystal structures. Unilamellar vesicles, dispersed lamellar liquid crystalline phase particles, are also visible in Fig. 6. Increased polymerto-monoolein ratios lead to the formation of larger ratios **Cubosomes: Bicontinuous Liquid Crystalline Nanoparticles**

ORDER

REPRINTS

of vesicles to cubosomes. Such vesicles often form during the dispersion of bulk cubic phase, despite the fact that the lamellar phase is not an equilibrium phase at these conditions. Ultrasonication of bulk cubic phase produces mostly vesicles^[34] 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. Distinct from the dispersed vesicles, the surfaces of some of the cubosome particles have hemispherical vesicle structures that are thought to block the exposure of the hydrophobic bilayer at the termination of the cubosome edge structure but may also aid in colloidal stabilization of the cubosomes.^[35,37] Gustafsson et al.^[35] were able to shift the cubosome structure between the P-surface and the D-surface by varying Poloxamer 407 concentration. Although cubosomes have been feasibly produced in various laboratory settings for years, the desire to apply cubosomes in various commercial products spurs current research interest in cubosome production.

CUBOSOME MANUFACTURE

At its most basic, the production of cubosomes entails the formation of nanoparticles. Two distinct nanoparticle production routes exist: top–down and bottom–up techniques. Most cubosome 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. No systematic study exists of the effect of power input on bulk cubic phase dispersion, but existing rheological work offers some insight into the energy required to form cubosomes.

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. Studies^[38,39] of the P-surface and D-surface cubic phases formed by several didodecyldimethylammonium bromide (DDAB)-oil-water systems found the phases to behave as viscoelastic fluids that exhibit relatively constant modulus values until the lattice structures break down around 1-5%strain. Rupture of the 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. Warr and Chen^[40] 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.^[40] Warr and Chen^[40] also suggested but could not confirm that cubic phases may behave as lamellar phases do during dispersion with increasing shear: dispersed liquid crystalline particles form at intermediate shear rates, whereas a defect-free bulk phase re-forms at higher shear rates. At high oscillatory frequencies, cubic phases become highly elastic^[38] and may be more apt to fracture than deform. A worthwhile study would be the correlation of energy input with the cubic phase breakage mechanism most feasible for a given deformation rate. Different breakage mechanisms could correlate with cubosome yield.

Unfortunately, most existing studies provide little insight into the mechanisms underlying cubosome formation by energetic dispersion of bulk cubic phases. Comparison of the dispersions produced by sonication and high-pressure homogenization suggests the formation 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 originating bulk cubic phase,^[41] but after homogenization, the P-surface dominates, either because of the added polymer or other factors.^[34] Based on such studies, it has been suggested that high-energy dispersion processes destroy the structure of bulk cubic phases, creating vesicles that then must fuse their bilayers to form ordered cubosomes.^[41] However, no quantitative conclusion can be drawn without a systematic study of the effects of the fundamental parameters of a dispersion process. Fluid shear stresses, temperature changes induced by sonication, and pressure^[42,43] all affect cubic phase and thus cubosome formation in different ways.

More recent work has examined the development of bottom-up methods of cubosome formation, allowing cubosomes to form and crystallize from precursors on the molecular length scale. Almgren et al.^[30] 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 crystallize into cubosomes. Large-scale production of cubosomes and products containing them requires more robust processes. Spicer et al.^[36] developed a room temperature process to produce cubosomes by simply diluting monoolein-ethanol (or other hydrotrope) solutions with aqueous Poloxamer 407 solutions. The cubosomes have been shown to form by spontaneous emulsification,^[44] 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 produced than those by high-energy processes, but some vesicles are also produced.^[36] A second process was also developed to allow cubosome production from a powdered precursor.^[45] Spray-dried powders comprising monoolein



ORDER

Fig. 7 Cryo-TEM micrograph of cubosome formed by hydrating starch-encapsulated monoolein particles. (From Ref. [45]. © Kluwer Academic Publishers, 2002.)

coated with starch or dextran form cubosomes on simple hydration. Colloidal stabilization of the cubosomes is immediately provided by the polymers. An example of a cubosome formed in such systems is shown in Fig. 7. The large unfolded bilayers that form in such cases may be stabilized by polymer-induced osmotic, steric, or viscous forces,^[45] identical to the higher fraction of surface vesicles observed in the Poloxamer 407–Monoolein–water system.^[35]

SYSTEMS FORMING CUBOSOMES

The formation of cubosomes is possible in binary and ternary systems with a sufficiently large miscibility gap between the cubic phase and the solvent. Colloidal stabilization of cubosomes is good when Poloxamer 407 is used to provide steric stabilization against aggregation and coalescence, but other suitable polymers can be used as well. Earlier studies^[37] advocate mixing vesicles and cubosomes produced by fragmentation of bulk lamellar and cubic liquid crystalline phases in excess water, respectively. The coating of cubosomes with lamellar bilayer "caps" covers the cubic bilayer openings formed by fragmentation, preventing exposure of hydrocarbon chains to water and providing limited colloidal stability. Coating the cubosomes with solid crystalline bilayers provides superior colloidal stability to lamellar liquid crystalline coatings, although such rigid coatings limit the lowest diameter of the cubosomes to 100 nm, rather than

Fig. 8 Schematic of the typical ternary phase diagram exhibited by systems containing a lamellar phase-forming polar lipid, a cubic phase-forming polar lipid, and water. (From Ref. [37].)

the absolute minimum of 50 nm, because of curvature restrictions.^[37] In addition, coatings of the sponge phase have been proposed as a stabilizing coating for cubosomes.^[37] Figs. 8 and 9 show schematics of the two general forms of ternary phase diagrams exhibited by systems forming cubosomes. For ternary systems of lamellar phase-forming polar lipids, cubic phase-forming polar lipids, and water (Fig. 8), cubosomes form in the two-phase region of equilibrium between the cubic phase and water or solvent, whereas vesicles form in the two-phase region between the lamellar phase and water. The three-phase region between the lamellar, cubic, and water phases is likely to contain both vesicles and cubosomes. Similarly, for ternary

Fig. 9 Generalized ternary phase diagram for systems containing a cubic phase-forming polar lipid, a nonaqueous polar solvent, and water.

Fig. 10 Molecular structure of phytantriol, the second most commonly used amphiphile for forming cubosomes.

systems containing cubic phase-forming polar lipids, a nonaqueous polar solvent or an active ingredient (such as a drug), and water, a sponge phase often forms in the general area indicated in Fig. 9.^[46] In such cases, cubosomes form in the two-phase region of equilibrium between the cubic phase and water or solvent, whereas cubosomes, vesicles, and/or sponge phase particles may form in the multiphase regions between the cubic phase, sponge phase, lamellar phase, and water.^[36] Currently, the only well-documented instances of cubosome formation are in systems containing monoolein. There is no reason why other similar surfactant systems should not form cubosomes, provided that a sufficient miscibility gap exists between the bicontinuous cubic phase and the solvent. One molecule with great potential for cubosome formation is phytantriol (Fig. 10), a commonly used additive in consumer products such as sunscreens. Several patents have emerged citing the formation of cubosomes in phytantriol-water systems,^[47-49] but no published phase diagram is known. However, a simple swelling experiment offers insights into the aqueous phase behavior of phytantriol. Figs. 11 and 12

Monoolein-Water

50 µm

show partially polarized light micrographs of the interfaces formed on contacting a small droplet of monoolein (Fig. 11) and phytantriol (Fig. 12) with excess water. In both cases, interfaces that separate the amphiphile from the optically birefringent lamellar phase, and two distinct optically isotropic cubic phase regions are visible. Although such experiments are qualitative, the similarity between monoolein and phytantriol is striking and fortuitous, suggesting the need for more quantitative assessment (e.g., using the Diffusive Interfacial Transport (DIT) technique^[50]) of the phytantriol-water system. Some glycolipids^[51,52] also exhibit the cubic-water miscibility gap required for cubosome formation, and confirmation should be relatively simple. Ultimately, much of the future work on cubosomes will be driven by the most financially compelling applications.

887

APPLICATIONS

Cubic

Controlled-Release Drug Delivery

Controlled release of solubilized actives is the most popular application pursued by cubosome researchers, and excellent reviews exist of attempted delivery applications as well as pharmaceutical actives that have been solubilized in bulk cubic phase^[53] and cubosomes.^[54] Cubic phase is attractive for controlled release because of its small pore size (ca. 5–10 nm); its ability to solubilize hydrophobic, hydrophilic, and amphiphilic molecules; and

Cubic

Fig. 11 Polarized light micrographs of the aqueous phase progression exhibited by monoolein in excess water.

Fig. 12 Aqueous phase progression of phytantriol in excess water. The sequence of phases is qualitatively identical to that of monoolein (Fig. 11).

its biodegradability by simple enzyme action.^[55] Cubic phase is strongly bioadhesive^[56] and is thought to be a skin penetration enhancer,^[57] suggesting excellent compatibility with topical and mucosal deposition and delivery of active ingredients. Recent studies^[58,59] have emphasized 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. The tortuous structure of cubic phase lends itself well to slowing diffusive release of solubilized actives. Theory predicts the minimum reduction of a solute's free solution diffusivity by 33%.^[60] Experimental measurements of small molecule diffusivity in cubic phases give values on the order of 10⁻¹⁰ m²/sec.^[61] No commercial applications of cubic phase delivery vehicles are known other than a treatment developed for periodontal disease that is based on triglyceride-monoolein mixtures combined with the drug metronidazole.^[62] 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.

Cubosomes prepared in dispersions possess a nanometer-scale structure identical to bulk cubic phase, but the dispersion itself has a much lower, waterlike viscosity. Compared to liposomes or vesicles, cubosomes possess 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 too high a surface area for such performance, exhibiting instead burst release.^[63] 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.^[47-49] For example, oleic acid-containing cubosomes change from cubic phase to hexagonal phase with a drop in pH, indicating a relatively rapid response of the cubosome interior to the outer fluid environment.^[64,65] Although the above controlled-release limitations exist for small molecule solutes and unmodified cubosomes, other routes may still exist for controlled-release applications of cubosomes. For example, large poly(amidoamine) dendrimer molecules exhibit a $100 \times$ reduction in free diffusivity when entrapped in cubic phases.^[66] Modification of the cubic phase environment is also a potential method of regulating solute transport. Puvvada et al.^[67] find that aqueous alginate molecules can be gelled in cubic phase pores by ionic strength changes, increasing local viscosity and drastically slowing active release. Other studies have found that the partitioning and release kinetics of ionic solutes in cubic phases are significantly altered by incorporating oppositely charged surfactants into the cubic bilayers.^[68,69] In such cases, the functionalizing surfactant must be chosen carefully to avoid

Cubosomes: Bicontinuous Liquid Crystalline Nanoparticles

Materials Synthesis

From a materials science perspective, 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 polymerization or reaction to form solids from precursors that are solubilized 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,^[70] used for catalytic processing of petroleum. Yang et al.^[71] successfully carried out polymerization inside cubosomes, yielding a solid nanostructured particle with cubic symmetry. Such particles hold promise for use in photonic and semiconductor applications.^[72] Lu et al.^[73] have developed novel aerosol processes that create particles with nanometerscale structure by evaporation of solvent from isotropic phase liquid droplets, simultaneously driving them into cubic phase structures and solidifying the particles. As sophistication in the cubic phase template area builds, optimization of the structures will be a leading interest area. Along these lines, Larson^[74] suggests the possibility of aligning the cubic phases by steady or large-amplitude oscillatory shearing prior to templating, forming materials with unique and highly anisotropic properties. Beautiful structures have been formed using careful growth of faceted cubosomes in the C12E2-water system,^[75] offering future promise of multiple-decade lengthscale control over the morphology of particles formed from such templates.

OPEN PROBLEMS

The creation of cubosomes and other liquid crystalline nanoparticles is still an open and active area of research. Numerous applications as well as fundamental insights into behavior and performance regularly emerge. As a result, many open problems remain to be examined and solved by intrepid researchers, including some described in the above sections. An especially interesting area is the formation of dispersions of other liquid crystalline phases besides cubosomes. Studies of such dispersions, excluding vesicles, are sparse. Dispersions of hexagonal liquid crystalline phase^[35] and sponge phase^[76] have been reported, usually in coexistence with cubosomes. Such dispersion mixtures may provide multiple modes of controlled re-

lease, whereas their structures are compelling templates for solid nanoparticle synthesis, but more work is needed. As more becomes known about bicontinuous mesh and intermediate phases,^[77,78] it may become possible to find

As more becomes known about bicontinuous mesh and intermediate phases,^[77,78] it may become possible to find stable, dispersed forms of these structures as well, liberally broadening liquid crystalline nanoparticle applications. Probably the most compelling direction of research into these systems seeks to understand the interactions between bicontinuous structures and biological systems in general. Enormous potential exists for the expansion of the current understanding of biological processes and medical treatment.

CONCLUSION

REPRINTS

ORDER

Cubosome nanoparticles formed from cubic liquid crystalline phases are a unique and intriguing self-assembled material with enormous potential in areas as diverse as medicine, materials science, and consumer products. The relatively recent discovery of cubosomes has spurred a broad level of investigation that, as proposed applications become financially attractive, will continue to narrow and fill in many of the current gaps in our knowledge of cubosome formation and performance. Interdisciplinary research in engineering, biology, medicine, and chemistry will be especially crucial to tie together existing cubosome research and to provide a consistent understanding of these fascinating particles.

ACKNOWLEDGMENTS

I am indebted to my collaborators at the Procter & Gamble Company, Matthew Lynch and Janet Burns (Corporate Research), and Kristin Hayslip, Bill Small, and David Piatt (Corporate Engineering). Invaluable advice and mentoring have been and continue to be received from Stig Friberg (Clarkson University, retired), Eric Kaler (University of Delaware), Robert Prud'homme (Princeton University), and Robert Laughlin (Procter & Gamble Company, retired). The support of management at the Procter & Gamble Company, in particular Richard Owens, has been crucial throughout our work in the area of cubosomes.

REFERENCES

 Luzzati, V.; Husson, F. The structure of the liquidcrystalline phases of lipid-water systems. J. Cell Biol. **1962**, *12*, 207–219.

- Luzzati, V.; Tardieu, A.; Gulik-Krzywicki, T.; Rivas, E.; Reiss-Husson, F. Structure of the cubic phases of lipid–water systems. Nature 1968, 220, 485–488.
- Fontell, K.; Mandell, L.; Ekwall, P. Some isotropic mesophases in systems containing amphiphilic compounds. Acta Chem. Scand. 1968, 22, 3209– 3223.
- Gunning, B.E.S. The Greening Process in Plastids:
 The Structure of the Prolamellar Body. In *Protoplasma*; Höfler, K., Porter, K.R., Eds.; Springer-Verlag: New York, 1965; 111–139.
- Lutton, E.S. Phase behavior of aqueous systems of monoglycerides. J. Am. Oil Chem. Soc. 1965, 42, 1068–1070.
- Sjöblom, J.; Stenius, P.; Danielsson, I. Phase Equilibria of Nonionic Surfactants and the Formation of Microemulsions. Phase Equilibria of Nonionic Surfactants and the Formation of Microemulsions. In *Nonionic Surfactants: Physical Chemistry*; Schick, M.J., Ed.; Marcel Dekker: New York, 1987; Vol. 23, 369–434.
- Angelov, B.; Ollivon, M.; Angelova, A. X-ray diffraction study of the effect of the detergent octyl glucoside on the structure of lamellar and nonlamellar lipid/water phases of use for membrane protein reconstitution. Langmuir **1999**, *15*, 8225–8234.
- Lindblom, G.; Larsson, K.; Johansson, L.; Fontell, K.; Forsen, S. The cubic phase of monoglyceride– water systems. Arguments for a structure based upon lamellar bilayer units. J. Am. Chem. Soc. **1979**, *101* (19), 5465–5470.
- Larsson, K.; Fontell, K.; Krog, N. Structural relationships between lamellar, cubic, and hexagonal phases in monoglyceride-water systems. Possibility of cubic structures in biological systems. Chem. Phys. Lipids **1980**, *27*, 321–328.
- 10. Lovett, D. *Demonstrating Science with Soap Films*; Institute of Physics: Bristol, 1994.
- 11. Schoen, A.H. Infinite Periodic Minimal Surfaces Without Self-Intersections; NASA Technical Note D-5541; NASA, 1970.
- 12. Scriven, L.E. Equilibrium bicontinuous structure. Nature **1976**, *263*, 123–125.
- 13. Longley, W.; Mcintosh, T.J. A bicontinuous tetrahedral structure in a liquid-crystalline lipid. Nature **1983**, *303*, 612–614.
- 14. Larsson, K. Two cubic phases in monoolein–water system. Nature **1983**, *304*, 664.
- Hyde, S.T.; Andersson, S.; Ericsson, B.; Larsson, K. A cubic structure consisting of a lipid bilayer forming an infinite periodic minimal surface of the gyroid type in the glycerolmonooleat–water system. Z. Kristallogr. **1984**, *168*, 213–219.

- 16. Qiu, H.; Caffrey, M. The phase diagram of the monoolein/water system: Metastability and equilibrium aspects. Biomaterials **2000**, *21*, 223–234.
- 17. Buchheim, W.; Larsson, K. Cubic lipid–protein– water phases. J. Colloid Interface Sci. **1987**, *117* (2), 582–583.
- Landh, T. Phase behavior in the system pine oil monoglycerides–Poloxamer 407–water at 20°C. J. Phys. Chem. **1994**, *98*, 8453–8467.
- 19. Von Schnering, H.G.; Nesper, R. Nodal surfaces of Fourier series: Fundamental invariants of structured matter. Z. Phys., B **1991**, *83*, 407–412.
- Anderson, D.M. Periodic surfaces of prescribed mean curvature. Adv. Chem. Phys. 1990, 77, 337– 396.
- 21. Hyde, S.; Andersson, A.; Larsson, K.; Blum, Z.; Landh, T.; Lidin, S.; Ninham, B.W. *The Language* of Shape, 1st Ed.; Elsevier: New York, 1997.
- 22. Schwarz, U.; Gompper, G. Bicontinuous surfaces in self-assembling amphiphilic systems. Lect. Notes Phys. **2002**, *600*, 107–151.
- 23. Patton, J.S.; Carey, M.C. Watching fat digestion. Science **1979**, *204*, 145–148.
- 24. Lindstrom, M.; Ljusberg-Wahren, H.; Larsson, K.; Borgstrom, B. Aqueous lipid phases of relevance to intestinal fat digestion and absorption. Lipids **1981**, *16* (10), 749–754.
- 25. Larsson, K. Cubic lipid–water phases: Structures and biomembrane aspects. J. Phys. Chem. **1989**, *93*, 7304–7314.
- Meyer, H.W.; Richter, W.; Gumpert, J. Periodically curved bilayer structures observed in hyphal cells or stable L-form cells of a *Streptomyces* strain, and in liposomes formed by the extracted lipids. Biochim. Biophys. Acta **1990**, *1026*, 171–178.
- Andersson, S.; Jacob, M.; Ladin, S.; Larsson, K. Structure of the cubosome—A closed lipid bilayer aggregate. Z. Kristallogr. 1995, 210 (5), 315–318.
- Jacob, M.; Andersson, S. *The Nature of Mathematics and the Mathematics of Nature*; Elsevier: Amsterdam, 1998.
- Bellare, J.R.; Davis, H.T.; Scriven, L.E.; Talmon, Y.J. Controlled environment vitrification system: An improved sample preparation technique. Electron Microsc. Technol. **1988**, *10*, 87–98.
- Almgren, M.; Edwards, K.; Gustafsson, J. Cryotransmission electron microscopy of thin vitrified samples. Curr. Opin. Colloid Interface Sci. 1996, 1 (2), 270–278.
- 31. Gulik-Krzywicki, T.; Delacroix, H. Combined use of freeze–fracture electron microscopy and x-ray diffraction for the structure determination of threedimensionally ordered specimens. Biol. Cell **1994**, *80*, 193–201.

- 32. Gulik-Krzywicki, T.; Aggerbeck, L.P.; Larsson, K. The Use of Freeze–Fracture and Freeze–Etching Electron Microscopy for Phase Analysis and Structure Determination of Lipid Systems. In *Surfactants in Solution*; Mittal, K., Lindman, B., Eds.; Plenum Press: New York, 1984; 237–257.
- Neto, C.; Aloisi, G.; Baglioni, P.; Larsson, K. Imaging soft matter with the atomic force microscope: Cubosomes and hexosomes. J. Phys. Chem., B 1999, 103 (19), 3896–3899.
- Gustafsson, J.; Ljusberg-Wahren, H.; Almgren, M.; Larsson, K. Cubic lipid–water phase dispersed into submicron particles. Langmuir **1996**, *12* (20), 4611–4613.
- 35. Gustafsson, J.; Ljusberg-Wahren, H.; Almgren, M.; Larsson, K. Submicron particles of reversed lipid phases in water stabilized by a nonionic amphiphilic polymer. Langmuir **1997**, *13*, 6964–6971.
- Spicer, P.T.; Hayden, K.L.; Lynch, M.L.; Ofori-Boateng, A.; Burns, J.L. Novel process for producing cubic liquid crystalline nanoparticles (cubosomes). Langmuir 2001, 17 (19), 5748–5756.
- Ljusberg-Wahren, H.; Nyberg, L.; Larsson, K. Dispersion of the cubic liquid crystalline phase— Structure, preparation, and functionality aspects. Chim. Oggi **1996**, *14* (6), 40–43.
- Radiman, S.; Toprakcioglu, C.; Mcleish, T. Rheological study of ternary cubic phases. Langmuir 1994, 10, 61–67.
- Jones, J.L.; Mcleish, T.C.B. Concentration fluctuations in surfactant cubic phases: Theory, rheology, and light scattering. Langmuir 1999, 15 (22), 7495– 7503.
- Warr, G.G.; Chen, C.-M. Steady Shear Behavior of Ternary Bicontinuous Cubic Phases. In *Structure* and Flow in Surfactant Solutions; Herb, C.A., Prud'homme, R.K., Eds.; American Chemical Society: Washington, 1994; Vol. 578, 306–319.
- Siekmann, B.; Bunjes, H.; Koch, M.H.J.; Westesen, K. Preparation and structural investigations of colloidal dispersions prepared from cubic monoglyceride-water phases. Int. J. Pharm. 2002, 244 (1– 2), 33–43.
- Squires, A.M.; Templer, R.H.; Seddon, J.M.; Woenckhaus, J.; Winter, R.; Finet, S.; Theyencheri, N. Kinetics and mechanism of the lamellar to gyroid inverse bicontinuous cubic phase transition. Langmuir **2002**, *18* (20), 7384–7392.
- 43. Pisani, M.; Bernstorff, S.; Mariani, P. Pressure induced cubic-to-cubic phase transition in monoolein hydrated system. J. Phys. Chem., B **2001**, *105* (15), 3109–3119.
- 44. Spicer, P.T. Cubosome Formation via Dilution— Kinetic Effects and Consumer Product Implications.

In *Mesoscale Phenomena in Liquid Systems*; Case, F., Alexandridis, P., Eds.; ACS:, 2003.

- 45. Spicer, P.T.; Small, W.B.; Lynch, M.L.; Burns, J.L. Dry powder precursors of "soft" cubic liquid crystalline nanoparticles (cubosomes). J. Nanopart. Res. **2002**, *4* (4), 297–311.
- Engström, S.; Alfons, K.; Rasmusson, M.; Ljusberg-Wahren, H. Solvent-induced sponge (L₃) phases in the solvent-monoolein-water system. Prog. Colloid Polym. Sci. **1998**, *108*, 93–98.
- 47. Ribier, A.; Biatry, B. Oily Phase in an Aqueous Phase Dispersion Stabilized by Cubic Gel Particles and Method of Making. US Patent 5,756,108.
- 48. Biatry, B. Cosmetic and Dermatological Emulsion Comprising Oily and Aqueous Phase. Eur. Pat. Appl. 968,704.
- 49. Biatry, B. Use of Phytantriol as Anti-Pollution Agent in a Cosmetic Composition. Eur. Pat. Appl. 1,161,938.
- 50. Lynch, M.L.; Kochvar, K.A.; Burns, J.L.; Laughlin, R.G. Aqueous phase behavior and cubic phasecontaining emulsions in the $C_{12}E_2$ -water system. Langmuir **2000**, *16* (7), 3537–3542.
- Hato, M.; Minamikawa, H.; Tamada, K.; Baba, T.; Tanabe, Y. Self-assembly of synthetic glycolipid/ water systems. Adv. Colloid Interface Sci. 1999, 80 (3), 233–270.
- 52. Attard, G.S.; Blackaby, W.P.; Leach, A.R. The aggregation behaviour of two structurally isomeric glycolipids. Chem. Phys. Lipids **1994**, *74*, 83–91.
- 53. Shah, J.C.; Sadhale, Y.; Chilukuri, D.M. Cubic phase gels as drug delivery systems. Adv. Drug Deliv. Rev. **2001**, *47*, 229–250.
- Drummond, C.J.; Fong, C. Surfactant self-assembly objects as novel drug delivery vehicles. Curr. Opin. Colloid Interface Sci. 2000, 4, 449–456.
- Borne, J.; Nylander, T.; Khan, A. Effect of lipase on monoolein-based cubic phase dispersion (cubosomes) and vesicles. J. Phys. Chem., B 2002, 106 (40), 10492–10500.
- Geraghty, P.B.; Attwood, D.; Collett, J.H.; Sharma, H.; Dandiker, Y. An investigation of the parameters influencing the bioadhesive properties of Myverol 18-99/water gels. Biomaterials 1997, 18, 63-67.
- Lee, J.; Kellaway, I.W. Combined effect of oleic acid and polyethylene glycol 200 on buccal permeation of [D-Ala₂, D-Leu₅]enkephalin from a cubic phase of glyceryl monooleate. Int. J. Pharm. 2000, 204, 137–144.
- Norlen, L. Skin barrier formation: The membrane folding model. J. Invest. Dermatol. 2001, 117, 823– 829.
- 59. Norlen, L. Skin barrier structure and function: The

single gel phase model. J. Invest. Dermatol. 2001, 117, 830-836.

- Anderson, D.M.; Wennerström, H. Self-diffusion in bicontinuous cubic phases, L₃ phases, and microemulsions. J. Phys. Chem. **1990**, *94*, 8683–8694.
- Mattisson, C.; Nylander, T.; Axelsson, A.; Zacchi, G. Diffusivity measurements using holographic laser interferometry in a cubic lipid–water phase. Chem. Phys. Lipids **1996**, *84*, 1–12.
- 62. Norling, T.; Lading, P.; Engstrom, S.; Larsson, K.; Krog, N.; Nissen, S.S. Drug delivery system based on a mixture of monoglycerides and triglycerides for use in treatment of periodontal disease. Proc. Int. Symp. Control. Release Bioact. Mater. **1997**, *23*, 294–295.
- Boyd, B. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. Int. J. Pharm. 2003, 260, 239–247.
- Nakano, M.; Sugita, A.; Matsuoka, H.; Handa, T. Small-angle x-ray scattering and ¹³C NMR investigation on the internal structure of "cubosomes." Langmuir **2001**, *17*, 3917–3922.
- Nakano, M.; Teshigawara, T.; Sugita, A.; Leesajakul, W.; Taniguchi, A.; Kamo, T.; Matsuoka, H.; Handa, T. Dispersions of liquid crystalline phases of the monoolein/oleic acid/pluronic F127 system. Langmuir **2002**, *18* (24), 9283–9288.
- Jeong, S.W.; O'Brien, D.F.; Oraedd, G.; Lindblom, G. Encapsulation and diffusion of water-soluble dendrimers in a bicontinuous cubic phase. Langmuir 2002, 18 (4), 1073–1076.
- Puvvada, S.; Qadri, S.B.; Naciri, J.; Ratna, B. Ionotropic gelation in a bicontinuous cubic phase. J. Phys. Chem. **1993**, *97*, 11103–11107.
- Lindell, K.; Engblom, J.; Jonstromer, M.; Carlsson, A.; Engstrom, S. Influence of a charged phospholipid on the release pattern of timolol maleate from cubic liquid crystalline phases. Prog. Colloid Polym. Sci. **1998**, *108*, 111–118.

- Lynch, M.L.; Ofori-Boateng, A.; Hippe, A.; Kochvar, K.; Spicer, P.T. Enhanced loading of water-soluble actives into bicontinuous cubic phase liquid crystals using cationic surfactants. J. Colloid Interface Sci. 2003, 260, 404–413.
- Kresge, C.T.; Leonowicz, M.E.; Roth, W.J.; Vartuli, J.C.; Beck, J.S. Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. Nature **1992**, *359* (6397), 710–712.
- Yang, D.; O'Brien, D.F.; Marder, S.R. Polymerized bicontinuous cubic nanoparticles (cubosomes) from a reactive monoacylglycerol. J. Am. Chem. Soc. 2002, 124 (45), 13388–13389.
- 72. Yang, J.; Ratna, B. Formation of Nanocrystalline Semiconductor Particles Within a Bicontinuous Cubic Phase. US Patent Appl. 841957.
- Lu, Y.; Fan, H.; Stump, A.; Ward, T.L.; Rieker, T.; Brinker, C.J. Aerosol-assisted self-assembly of mesostructured spherical nanoparticles. Nature 1999, 398 (6724), 223–226.
- Larson, R. The Structure and Rheology of Complex Fluids; Oxford University Press: New York, 1999; 590–610.
- Pieranski, P.; Sittler, L.; Sottaa, P.; Imperor-Clerc, M. Growth and shapes of a cubic lyotropic liquid crystal. Eur. Phys. J., E 2001, 5, 317–328.
- Gustafsson, J.; Nylander, T.; Almgren, M.; Ljusberg-Wahren, H. Phase behavior and aggregate structure in aqueous mixtures of sodium cholate and glycerol monooleate. J. Colloid Interface Sci. 1999, 211, 326–335.
- 77. Hyde, S.T. Interfacial architecture in surfactantwater mixtures: Beyond spheres, cylinders and planes. Pure Appl. Chem. **1992**, *64* (11), 1617– 1622.
- 78. Hyde, S.T. Microstructure of bicontinuous surfactant aggregates. J. Phys. Chem. **1989**, *93*, 1458– 1464.

MARCEL DEKKER, INC

270 Madison Avenue, New York, New York 10016