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Dry powder precursors of cubic liquid crystalline nanoparticles (cubosomes*)

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Abstract

Cubosomes are dispersed nanostructured particles of cubic phase liquid crystal that have stimulated significant research interest because of their potential for application in controlled-release and drug delivery. Despite the interest, cubosomes can be difficult to fabricate and stabilize with current methods. Most of the current work is limited to liquid phase processes involving high shear dispersion of bulk cubic liquid crystalline material into sub-micron particles, limiting application flexibility. In this work, two types of dry powder cubosome precursors are produced by spray-drying: (1) starch-encapsulated monoolein is produced by spray-drying a dispersion of cubic liquid crystalline particles in an aqueous starch solution and (2) dextran-encapsulated monoolein is produced by spray-drying an emulsion formed by the ethanol–dextran–monoolein–water system. The encapsulants are used to decrease powder cohesion during drying and to act as a soluble colloidal stabilizer upon hydration of the powders. Both powders are shown to form (on average) 0.6 μm colloidally-stable cubosomes upon addition to water. However, the starch powders have a broader particle size distribution than the dextran powders because of the relative ease of spraying emulsions *versus* dispersions. The developed processes enable the production of nanostructured cubosomes by end-users rather than just specialized researchers and allow tailoring of the surface state of the cubosomes for broader application.

Introduction

Aqueous surfactant systems can self-assemble into thermodynamically stable bicontinuous cubic liquid crystalline phases (Fontell et al., 1968; Luzzati, 1968). One of the most studied binary systems, monoolein–water, forms two types (diamond and gyroid; Larsson, 1983) of an optically clear, solid-like (Jones & McCleish, 1999) bicontinuous cubic phase at water contents between 20% and 40% (w/w) at room temperature (Lutton, 1965; Hyde et al., 1984; Laughlin, 1994; Briggs et al., 1996). Such phases are composed of porous matrices (pores $\sim$ 5 nm) built from bilayers contorted into infinite periodic minimal surfaces (Hyde et al., 1984; Mackay, 1985). Figure 1 shows calculated representations of a sub-unit of the 'diamond' bicontinuous cubic phase geometry, the combination of several sub-units to form a finite cubosome particle, and the same cubosome 'closed off' by a cube surface. The shapes in Figure 1 are calculated using nodal surface approximations of the periodic minimal surface (von Schnering & Nesper, 1991; Andersson et al., 1995; Jacob & Andersson, 1998) and are useful for visualizing the bicontinuous structure of the cubic phases. The term 'bicontinuous' refers to the separation of two distinct (continuous but non-intersecting) hydrophilic regions by the bilayer (Scriven, 1976). A variety of surfactants and other amphiphilic molecules also form bicontinuous cubic liquid crystalline phases.

*The word 'cubosome' is now a USPTO registered trademark of GS Development AB Corp., Sweden.

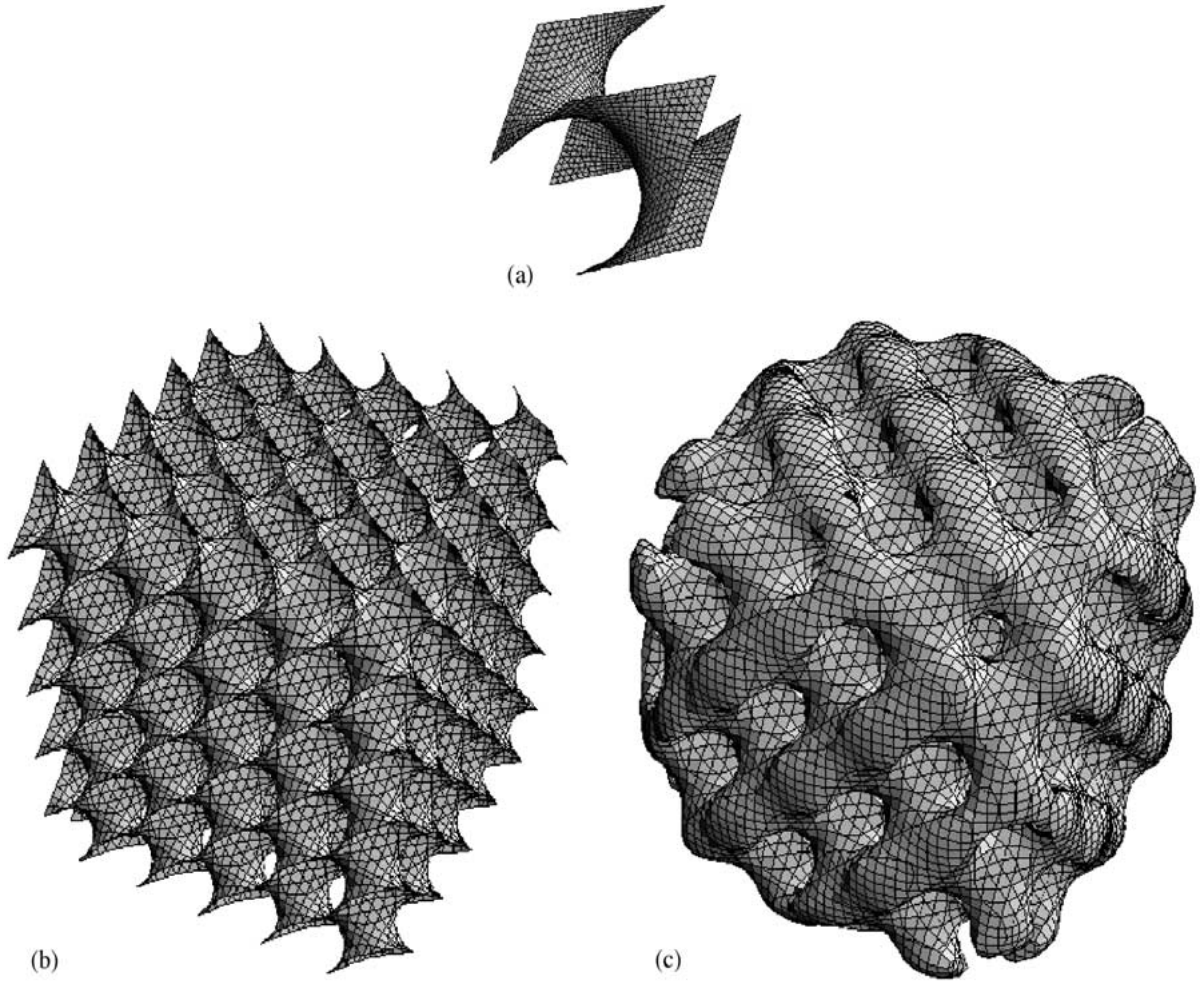

Figure 1. Calculated representation of the hierarchy of a cubosome, starting with the double diamond sub-unit (a), the lattice structure of a cubosome (b), and the lattice surface sealed off with a cube (c). In each case, the calculated surface represents a lipid bilayer that separates two continuous but non-intersecting aqueous (hydrophilic) regions.

The self-assembly of amphiphilic molecules to form bicontinuous cubic liquid crystalline materials is an active research topic (Hyde et al., 1997), especially in the areas of controlled-release (Engström et al., 1992) and drug delivery (Drummond & Fong, 2000). The bicontinuous water and oil channels allow for simultaneous incorporation of water- and oil-soluble actives as well as other amphiphiles. The pore structure provides a tortuous diffusion pathway for controlled-release (Anderson & Wennerström, 1990; Engström et al., 1992) and cubic phase liquid crystals are biocompatible, digestible (Patton & Carey, 1979; Lindström et al., 1981), and bioadhesive (Nielsen et al., 1998).

Three macroscopic forms of cubic phase are typically treated in the scientific and patent literature: precursor, bulk, and particulate (i.e., cubosomes). Precursor materials are usually liquid and form cubic phase only in response to some stimulus, like dilution (Czarnecki & Williams, 1993; Spicer et al., 2001). Bulk forms of cubic phase are viscous liquid crystalline materials, usually hydrated monoolein, often with a drug incorporated in their structure (Landh & Larsson, 1993). Cubosome particles have the advantage of increased surface area and aqueous cubosome dispersions have much lower viscosity than bulk cubic gel. When cubosomes are produced, it is typically by high-energy dispersion of bulk

cubic gel (Gustafsson et al., 1996; 1997), followed by colloidal stabilization using polymeric stabilizers (Landh, 1994). After formation of the cubosomes, the dispersion is applied to some target environment (i.e., body tissue) and active ingredient diffuses out in a controlled-release fashion.

In spite of active research into cubic phase materials, there is little published work on manufacture and processing of nanometer- and micron-scale cubosome particles. An exception is the development of a process spontaneously forming cubosomes via dilution of the monoolein–ethanol–water system (Spicer et al., 2001), avoiding the more common high-energy dispersion of bulk cubic gel (Ljusberg-Wahren et al., 1996). The hydrotrope process allows the creation of concentrated liquid precursors that spontaneously produce cubosomes upon dilution. Such a process could be applied to applications requiring formation of cubosomes *in situ*. However, for many applications it is desirable to work with dry powder or granular materials rather than with liquid phase products. Powders that spontaneously form cubosomes upon hydration avoid the need for transport and processing of bulk water and access a wider range of applications (e.g., drug delivery via inhalation).

Kim et al. (2000) attempted to create powdered cubosome precursors using freeze-drying, a more expensive process than spray-drying. No cryo-TEM or X-ray scattering data (necessary to verify cubosome formation) were presented, however, so it is unclear whether Kim et al. (2000) produced cubosomes. Powdered precursors of drug-containing vesicles have been prepared by freeze-drying (Szoka et al., 1998) for delivery of DNA and proteins by inhalation. However, vesicles are spherical, lamellar liquid crystalline shells that are more shear-sensitive and have less bilayer area per volume than cubosomes and are thus less robust in some applications. Anderson (1999) applied for patent protection of cubic liquid crystalline materials coated by metals as controlled delivery and uptake devices, but these require the presence of fully hydrated cubic phase at their cores. Finally, solid lipid nanoparticle powders have been formed via spray-drying (Freitas & Müller, 1998), but are comprised of lipids like triglycerides that do not typically form liquid crystals.

This work describes the production, via spray-drying, of dry powder precursors that can incorporate active ingredients and spontaneously form colloidally-stabilized cubosomes upon hydration. Because simple freeze- or spray-drying of molten monoolein (a waxy solid at room temperature) produces sticky agglomerates and not discrete, flowable powders, the monoolein is combined with an aqueous starch solution to form (upon spray-drying) monoolein encapsulated in a dry starch shell. The starch-encapsulated monoolein is then easily hydrated but in dry form remains powdered and non-cohesive. Although this technique is practical, monoolein combined with an aqueous starch solution can be difficult to spray-dry continuously because cubic phase forms immediately upon monoolein hydration. An additional dispersion step is required for efficient spray-drying. For this reason, a second process is also developed by applying the hydrotrope method of Spicer et al. (2001) to allow easy spray-drying of the solution. Ethanol is used here to efficiently dissolve the cubic liquid crystalline phase. A substitution of dextran for starch (as film-former) is also required as starch is insoluble in ethanol. Upon ethanol addition to the monoolein–dextran–water system, a low-viscosity emulsion forms that is easily spray-dried to remove water and ethanol. The dry particles produced by both techniques are, on (volume-weighted) average, 24 μm in diameter and when hydrated form, on average, 0.6 μm cubosomes that are colloidally-stabilized by the surface coating of hydrated polymer.

Experiment

Materials

Monoolein (Figure 2) is obtained from Danisco A/S (Dimodan MO90K, Denmark) and determined by gas chromatography to contain 90% (w/w) monoglyceride. There are minor fractions of diglycerides, triglycerides and a monostearin glycol present, with free fatty acids present at a level of 0.5% (w/w). Two different starches (Figure 2) hydrophobically-modified with octenyl succinate groups (HI-CAP 100 and CAPSUL-E, National Starch and Chemical) are used to encapsulate the monoolein during spray-drying. The average degree of substitution of the modified starches is 0.02, meaning one octenyl succinate group is present for every 50 anhydroglucose units. The starches are industrial grade with an average molecular weight of 335,000 (HI-CAP 100) and 84,000 g/mol (CAPSUL-E) and contain minor amounts of corn sugar and maltodextrin solids as well. Dextran, produced by *Leuconostoc mesenteroides* Strain No. B-512, is purchased from Sigma Chemical and has a reported average molecular weight of ∼35,700 g/mol. The dextran used in

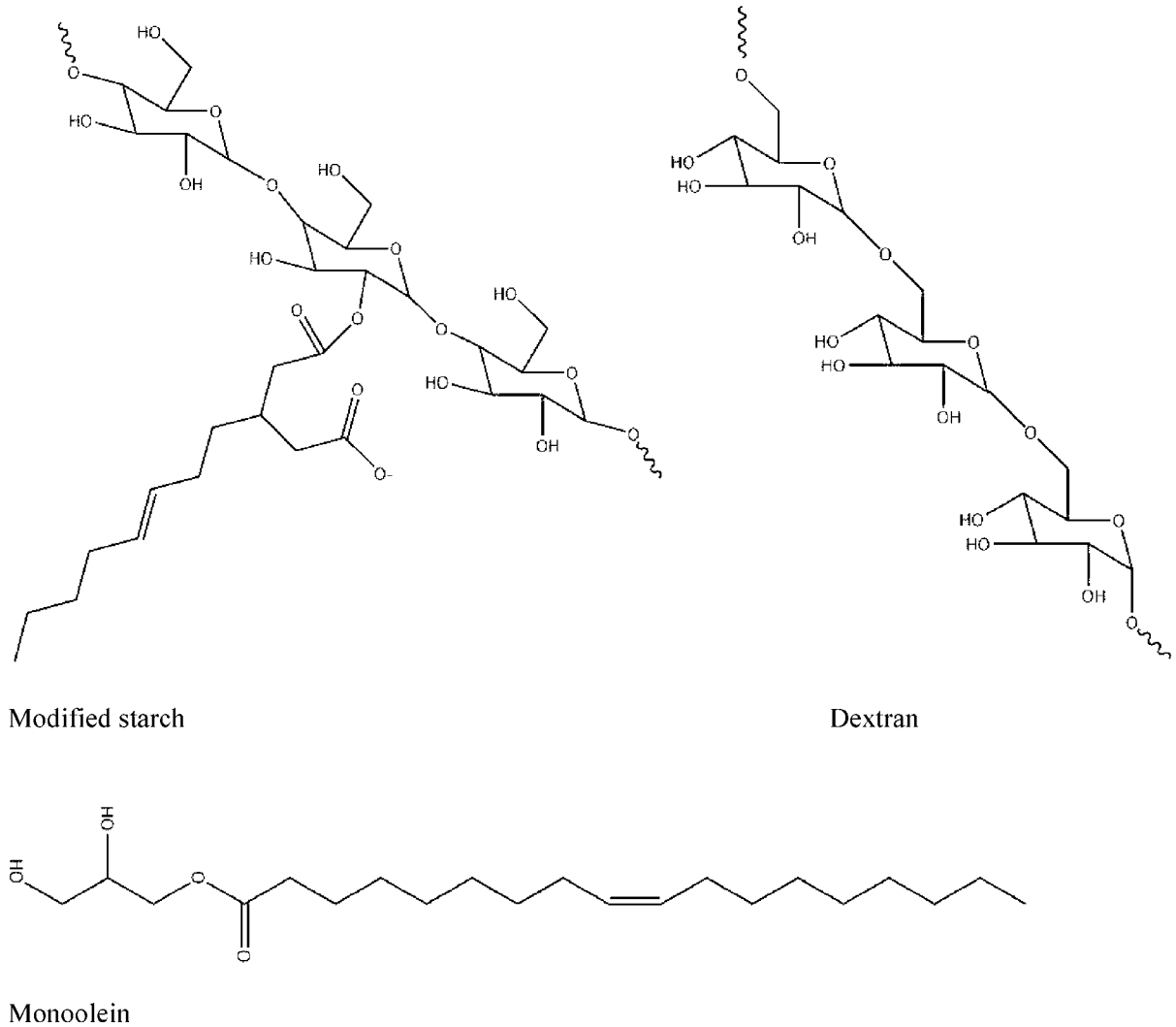

Figure 2. Diagram of the structure of the hydrophobically-modified starch film-former, dextran film-former, and the monoolein that is encapsulated during spray-drying.

all experiments contains branching units of different length (fewer than 100 monomers per branch) distributed randomly along the 1,6-α-linked main backbone (Lapasin & Pricl, 1995). Dextran solutions are used immediately with ethanol present to avoid microbial growth. All water is de-ionized using Millipore Water systems.

Spray-drying

Spray-drying experiments are performed using a Pulvis Basic Unit (Yamato International, Michigan, USA) consisting of a cylindrical chamber with a cyclone collector at the air exit (Figure 3). A twin-fluid nozzle with a liquid orifice size of 0.1 cm is used to feed the liquid to be spray-dried into the top of the spray-dryer body. Air at a pressure of 300 kPa is pumped through a 0.25-cm annular air orifice. Drying occurs via contact of the feed liquid with heated, drying air at 200°C that flows down past the nozzle. The short residence time of the spray-drier should prevent any oxidation of the monoolein at the elevated temperatures. When cubic liquid crystalline material forms in the feed liquid, as in the case of the monoolein–starch–water system, high shear is applied to disperse the high-viscosity

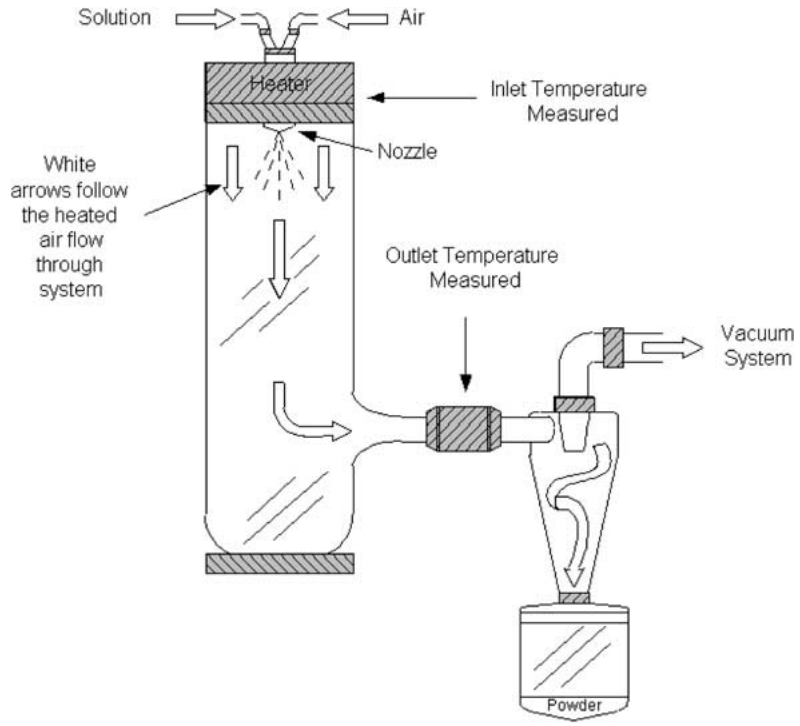

Figure 3. Schematic of the spray-dryer apparatus used to produce starch–monoolein and dextran–monoolein powders.

cubic gel. The resulting dispersion is pumped through the liquid side of the twin-fluid atomizer at a rate of 15 ml/min, with slight adjustments being made to keep the temperature of the exit air in the system at 90–95°C. The dextran–monoolein–ethanol–water system is spray-dried under similar conditions but at a lower flow rate (4 ml/min) and higher exit air temperature (130°C) to promote evaporation of most of the ethanol with the water.

Scanning electron microscopy (SEM)

Samples are prepared by mounting the powders to silver studs with carbon conductive double-sided tape. The samples are coated with a gold film under vacuum for 2 min. The specimens are transferred to an ISI ABT SX-40A scanning electron microscope and digital images captured.

Cryo-transmission electron microscopy (Cryo-TEM)

Samples are prepared in a controlled environment vitrification system (CEVS) as described by Bellare et al. (1988). In particular, for this work, a 3-μl drop of solution is placed on a carbon-coated porous-polymer support film mounted on a standard 300-mesh TEM grid (Ted Pella, Inc.; Redding, CA, USA). The drop is blotted with filter paper until it is reduced to a thin film (10–200 nm) spanning the holes (2–8 μm) of the support film. The sample is then vitrified by rapidly plunging it through a synchronous shutter at the bottom of the CEVS into liquid ethane at its freezing point. The vitreous specimen is transferred under liquid nitrogen into a Philips CM120 transmission electron microscope for imaging. The temperature of the sample is kept under −170°C throughout the examination. Vitrification of aqueous liquid crystalline samples allows the observation of hydrated structures without the interference of ice crystal formation.

Small-angle X-ray scattering (SAXS)

SAXS is performed on samples with CuKα radiation ($\lambda = 0.154$ nm) generated with a Rigaku RU-300 rotating anode. The generator is operated at 40 kV and 40 mA with a 0.2 × 0.2 mm focal size (a 0.2 × 2-mm filament run in point mode). The patterns are collected

with the Siemens two-dimensional small angle scattering system which consists of the HI-STAR wire detector and Anton Parr HR-PHK collimation system. Collimation is achieved with a single 100-mm diameter pinhole 490 mm from the focal spot. The size of the focal spot restricts beam divergence. A 300-mm guard pinhole is placed 650 mm from the focal spot, just in front of the sample. The detector is placed a distance of 650 mm from the sample. Ni filters were used to eliminate the Kβ radiation. Because of the small beam size and large sample-to-detector distance, two-dimensional profiles can be obtained with a minimum of instrumental smearing, so no smearing corrections are employed.

The effect of adding starch to the cubic liquid crystalline phase is determined using SAXS patterns to confirm the structure. For the levels of monoolein and water used, the liquid crystal is determined to be in I_{a3d} symmetry. The symmetry provides six strong reflections assigned to the following Miller indices: [211], [220], [321], [400], [420], and [332]. For cubic phase symmetry, a plot of peak position *versus* $(h^2+k^2+l^2)^{1/2}$ generates a straight line with a slope inversely proportional to the lattice parameter (Ivanova et al., 2000). For all determinations, the linear least-squares correlation coefficient is found to be greater than 0.9999, indicating excellent confirmation of the assigned cubic phase symmetry.

Particle size distribution measurement

Determination of the particle size distribution of the cubosome dispersions is carried out using laser diffraction (Horiba LA-910) to characterize both the spray-dried powders and the aqueous dispersions formed upon hydration of the powders. Diffraction analysis is performed on cubosome dispersions in a re-circulation loop with the lowest ultrasonic setting applied. Dry powders are analyzed using dispersion in a non-solvent (i.e., starch powders in isopropyl alcohol) or the dry powder feeder attachment of the Horiba that allows powder feeding by vibration and air suction to allow the powders to fall through the laser light beam (in the case of the dextran–monoolein powders). The relative refractive index used to size the hydrated powders (i.e., cubosomes) is 1.02 based on literature values for water ($n \sim 1.33$) and monoglyceride ($n \sim 1.36$) as well as agreement with microscopy data. Starch powder dispersions in isopropyl alcohol are sized using a relative refractive index of 1.16.

Dispersion preparation

The starch–monoolein–water system is sheared to produce cubosome dispersions using an Ika Ultra-Turrax T-50 rotor stator mill at 13,500 rpm. The monoolein is first melted and then added to a solution of starch that has been prepared previously, then high shear is applied to disperse the cubic phase that forms upon hydration.

Polarized light microscopy

Microscopic observations of dispersions and emulsions are recorded using a Zeiss Axioscop microscope with a Nikon Coolpix 995 digital camera attached.

Moisture determination

The amount of moisture and ethanol remaining following spray-drying is determined using a combination of Karl Fischer titration and thermal gravimetric analysis (Hi-Res TGA 2950, TA Instruments).

Results and discussion

The objective of this work is the creation of dry powders that form cubosomes upon addition to water. Because monoolein is a waxy solid, an encapsulation ingredient is used to prevent powder cohesion, as would occur for monoolein by itself. Hydrophobically-modified starch is chosen to encapsulate the monoolein because of its cold-water solubility, emulsifying behavior, encapsulation properties, and approval for food use (Trubiano, 1986). Starches are known to form inclusion compounds with monoglycerides via incorporation of the hydrophobic tail of the lipid into the helical hydrophobic region of the starch (Eliasson, 1993). The modified starch used here, however, is not expected to form such complexes because of interference by the functional groups. Experimental equilibration of fully hydrated starch with monoolein confirms the lack of complex formation. Unless otherwise specified, results given are for the low molecular weight starch.

Starch–monoolein–water phase behavior

The starch–monoolein–water system can be characterized using a pseudo-ternary (both the starch and monoolein are not pure) equilibrium phase

diagram. The phase diagram allows planning and communication of the spray-drying and hydration processes as well as demonstration of starch effects on the system phase behavior. The phase behavior of this system is especially important given the desirability of the cubic liquid crystalline phase for controlled-release. Figure 4 shows the phase diagram determined for the starch–monoolein–water system on a weight basis. Phases are classified by appearance, rheology, optical properties (e.g., optical birefringence), and literature references. All characterization is carried out at 25°C unless otherwise noted. Certain areas of the phase diagram were studied in more detail than others: all three binary systems, the upper tolerance of starch addition by the cubic phases (i.e., the lower third of the triangle), and the region of cubic liquid crystalline particle formation (i.e., the tie-lines between L_1 phase and cubic phase). Each side of the triangle corresponds to the respective binary system. The system exhibits four single-phase regions: L_1 (isotropic liquid), $V_2^{(1)} - P_{n3m}$ (Q^{224}, P_{n3m} diamond bicontinuous cubic liquid crystalline), $V_2^{(2)} - I_{a3d}$ (Q^{230}, I_{a3d} gyroid bicontinuous cubic liquid crystalline), and D (lamellar liquid crystalline), in agreement with literature on the monoolein–water phase diagram (Lutton, 1965; Hyde et al., 1984; Laughlin, 1994; Briggs et al., 1996; Qiu & Caffrey, 2000). The monoolein–starch system is all two-phase while the starch–water system reflects the starch solubility limit of 40% (w/w). Cubosomes form in the miscibility gap between the $V_2^{(1)}$ region and the starch–water (L_1) solution, indicated by the dotted tie-lines in Figure 4, as cubosomes are merely cubic liquid crystalline material in equilibrium with L_1. In Figure 4, at a constant water-to-lipid ratio, the $V_2^{(1)}$ phase can tolerate only about 5% (w/w) starch before the single phase region ends and dehydrated starch crystallites are present. Superimposed on the $V_2^{(2)}$ phase region in Figure 4 are lattice parameter values determined by SAXS for various levels of starch in cubic gel. Above 5% (w/w), the starch formed crystalline precipitates in the gel and measurements were performed on the gel only. It is clear from Figure 4 that the gyroid lattice parameter decreases with increasing starch levels, probably as a result of dehydration of the cubic gel by the starch. The dehydration observed for bulk cubic gel is not expected to affect the formation of cubosomes, as cubosomes form in the region of excess water where dehydration is not a concern.

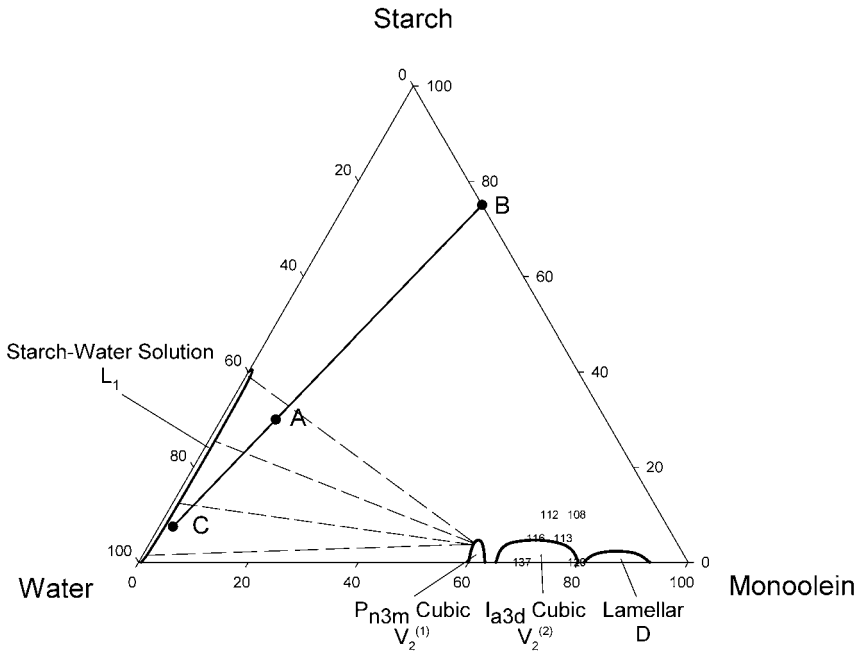

Figure 4. Pseudo-ternary phase diagram of the starch–monoolein–water system. In addition to four single phase regions, the diagram shows the lattice parameter (in Angstroms) of the gyroid cubic unit cell as a function of starch addition. Also shown is the process trajectory for production of starch–monoolein powders and hydrated cubosomes.

Starch–monoolein powder production

The phase diagram in Figure 4 is also useful to communicate the process used to create powdered cubosome precursors via spray-drying and their hydration to form cubosomes. The initial composition pumped into the spray-drier (60% w/w water, 30% starch, and 10% monoolein) is indicated in Figure 4 by point A. Drying represents removal of water, a straight trajectory along a line connecting the water apex and the beginning composition (i.e., point A). Upon complete drying, the powder composition is indicated by point B in Figure 4. Finally, hydration of the powders by addition to water results in another trip along the process trajectory in Figure 4 to point C (2.5% (w/w) monoolein, 7.5% starch, and 90% water), where tielines indicate the presence of about 5% (w/w) cubosomes. As seen in Figure 4, cubic phase gel is present in equilibrium with aqueous starch solution at composition A, requiring dispersion into coarse cubosomes prior to spray-drying. Figure 5 shows microscopic views of the dispersion (formed by high shear treatment of the cubic gel–aqueous starch system) of coarse ($\sim$1 μm) cubosome particles to be spray-dried. The hydrophobically-modified starch in this case acts as a colloidal stabilizer, preventing dense agglomeration of the cubosomes. Assuming total removal of the added water during spray-drying, the resultant powder contains 25% (w/w) monoolein and 75% starch (point B in Figure 4), but thermal gravimetric measurements indicate a final moisture content of 5% (w/w).

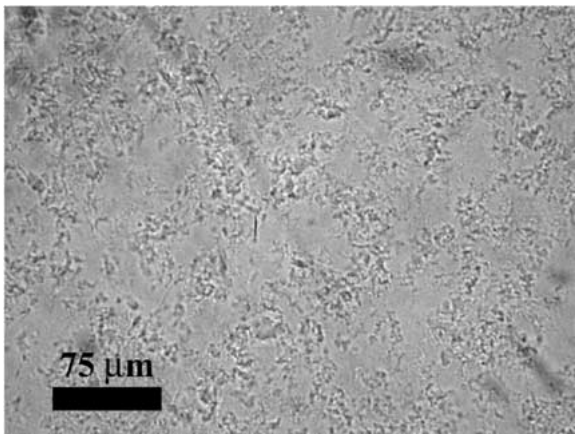

Figure 5. Dispersion of cubic liquid crystalline particles in an aqueous starch solution environment prior to spray-drying. The dispersion is viscous but able to be pumped through the spray-drier.

The presence of cubic gel in the dispersion to be spray-dried can affect performance during large-scale processing. Drying occurs as the dispersion is sprayed into droplets and moisture rapidly evaporates by convective heating. The cubic gel particles in the dispersion form the nucleus of many of the sprayed droplets, surrounded by aqueous starch solution. As drying occurs, the starch forms a coating on the cubic gel particle, encapsulating it. Because the cubic gel itself contains 40% (w/w) water, some drying must also occur at the core of the particles and will affect the final powder properties. Two different starches with high(335,000) and low(84,000) molecular weight are used to produce dry powder cubosome precursors. In a typical bench-scale experiment, when the high molecular weight starch is used, upon shut down the dryer walls are coated with a significant layer of powder. The deposited wall layer accounts for about half of the possible solids recovered. The large amount of powder deposition is likely the result of powder tackiness caused by incomplete encapsulation. When the low molecular weight starch is used to encapsulate the monoolein, powder recovery is high (78% w/w) and little deposition occurs. The deposition efficiency's dependence on starch molecular weight is related to the elasticity of the aqueous starch film formed during drying. Films formed by the higher molecular weight starch are expected to be more rigid and less efficient at encapsulation, allowing residual moisture to stick the powders to the dryer wall.

The powder structures are decidedly different depending on the type of polymer used as an encapsulant. Figure 6 shows a representative SEM image of spray-dried monoolein–starch particles produced by the above method using the high molecular weight (335,000) starch. The particles in Figure 6 have a multiple-mode primary particle size distribution of larger (20 μm) and smaller (micron-scale) capsules. In Figure 6, the starch has formed numerous shells, as evidenced by the particle resembling a bowling ball (the holes formed as evaporating liquids escaped). The high molecular weight starch forms a film too rigid to allow rapid gas escape and shrinkage to form perfect capsules, supporting the above explanation for the increased powder deposition when high molecular weight starch is used. The powders in Figure 6 appear similar in size to the cubic gel particles in Figure 5. Some shearing occurs during spraying as the dispersion passes through the nozzle, but the average particle size is not expected to significantly reduce. When a lower molecular weight (84,000) starch is used to form dry powder cubosome precursors, the

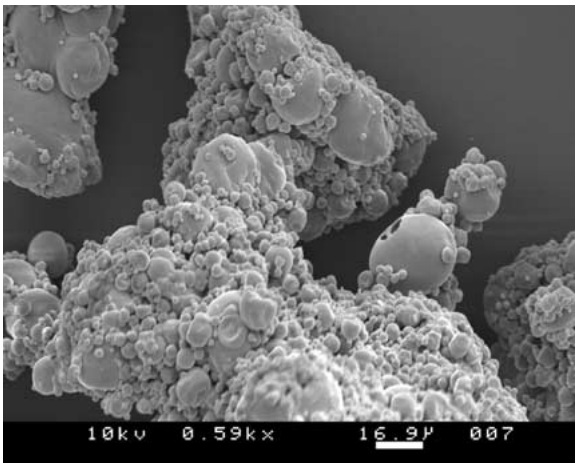

Figure 6. SEM photograph of starch–monoolein powders produced by spray-drying of cubic liquid crystalline particles in a high molecular weight starch solution. The particles are composed of large and small starch-encapsulated monoolein pieces that form cubosomes upon hydration. The rigid capsules formed are indicative of a stiffer, higher molecular weight starch film-former.

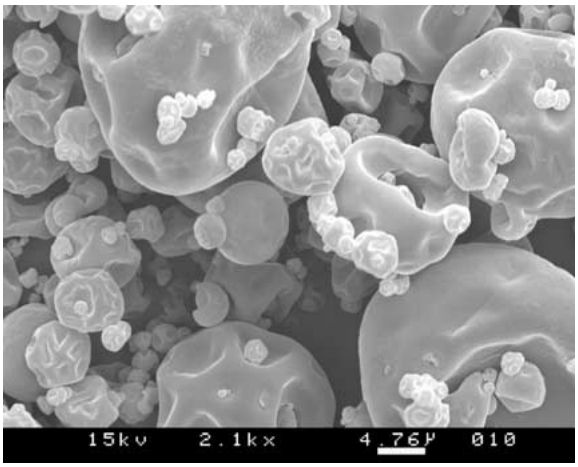

Figure 7. SEM photograph of starch–monoolein powders produced using low molecular weight starch film-former. In contrast to the powders in Figure 6, the powders produced using the lower molecular weight starch display the classic dimpling observed in spray-dried powders produced with a more elastic film-former.

resultant powders (Figure 7) are significantly different from those in Figure 6. In Figure 7, the powders exhibit the classical shrinkage of a film-forming polymer permeable to evaporating gases but sufficiently elastic to also provide encapsulation. Judging from the appearance of the powders in Figure 6 and especially Figure 7, the monoolein is encapsulated best when low molecular weight starch is used. A uniform

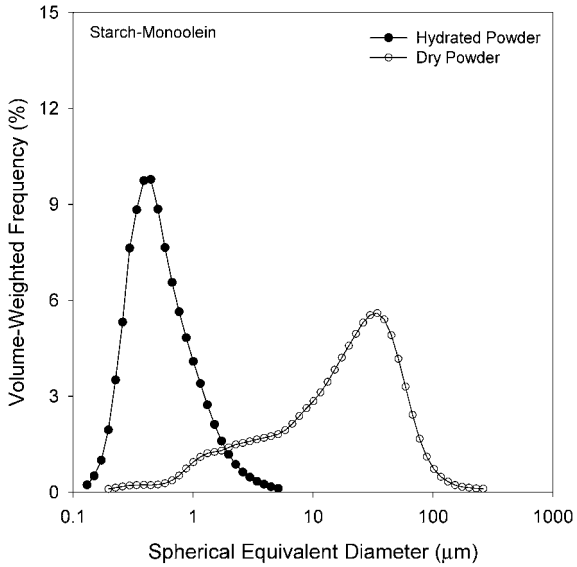

Figure 8. Particle size distribution of the dry and hydrated monoolein–water–starch powder as measured by laser diffraction. Three distinct modes are apparent for the dry powder. When hydrated, the starch dissolves quickly and the remaining particles are small monoolein–starch particles that hydrate to form cubosomes.

starch coating allows formation of stable cubosome dispersions. Upon addition of the powders to water, the starch shell dissolves to form a stabilizing surface coating on the monoolein, which then hydrates to form a cubosome. Quantitative volume-weighted particle size distributions of the starch–monoolein dry powder and dispersions are shown in Figure 8. The powder size distribution in Figure 8 has three distinct modes covering a range from 1 to about 200 µm. The two larger modes are representative of the particles formed by pre-existing cubic gel particles that were spray-dried, while the smallest mode represents fragments of dried cubosomes and empty starch shells. The process above demonstrates the feasibility of forming dry powders with the potential to form cubosomes upon hydration.

Cubosomes from hydrated starch–monoolein powders

The hydration and dispersion properties of the powders are investigated to characterize the powder utility. Upon addition of the above powders to water, a translucent dispersion is formed almost immediately with gentle stirring. No large agglomerates are evident from visual inspection, and optical microscopic observations reveal a uniform dispersion of sub-micron particles

with regular shapes, indicating a high degree of colloidal stabilization. Upon addition of the powders to water, the powder size distribution in Figure 8 shifts to a narrower size range from 0.1 to 5 μm with an overall volume-weighted mean size of 0.6 μm. It is worth emphasizing that colloidally-stable dispersions of nanostructured cubosomes are created by simply adding the starch–monoolein powders to water. Block copolymers are usually added to stabilize cubosomes formed by high-energy dispersion of bulk cubic gel (Landh, 1994; Gustafsson et al., 1996; 1997), as the sticky gel particles readily agglomerate. The starch used to prevent cohesion among the powders also acts to stabilize the hydrated powders against agglomeration; test dispersions remain un-agglomerated for at least six months.

A more detailed characterization of the cubosome particles formed by starch–monoolein powder hydration is possible using the three cryo-TEM images in Figure 9. The cubic liquid crystalline region of the particles is evident from the cubic lattice of hydrophobic channels (white dots) and the distinct corners, similar to the calculated approximation in Figure 1. In Figure 9, two of the three featured cubosome particles exhibit a hemispherical surface vesicle (lamellar liquid crystalline bilayer) several times larger than the parent cubosome. The vesicular structure on the cubosome surface is likely the result of an unfolding of the outer bilayer of the cubosome, rather than attachment of a separate vesicle particle. A three-dimensional schematic representation of a cubosome with an unfolded integral bilayer is also shown in Figure 9, emphasizing the cubic and spherical nature of the two particle regions. Although both the cubic and lamellar regions of the particle are constructed from lipid bilayers, the differences are clear as the lamellar region is simply a single hemispherical bilayer shell while the cubic region possesses much greater internal bilayer density (because the bilayers are contorted to pack more efficiently). Surface vesicles are often observed on cubosome surfaces, even when the lamellar liquid crystalline phase is not an equilibrium phase, and have been proposed as a thermodynamic means of avoiding aqueous exposure of lipid hydrocarbon chains when the cubic phase is fragmented during dispersion (Ljusberg-Wahren et al., 1996). Surface vesicles appear at the corners of cubosomes as this is the point where three orthogonal crystal planes meet and it is difficult to terminate the structure (Gustafsson et al., 1996). Also visible in Figure 9 are larger fragments of bulk cubic phase that have adhered to the EM grid. The appearance of the cubosomes in Figure 9 is similar but distinct (larger lamellar-to-cubic ratio) when compared to cubosomes usually observed in the monoolein–water system (Gustafsson et al., 1996; 1997; Almgren et al., 2000) as well as the ethanol–monoolein–water system (Spicer et al., 2001). The simplest explanation is that these cubosomes can be formed by simply mixing powder with water, so the high shear usually necessary to fragment bulk cubic phase is not needed and does not reduce the surface vesicle size.

Spray-drying dextran–monoolein–ethanol–water systems

The starch–monoolein powders perform well at producing cubosomes by simple hydration, but require spray-drying a dispersion of viscous cubic liquid crystalline gel particles. It is also of interest to develop a process for producing these particles that avoids spray-drying of cubic gel dispersions. Ethanol is known to act as a hydrotrope (Pearson & Smith, 1974) and dissolve viscous cubic liquid crystalline gel to form a low-viscosity liquid and ease processing (Spicer et al., 2001). Re-application of the hydrotrope effect in the spray-drying process may greatly ease manufacture of dry powder cubosome precursors by avoiding formation of the cubic gel dispersion in Figure 5. Other changes in the formula are also required to accommodate the ethanol. A polymer is needed for encapsulation of the monoolein, as the insolubility of starch in ethanol prevents its use. Dextran, however, has finite solubility in both ethanol and water (Neuchl & Mersmann, 1995) and is well suited for application here. The material to be spray-dried contains 37.5% water, 25% dextran, 22.5% ethanol, and 15% (w/w) monoolein. Excluding the dextran, the ternary 50% water–30% ethanol–20% (w/w) monoolein system is a low-interfacial-tension emulsion of two phases rich and lean in monoolein (Spicer et al., 2001). The quaternary system is prepared by dissolving the dextran in the water and the monoolein in the ethanol (each forms an optically isotropic solution), combining the two solutions, and mixing for 15 min. Once mixed, the quaternary system forms an emulsion of two distinct phases: one optically isotropic and one optically birefringent (Figure 10). In Figure 10, the two images show the emulsion that is spray-dried to make the dextran–monoolein powders under unpolarized (upper) and polarized (lower) light. When viewed between crossed polarizers, the birefringent phase exhibits the ‘oily streak’ lamellar liquid crystalline texture (Rosevear, 1968) upon minimal

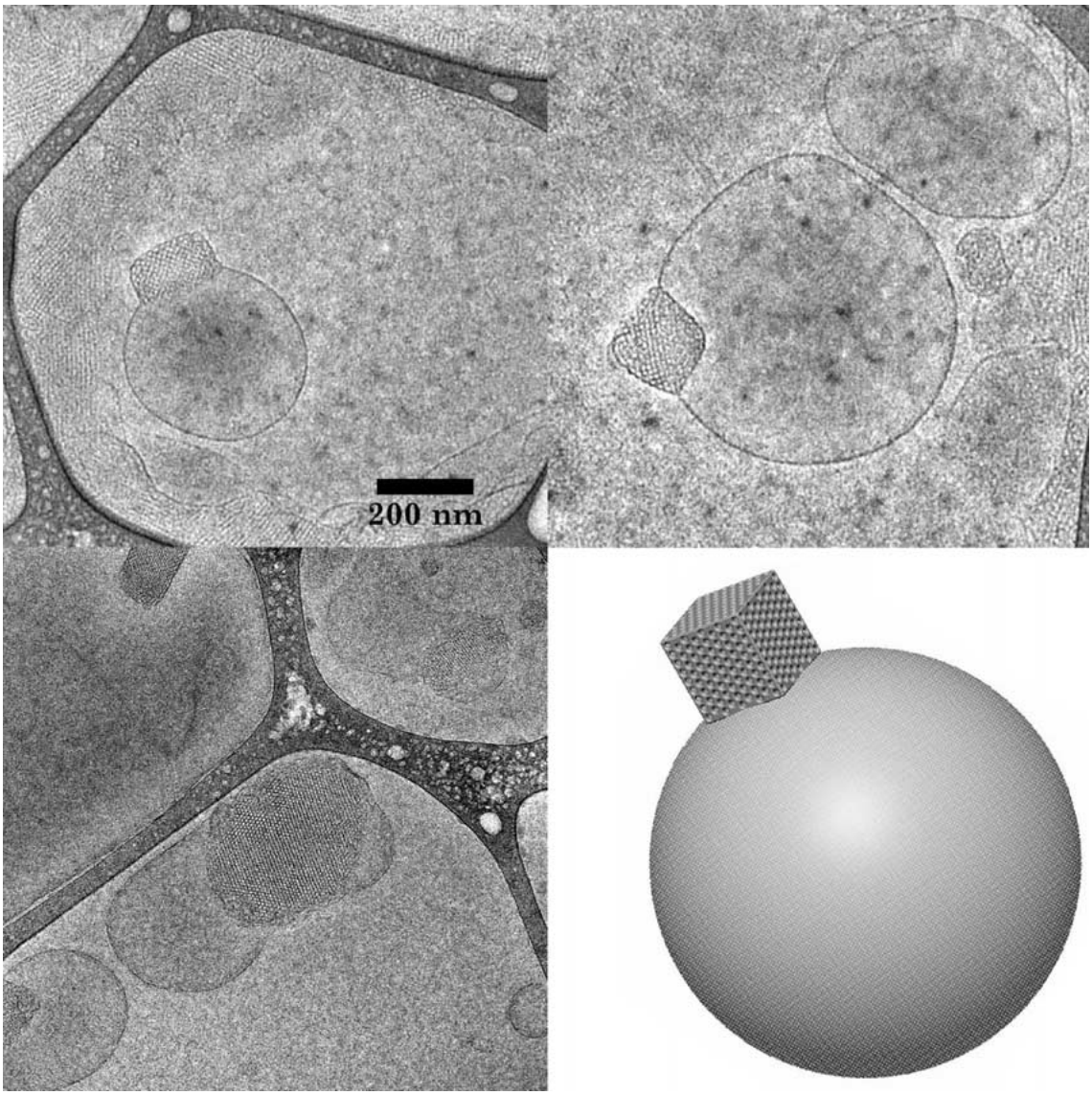

Figure 9. Cryo-TEM photographs of three different cubosome particles (with large surface vesicles) that form upon hydration of the starch–monoolein powders. Also visible on the edges of the grid surrounding the particles are regions of cubic liquid crystalline gel that are not dispersed into discrete cubosomes. The bottom right hand corner shows a three-dimensional generalized cartoon of the cubosome particles (with large surface vesicle) produced by hydrating the starch–monoolein particles in Figures 6 and 7.

shearing (Figure 10). Lamellar phases are known to be remarkable emulsion stabilizers, as they have a tendency to exist at interfaces and create a viscous barrier to coalescence (Laughlin, 1994).

The powders produced by spray-drying dextran–monoolein–ethanol–water emulsions are shown in Figure 11 under two levels of SEM magnification. The powders are agglomerated clusters of small capsules, likely dextran encapsulating monoolein and some ethanol/water. As in the case of the high molecular weight starch, the particles are encapsulated by a more rigid molecule that produces a less elastic film (dextran has a greater degree of branching than the starch) than is typically desired for spray-drying. The powder size distribution is shown in Figure 12. The dry powders span a particle size range from 1 to 100 μm, with a volume-weighted mean size of 26 μm. The size distribution is unimodal and much narrower than that

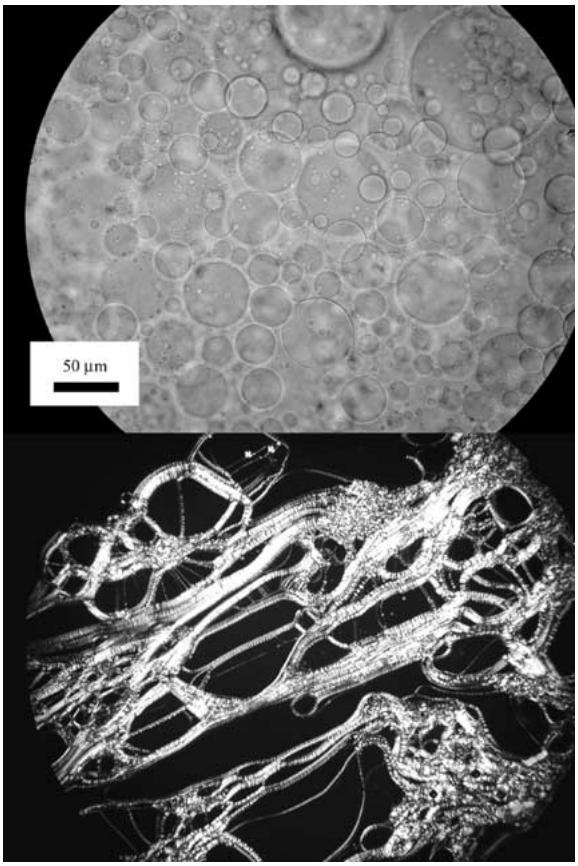

Figure 10. Unpolarized (top) and polarized (bottom) light microscopy views of precursor emulsion that is spray-dried to form dextran–monoolein powders. The continuous phase exhibited classical 'terraced drop' lamellar liquid crystalline textures when sheared and viewed under polarized light (bottom).

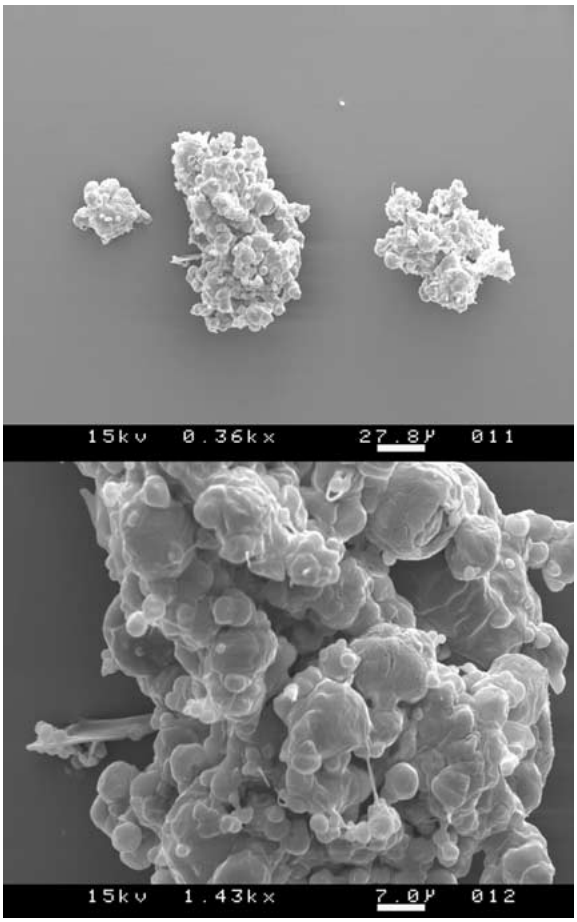

Figure 11. Two SEM photographs of spray-dried dextran–monoolein particles. Agglomerates, 20–50 µm in diameter, are formed that are composed of smaller primary particles 1–10 µm in diameter. These particles are believed to be dextran shells encapsulating monoolein.

of the starch–monoolein powders as a result of the relative ease of spraying an emulsion *versus* a dispersion of viscous cubic gel particles. The total solids yield from the dryer (assuming complete water and ethanol evaporation) is 62% (w/w) and no appreciable deposition on the walls of the dryer occurred, likely as a result of the ethanol's hydrotropic properties. The application of the hydrotrope method to spray-drying significantly eases processing. Thermal gravimetric analysis indicates the presence of 16% (w/w) volatile materials remaining in the powders following drying, of which 3% is water and 13% is ethanol. The volatile content remains constant for several months, indicating (along with the lack of deposition) good encapsulation of the ethanol and monoolein by the dextran. Depending on the application, the powders can be produced with varying amounts of ethanol by tailoring the film properties (Lee et al., 1999) in order to take advantage (during hydration) of the nucleation of small cubosomes from monoolein–ethanol solution (Spicer et al., 2001).

Cubosomes from hydrated dextran–monoolein powders

The ternary phase behavior of the dextran–monoolein–water system is qualitatively similar to the starch–monoolein–water system shown in Figure 4. Dextran is soluble in water up to about 65% (w/w), and the bicontinuous cubic phase extends up to dextran concentrations of about 1%. Cubic phase is found to be in equilibrium with dextran solution over a composition range similar to the starch system, indicating the

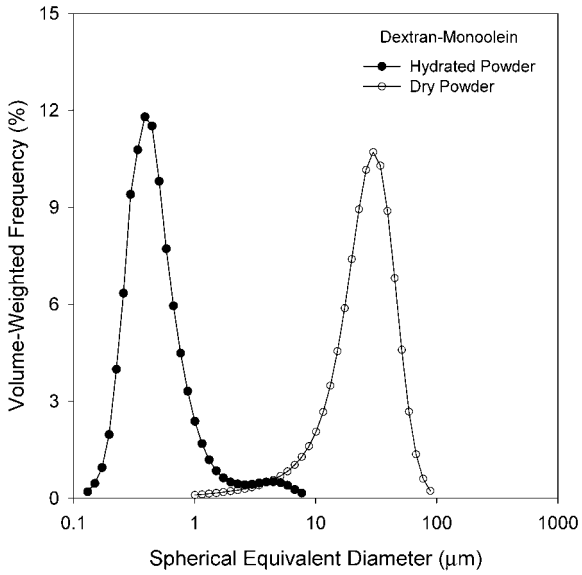

Figure 12. Particle size distribution of the dextran–monoolein powders in dry (open circles) and aqueous dispersion (filled circles) form.

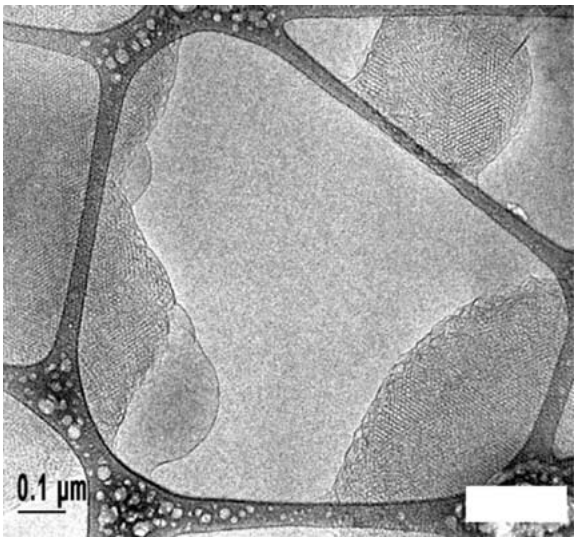

Figure 13. Cryo-TEM of hydrated dextran–monoolein particles with distinct cubic liquid crystalline structure and, in one case, a surface vesicle.

ability to form cubosomes from dextran–monoolein powders. When the dextran powders are hydrated, a dispersion is formed easily with little stirring, similar to the starch–monoolein powders. The particle size distribution of the hydrated powders in Figure 12 (filled circles) exhibits two modes: one ranging from 0.1 to 1 μm and the second (likely agglomerates) extending up to 10 μm with an overall volume-weighted mean size of 0.6 μm, identical to the cubosomes formed by the starch–monoolein powders. By comparison with the very stable hydrated starch powders, the hydrated dextran powders tend to loosely agglomerate over time, but are easily re-dispersed. Hydrophobically-modified starch is expected to perform better as a colloidal stabilizer than dextran as a result of its favorable balance of hydrophobicity and hydrophilicity, whereas dextran is primarily hydrophilic and does not significantly interact with vesicle lipid bilayers (Vereykan et al., 2001). Nevertheless, the dextran provides adequate steric resistance to cubosome agglomeration. Figure 13 shows a cryo-TEM image of several cubosome particles formed by hydration of the dextran–monoolein powders. The cubosomes exhibit regular cubic lattices and one has a surface vesicle similar to those in Figure 9. The surface texture on some of the cubosomes is more disordered than the core of the particles, possibly as a result of dextran interactions. Again similar to the starch–monoolein powders, the dextran–monoolein powders make it possible to produce cubosomes by simple powder hydration. High shear dispersion of bulk cubic gel is therefore avoided and standard unit operations (i.e., spray-drying and powder incorporation) can be used for the powder manufacture.

In addition to the processing benefits of encapsulating polymers, surface-modification benefits can also be envisioned. Dextran is often used in biomedical applications for prevention (by steric mechanisms) of protein and biomolecule adsorption on surfaces (Jönsson et al., 1998). The dextran layer on the surface of the cubosome particles may perform similarly by elastically resisting the close approach of proteins and by entropic repulsion of the proteins in order to avoid loss of the dextran's conformational freedom. In drug delivery applications the dextran coating could help prevent undesirable protein interactions with the cubosomes. The powder precursor process thus also enables the production of cubosomes with tailored surface function, all by simply adding powders to water. Dextran also affects surfactant phase behavior; bicontinuous microemulsion phases decrease in volume by dextran-driven osmotic water loss, but only when the dextran molecules are too large to be incorporated into the water channels of the microemulsion (Kabalnov et al., 1994). Comparison of the bicontinuous cubic phase water channel dimension (∼50 Å) and the mean end-to-end distance for the dextran used (∼350 Å) indicates the dextran is unlikely to be incorporated into the

cubosomes upon hydration and may have some destabilization effect. We have not extensively investigated this effect here.

Conclusions

It has been demonstrated that two different types of dry powder cubosome precursors may be produced by spray-drying aqueous polymer–surfactant systems. The first type of powders, monoolein encapsulated with hydrophobically-modified starch, is produced by spray-drying cubic phase particles dispersed in aqueous starch solution. Characterization of the starch powders by SEM indicates uniform encapsulation of the monoolein by the starch and the dependency of the powder size distribution on the size distribution of the dispersed particles that are spray-dried. The starch coating is found to both prevent powder cohesion and, upon hydration, confer colloidal stability to the cubosome nanostructured particles that form. Low molecular weight starch is optimal from a film-forming and powder quality perspective. Starch powders form a broad size distribution from 0.2 to 200 μm while the cubosomes formed by powder hydration are 0.1–5 μm in size with a volume-weighted mean of 0.6 μm. Cryo-TEM images of the cubosomes produced by starch powder hydration reveal nanostructured particles with large unfolded bilayers on their surfaces. Because the starch–monoolein powders are produced by spray-drying a dispersion, powders are also produced by spray-drying an emulsion formed by the dextran–monoolein–ethanol–water system, avoiding the need for a high shear dispersion step prior to spray-drying. The dextran system produces a narrower powder size distribution as a result of the relative ease of dispersing and spraying an emulsion. Hydration of the dextran powders also produces cubosomes, with a similar size distribution to the hydrated starch powders. The use of a powder precursor to form cubosomes by simple addition to water widens the range of applications for cubosomes by making their production and use in products accessible to manufacturing and formulation personnel instead of being limited to specialized researchers. In addition, the powder encapsulation process enables tailoring of the surface function of the cubosomes to achieve desirable colloidal and functional effects.

Application of the powders produced here will, to some degree, be limited by the large proportion of polymer required for encapsulation (∼75% w/w for starch and ∼60% for dextran) that will limit the amount of active material that can be incorporated for subsequent delivery. Assuming (as an upper feasible limit) a 10% w/w dispersion of starch-stabilized cubosomes is desired and a 1 : 1 ratio of monoolein to active is used, the maximum weight percent of active in the dispersion is 1.25%, useful only for high value-added materials like pharmaceuticals, vitamins, flavors, or scents. However, the primary applications of cubosomes are of a medical and pharmaceutical nature, so the powders described above could still be applied. Future work will investigate the controlled-release and targeted delivery properties of the cubosomes produced by powder precursors.

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