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CUBOSOME PROCESSING
Industrial Nanoparticle Technology Development

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Cubosomes are nanoparticles but instead of the solid particles usually encountered, cubosomes are self-assembled liquid crystalline particles with a solid-like rheology that provides unique properties of practical interest. The discovery of cubosomes is a unique story and spans the fields of food science, differential geometry, biological membranes, and digestive processes. Despite the early realization of their potential, the manufacture of cubosomes on a large scale embodied difficulty because of their complex phase behaviour and viscous properties. This article reviews the development of several processes for practical manufacture and use of cubosomes in the consumer product industry, the intellectual property that resulted, and the eventual use of the patented technology.

Keywords: cubosome; nanoparticle; liquid crystal; self-assembly.

When asked to give a plenary talk at the UK Particle Technology Forum (PTF) on cubosomes I was simultaneously thrilled (my first plenary!) and nonplussed (have I anything new left to say on the subject?). The best approach in such a case is to think broadly and find a novel path that meets the objective. The cubosome project consumed nearly 3 years of my life at Procter and Gamble (P&G) and was a fascinating, fun pursuit. But unlike some academic research topics with decade time scales, industrial interests can be relatively short-lived. As a result, I had not done active research on cubosomes in 2 years when I came to UCL for the PTF. Nevertheless, although the technical work had been published, the background story of the work we did on cubosomes at P&G had not been told because of proprietary considerations. Given the 'Bridging the Chemistry/Chemical Engineering Interface' theme of the PTF in 2004, our work on cubosomes was especially relevant as it entailed collaboration between Matt Lynch, a chemist in P&G Corporate Research, and me, a chemical engineer in P&G Corporate Engineering. Also, the UK PTF places a strong emphasis on student involvement, and an overview of P&G's work on cubosomes provides unique insight into the type of work engineers with a background in Particle Technology can perform during industrial careers. Finally, in this age of excitement about nanotechnology, start-up companies, and invention, it is useful to speak frankly about the circuitous path often followed during industrial technology invention, process development, and the unexpected outcomes of such pursuits. Professor Seville's article should also be consulted for perspective on particle technology start-up companies and the associated financial rewards.

Cubosomes are nanoparticles, more accurately nanostructured particles (for a more detailed review, see Spicer, 2003), of a liquid crystalline phase with cubic crystallographic symmetry formed by the self-assembly of amphiphilic or surfactant-like molecules (Figure 1). That's right, although cubosomes are nanoparticles, they are not solids. However, the cubic phases are unique in that they possess very high solid-like viscosities because of their intriguing bicontinuous structures [meaning they enclose two distinct regions of water separated by a contorted bilayer of surfactant (Scriven, 1976)]. As a result cubic phases can be fractured and dispersed to form particulate dispersions that are colloidally, if not thermodynamically, stable for long times. Certain surfactants will spontaneously form cubic phases when combined with water above a certain level and the first determination of their molecular structure was ingeniously carried out by Luzzati and Husson (1962), Luzzati *et al.* (1968), Larsson (1983) and Hyde *et al.* (1984) between 1960 and 1985. Kåre Larsson is unanimously credited with discovering that these phases can exist as dispersed particles as well as in the bulk (Larsson, 1989), an observation made from studies of human fat digestion (Patton and Carey, 1979). Larsson's trailblazing work earned him the Rhodia European Prize for colloid and interface work in 2001. So you see that discovery, even in an exciting area like nanoparticles, can come at unexpected moments within seemingly unrelated areas of study. Larsson named the particles he discovered 'cubosomes' to reflect their cubic molecular crystallographic symmetry and their similarity to liposomes (also known as vesicles: dispersed nanoparticles of lamellar liquid crystalline phase). Because cubosomes can form from biological lipids like monoglycerides, can

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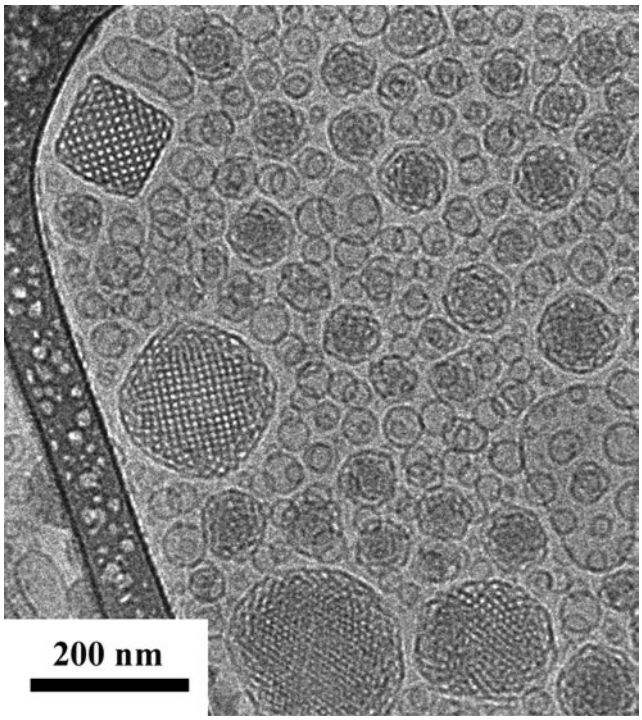

Figure 1. Cryotransmission electron microscope (cryo-TEM) image of cubosomes. Cubosomes are the square and rounded particles with internal cubic lattices visible. Also seen are vesicles, a less structured liquid crystalline particle similar to biological cell membranes.

solubilize numerous biologically active molecules including proteins (Buchheim and Larsson, 1987), and possess a tortuous microstructure (Anderson and Wennerström, 1990), their application as drug (and other active ingredient) delivery vehicles was pursued (Lawrence, 1994).

In 1999 P&G had already begun applying technologies that blended aspects of pharmaceutical products (delivery to and through skin) and consumer products (emphasis on softening and conditioning of skin rather than physiological effects). Cubosomes were an excellent example because they allowed a wide variety of active ingredients to be delivered, their use required knowledge of surfactant aqueous phase behaviour, and the surfactant most commonly used to form cubosomes, glycerol monoolein, is a food-grade material that is inexpensive and broadly safe for use. We became aware of the unique properties of cubosomes through the efforts of Prof Barry Ninham of Australian National University and Prof Stig Friberg of Clarkson University who had both worked for years with the Australian and Swedish researchers at the centre of the now-classic work that highlighted the cubic phases' relevance to areas as diverse as differential geometry, material science, plant biology, and even the origins of life on Earth (Hyde *et al.*, 1997).

As industrialists and engineers we realized that, although cubosomes were intriguing with fascinating properties and potential, their flexible, efficient, and economical manufacture would be difficult (if not impossible) using the technology and understanding available at the time. The published (Ljusberg-Wahren *et al.*, 1996) processes for cubosome production relied on the top-down approach to nanoparticle production: make the extremely viscous bulk cubic phase by hydrating molten lipid mixed with block copolymer (for steric stabilization of the resulting particles) and then use very high energy (ultrasound or high pressure homogenization were most commonly cited) to disperse the cubic phase into nanoparticles. The problem of course is that such unit operations can require multiple passes to achieve the desired particle size distribution and the high energy input can be an obstacle to using many temperature-sensitive active ingredients. The assignment was thus easily stated: develop a process to produce cubosomes that did not require high energy processing. The crucial aspect of the work rested on the application of particle technology: although cubosomes were perceived as exotic nanoparticles because of their liquid crystalline nature, they could be treated the same as solid nanoparticles because of their high viscosity. As a result, our objective became the production of cubosomes using a bottom-up technique, starting from a molecular solution rather than a bulk material.

Similar approaches had been applied to form vesicles (Friberg *et al.*, 1997) by using a hydrotrope solvent to dissolve the viscous liquid crystalline bulk phase (and avoid its formation entirely) but then add excess water to reduce the liquid crystalline phase's solubility and crystallize it as discrete particles. The key to making such a process work is the use of a hydrotrope, defined as a material with hydrophilic and hydrophobic character but no surfactant properties, instead of a solvent. Use of a solvent would have prevented the liquid crystalline phase from forming even after dilution, whereas the hydrotrope only prevents liquid crystal formation at high concentrations. Design of such a process requires knowledge of the full system phase behavior, so after some trial and error the ternary ethanol–monoolein–water system was chosen and its phase diagram constructed (Spicer *et al.*, 2001). Figure 2 shows the full phase diagram used to design the cubosome dilution process. Single phase regions are labelled, indicating the formation of two cubic phases and a lamellar liquid crystalline phase, all viscous liquid crystals. Cubosomes form between a cubic phase and the water–ethanol mixture, where two-phase cubic-fluid equilibrium exists. The single

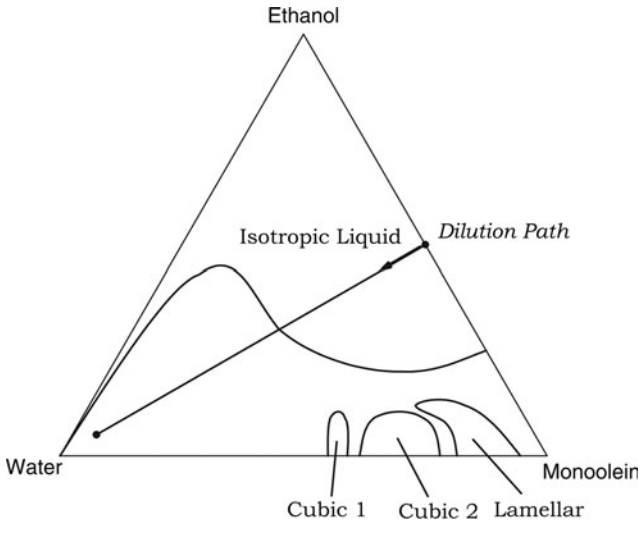

Figure 2. Ternary phase diagram for a typical process forming cubosomes via dilution.

phase low viscosity isotropic liquid at ethanol concentrations above 10% is the precursor that, upon dilution, forms cubosomes spontaneously without application of high energy dispersion processes. Such a dilution process is shown schematically in Figure 2 as a straight line drawn from the starting composition (e.g., 50% ethanol and 50% monoolein) to the water apex of the diagram. The strength of such an approach to process design is the ability to predict the starting and ending phases present, their composition, and their properties, such as low viscosity in the case of the isotropic liquid phase. The process we developed thus allows the creation of cubosomes while entirely skipping the creation of a bulk cubic phase that would require high energy to disperse. Comparison of the cubosomes produced by this process with those made by multiple-pass homogenization indicates little difference with vastly improved production rates (Spicer *et al.*, 2001). Once the liquid process was developed, discussions with P&G formulators indicated some systems require a low-to-no-water form of the cubosomes to allow addition to existing formulas without accompanying water volume. The objective was then shifted to the creation of a powdered precursor that forms cubosomes spontaneously upon hydration.

Although it is theoretically possible to simply fragment the solid monoolein into small particles, its sticky nature required additional modification to prevent powder cohesion. The concept developed from there to attempt spray-drying of a dispersion of cubosomes with modified starch also present to encapsulate the monoolein and protect against powder cohesion (Spicer *et al.*, 2002). The process worked relatively well, producing hollow starch shells containing small fragments of monoolein, separated by starch, as seen in Figure 3. When rehydrated, the powders formed cubosomes readily without shear, with the hydrated starch performing double duty as a steric stabilizer of the cubosomes. Although the process worked, it had perversely returned us to making a cubosome dispersion for spray-drying via high energy processing and this dispersion sometimes clogged the spray nozzle. A second process was developed to avoid these problems by again developing a map of the phase behaviour with ethanol present to prevent cubic phase formation. Now an emulsion of ethanolic monoolein solution in aqueous dextran solution could be easily spray-dried, evaporating the water and ethanol to form dextran shells with incorporated monoolein. As before, upon hydration the powders formed cubosomes without the need for high energy processing to form nanoparticles. We now had two distinct processes for producing cubosomes that do not involve small-scale high energy treatments and are more easily scaled up to international consumer product levels. Although much of the focus in cubosome research was on application as controlled release vehicles, our interest was on the unique bioadhesive properties of cubic phase (Nielsen *et al.*, 1998) and cubosomes' similarity to structures in human skin (Norlén, 2001). Indeed, simple diffusion considerations and later experimental verification showed that cubosomes do not offer controlled release benefits on their own (Boyd, 2003). In addition, the large amount of water present during cubosome formation made water-soluble actives difficult to load into the cubic phase. So for maximum usefulness of the technology Matt Lynch developed a process for modifying native cubic phases with charged or long-chain molecular 'anchors' that increase the affinity of solubilized actives for the cubic phase, slowing release kinetics (Lynch *et al.*, 2003).

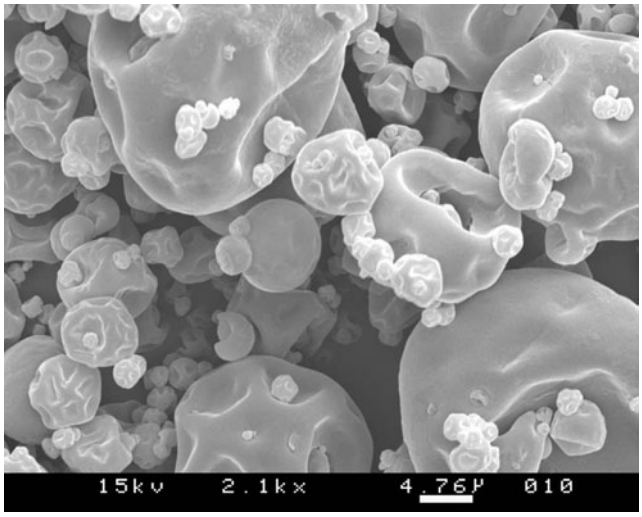

Figure 3. SEM image of the spray-dried starch powders that form cubosomes upon rehydration.

The technology portfolio described above was turned into a series of patent filings as we continued to work with business groups interested in applying cubosomes for product benefit. One common element of product and process development is that there are many reasons (often unrelated to technical validity or uniqueness) why a product ultimately never makes it to the marketplace. After several years of work the cubosome technology either never provided the product benefits sought or were a part of a product effort that was never completed for other reasons. Although other consumer product companies have signaled interest in applying cubosomes as well (Biatry, 2000), we are aware of no commercial products that incorporate cubosomes. We began to search for a better home for the technology than the consumer product industry. In the course of prototype testing, the University of Cincinnati (UC) Children's Hospital Skin Science Institute discovered some unique cubic phase behaviour on human skin and skin substitutes. Preliminary results indicate that cubic phases provide distinct benefits for electrical interrogation of the body through the skin as in electrocardiogram tests, possibly because of the similar bicontinuous structure of cubic phase and human skin (Hoath and Norlén, 2005). In addition, UC was working to develop a treatment for premature babies who are born without a fully developed stratum corneum (outer skin layer) and they indicated an interest in applying cubosomes in such a treatment. The decision was soon made to transfer the cubosome processing technology portfolio to UC and allow them to develop the technology in the medical area for the benefit of small children. Their work is proceeding and they have recently invented a synthetic vernix coating like the one a woman's body naturally forms to protect a child in the womb (Hoath *et al.*, 2003).

In conclusion, the path taken during the cubosome technology development looked nothing like what we imagined

back in 1999. However, the result is still something of which an engineer (and a scientist) can be proud. New processes for making unique nanoparticles and enhancing their properties were developed and supplied to doctors working to save the lives of some of society's most fragile members. Along the way some fascinating insights were gained into the behaviour of complex fluid materials and their equilibrium and kinetic properties, all well within the charter of chemical engineering practitioners today. Although the story is distinct from the high-risk high-reward world of high-tech startup companies, I would like to think that it shares something with that world's excitement of discovery and unexpected technology application as well as the satisfaction of contributing to something with the potential to improve society at large.

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