Bicontinuous Cubic Liquid Crystalline Phase and Cubosome[+] Personal Care Delivery Systems

by

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[+] The word "cubosome" is a USPTO registered trademark of GS Development AB Corp., Sweden.

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Introduction

Bicontinuous cubic phase liquid crystals are newly discovered exotic materials originally found in the most unassuming places. The original observations of cubic liquid crystalline phase came during the study of polar lipids, such as monoolein (Figure 1), that are used as food emulsifiers (Lindstrom et al., 1981; Andersson et al., 1995). The most studied binary system, monoolein-water, forms at least two types (diamond and gyroid; Larsson, 1983) of an optically clear, solid-like (Jones and McCleish, 1999) bicontinuous cubic phase at water contents between 20-40% (w/w) at room temperature (Lutton, 1965; Hyde et al., 1984; Laughlin, 1996; Briggs et al., 1996). Other aqueous surfactant systems self-assemble into thermodynamically stable bicontinuous cubic liquid crystalline phases as well (Fontell et al., 1968; Luzzati, 1968).

Bicontinuous cubic liquid crystalline materials are an active research topic (Hyde et al., 1997) because their unique structure lends itself well to controlled release applications. Amphiphilic molecules form bicontinuous water and oil channels, where “bicontinuous” refers to two distinct (continuous, but non-intersecting) hydrophilic regions separated by the bilayer (Scriven, 1976). This allows for simultaneous incorporation of water- and oil-soluble materials as well as amphiphiles. The phase structure provides a tortuous diffusion pathway for controlled release (Anderson and Wennerström, 1990, Engström et al., 1992) and lipid-based cubic phase liquid crystals are biocompatible, digestible (Patton and Carey, 1979; Lindström et al., 1981), and bioadhesive (Nielsen et al., 1998).

Cubosomes are discrete, sub-micron, nanostructured particles of bicontinuous cubic liquid crystalline phase (Figure 2). Cubosomes possess the same microstructure as the parent cubic phase but have much larger specific surface area and their dispersions have much lower viscosity than the bulk cubic phase. The relative insolubility of cubic phase-forming lipid in water allows cubosomes to exist at almost any dilution level, as opposed to most liquid crystalline systems that transform into micelles at higher levels of dilution. As a result, cubosomes can be easily incorporated into product formulations.

Cubosomes are typically produced by high-energy dispersion of bulk cubic phase (Gustafsson et al., 1996; 1997), followed by colloidal stabilization using polymeric surfactants (Landh, 1994). After formation of the cubosomes, the dispersion is formulated into a product and then applied to a substrate of interest, usually bodily tissue. Thereafter materials are either absorbed or released via diffusion.

The Eureka Moment

The thought to apply cubosomes in personal care products came to us following a lecture on the properties of surfactant liquid crystalline systems by Professor Stig Friberg, formerly of Clarkson University, now retired. The mention of a material capable of solubilizing high levels (~40% w/w) of proteins (Ericsson et al., 1983) is intriguing and suggests further potential for solubilization and controlled release of active ingredients.

Cubosome Applications

A common application for such new materials is as drug delivery vehicles. The first patent describing cubosome usage specifies numerous medical and controlled release applications (Landh and Larsson, 1996), although controlled release is usually possible only for bulk cubic phases (Boyd, 2003). Consequently, self-assembled surfactant phases have been extensively examined for compatibility with numerous medical active ingredients and their applications (Drummond and Fong, 2000).

The rapid expansion of the life-sciences industry is expected to drive previously “exotic” delivery vehicles and ingredients into broader marketplaces, such as personal care and consumer products (Enriquez and Goldberg, 2000). An application area under current development by L’Oreal is the use of cubosome particles as oil-in-water emulsion stabilizers and pollutant absorbents in cosmetics (Ribier and Biatry, 1995, 1996, 1998; Biatry, 2000, 2001; Afriat and Biatry, 2001). They discovered that a second amphiphile, phytantriol (Figure 3),

has an aqueous phase behavior sufficiently close to that of monoolein to form cubosomes under similar conditions. Even more recent patent activity by Nivea points to cubosome use in personal care product areas as varied as skin care, hair care, cosmetics, and antiperspirants (Schreiber and Albrecht 2002a, b; Schreiber and Eitrich, 2002; Schreiber et al., 2002). Despite recent activity, there remains a lack of the practical elements like manufacturing scalability and material customization that is necessary to lead formulators to consider using cubosomes in commercial products.

This chapter reviews our work on optimization of cubosome production, physicochemical properties of cubosome particles, and their clinical performance evaluation. The driving force for the work described in this chapter is the extension of existing cubosome technology to make possible their manufacture on large-scale, as well as developing techniques for adjustment of important cubosome physical properties. The first part of the work described herein is the development of a process for spontaneously forming cubosomes via dilution of the monoolein-ethanol-water system (Spicer et al., 2001). The process avoids the traditional, high-energy dispersion of bulk cubic phase used previously (Ljusberg-Wahren et al., 1996; Gustafsson et al., 1996; 1997). A second process we developed produces powder precursors that spontaneously form cubosomes upon hydration. The method avoids the need for transport and processing of bulk water and opens the door to a wider range of applications like drug delivery via inhalation (Spicer et al., 2002a). Both processes make cubosome manufacture more broadly possible than previous technology because they require only standard processing equipment and materials. A third development described in this work discusses enhancement of the properties of the native cubic phase using charged surfactants and polymers that strongly associate with solubilized active ingredients. Finally, the effects of the bicontinuous cubic phase on human skin are studied and the results compared to existing consumer skin treatments.

2. Liquid Cubosome Precursors

In view of the difficulty and expense of high-shear dispersion of viscous bulk cubic phase to form cubosomes, it is desirable to seek less aggressive processes of manufacture. High-energy processes can be expensive, difficult to scale-up, and harmful to fragile temperature-sensitive active ingredients like proteins. In some product applications, it is also advantageous for cubosomes to form only upon use, such as during hand washing or mouth rinsing. A strong driving force exists for development of a liquid phase precursor to cubosomes to avoid high-energy processing and produce them *in situ*. The hydrotrope dilution process is found to consistently produce smaller, more stable cubosomes. In concept, particles are formed by nucleation and growth, as employed in crystallization and precipitation processes. This is achieved by dissolving the monoolein in a hydrotrope, such as ethanol, that prevents liquid crystalline formation. Subsequent dilution of this mixture spontaneously "crystallizes" or precipitates the cubosomes. This is all done without the need for high shear, minimizing the risk of degrading the cubic liquid crystalline structure (Spicer et al., 2001). The liquid precursor process allows for easier scale up of cubosome preparations and avoids bulk solids handling and potentially damaging high energy processes.

The dilution process is easily visualized using a ternary phase diagram of a hydrotrope like ethanol, water, and monoolein (Figure 4). A large isotropic liquid (L_1) region exists at high ethanol concentrations, which has a low viscosity and is easy to prepare. In addition to the L_1 region, there are three single-phase liquid crystalline regions: the lamellar liquid crystalline phase, or L_α, and the two bicontinuous cubic liquid crystalline phases: the P_{n3m} (diamond) and the I_{a3d} (gyroid). The cubic phase liquid crystals can tolerate about 10% by weight of ethanol. Not all hydrotropes work this well, however, and Lynch and Spicer (2002) provide a list of the materials most compatible with monoolein. Most importantly, there is a large miscibility gap between the water apex and the cubic phase, where cubosomes form. Production of large and sub-micron cubosomes

merely requires adding water to monoolein-ethanol precursor solutions until the mixture falls into this miscibility gap.

The dilution pathway determines the size of the cubosomes. Consider Path A (Figure 4) that involves the dilution of an isotropic liquid (50% monoolein, 50% ethanol) with a polymer/water solution to form a colloidal dispersion of cubosomes in water (89% water, 5% monoolein, 5% ethanol, and 1% Poloxamer 407, which acts as a stabilizer to avoid aggregation of the cubosomes). This dilution pathway provides a fine dispersion of sub-micron cubosomes. Alternatively, a second path B results in formation of larger cubosomes via dilution from the region of the phase diagram where emulsions of both rich and lean isotropic liquid form. Such emulsions are also excellent precursors for cubosome dispersions because they can be easily dispersed and stabilized prior to cubosome formation. The precursor emulsion particle size distribution is easily tailored by low shear, then can be stabilized and diluted into the cubic-liquid equilibrium region to form cubosomes. In fact, a macroemulsion (70% water, 20% ethanol, 10% monoolein) diluted with an aqueous solution of Poloxamer 407 forms a cubosome dispersion (90% water, 6% ethanol, 3% monoolein, 1% polymer) by means of simple hand agitation. Spicer et al. (2001) found that additional shear beyond hand mixing aids in uniformly producing sub-micron cubosomes, although much less shear is required than for dispersion of bulk cubic phase without hydrotrope. Omission of the stabilizing polymer during dilution will obviously form bulk cubic phase instead of cubosomes, allowing additional applications.

3. Powdered Cubosome Precursors

Powdered cubosome precursors are powders composed of dehydrated surfactant coated with polymer. Such powders offer some process and performance advantages to liquid phase hydrotropic cubosome precursors. Hydration of the precursor powders forms cubosomes with a mean particle size of 600 nm, as confirmed by light scattering and cryo-TEM (Spicer et al.,

2002a,b). The lipids used to make cubosomes are waxy, sticky solids, rendering them unable to form small discrete particles. It is found that a water-soluble non-cohesive starch coating on the waxy lipid prevents agglomeration and allows control of particle size. Spray drying is an excellent process to produce these particles. Spray drying produces encapsulated particles from an emulsion of liquid droplets or a dispersion of solid particles in a concentrated aqueous polymer solution. The continuous and dispersed phases are sprayed through a nozzle to create suspension droplets that are contacted with a heated, dry air stream flowing in the opposite direction. Excess water immediately evaporates, leaving dry powder particles composed of the dispersed phase encapsulated by a shell of the formerly dissolved polymer. Spray-drying processes are easily scaled up and are already widely employed for manufacturing consumer products like detergents and foods. Further, the process provides an easy route to preload active into the cubosomes prior to drying. Finally, the polymer coating on the powder imparts surface properties to the hydrated cubosomes that can be tailored by proper selection of the encapsulating polymer.

The liquid feed to the spray-dryer can be tailored to adjust the resultant powder properties. The production of starch-coated cubosome powder precursors requires high shear treatment of monoolein in aqueous starch solution to form a coarse cubosome dispersion that is then pumped through a nozzle and dried. Full operating conditions are given in Spicer et al. (2002a). The initial composition pumped into the spray-drier is 60% w/w water, 30% starch, and 10% monoolein. Drying removes almost all water present and gravimetric tests of the powder generally indicate a final composition of about 4% w/w water, 72% starch, and 24% monoolein in the product powders. Although the relative fraction of starch is high (3:1 starch:monoolein), the level is necessary to preserve powder quality. Figure 5 compares SEM photographs of starch-encapsulated monoolein with a 3:1 (left hand side) and 1:1 (right hand side) starch:monoolein ratio. The powder with the 3:1 ratio exhibits good encapsulation of the monoolein and small particle size. However, the 1:1 ratio exhibits poor morphology and larger particle size. In the latter case, the bulk

powder is more cohesive as a result of poor encapsulation of the sticky monoolein by the starch.

The production of starch-coated powder precursors from a hydrotropic solution of monoolein emulsified in water makes the spray-drying step easier. Ethanol is known to act as a hydrotrope and dissolve viscous cubic liquid crystalline phase to form a low-viscosity liquid and ease processing (Spicer et al., 2001). Re-application of the hydrotrope effect in the spray-drying process avoids formation of a dispersion of cubosomes and eases spray drying. However, other changes in the formula are also required to accommodate the ethanol. A new polymer is needed for encapsulation of the monoolein, as the insolubility of starch in ethanol prevents its use. A useful alternative to the starch is dextran. The material to be spray-dried contains 37.5% water, 25% dextran, 22.5% ethanol, and 15% (w/w) monoolein. The quaternary system is prepared by first dissolving the dextran in the water and the monoolein in the ethanol. Thereafter the two solutions are combined and mixed. Once mixed, the quaternary system forms an emulsion of two distinct phases. One phase is optically isotropic and the other is optically birefringent. The emulsion of both phases has low viscosity and is easily spray dried.

The type of encapsulating starch also affects powder quality. Drying occurs as the dispersion is sprayed into droplets and moisture rapidly evaporates by convective heating. The cubosomes in the dispersion form the nucleus of many of the sprayed droplets, surrounded by aqueous starch solution. As drying proceeds, the starch remains and forms a coating on the cubic gel particle, thereby encapsulating it. Because the cubic phase itself contains 40% (w/w) water, some drying must also occur at the core of the particles. Low molecular weight starches (84,000 MW) produce superior powders when compared to those made using high (335,000 MW) molecular weight starches (Spicer at al., 2002a). Spicer et al. (2002b) provide a more comprehensive listing of feasible polymers and other materials for use as polymeric coatings to encapsulate cubosomes.

The application of the hydrotrope method to spray-drying for production of cubosome precursors significantly eases processing. Thermal gravimetric analysis indicates the presence of 16% (w/w) volatile materials remaining in the powders following drying. Of this fraction, 3% is water and 13% is ethanol. The volatile content remains constant for several months, indicating good encapsulation of both the ethanol and the monoolein by the dextran. Depending on the application, the powders can be produced with varying amounts of ethanol by tailoring the film properties of the polymer in order to take advantage (during hydration) of the nucleation of small cubosomes from monoolein-ethanol solution (Spicer et al., 2001).

The large proportion of polymer required for encapsulation (~75% w/w for starch and ~60% for dextran), limits the amount of active material 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%. Such a low level is useful only for high value-added materials like pharmaceuticals, vitamins, flavors, or scents. The process described demonstrates the feasibility of forming dry powders with the ability to form cubosomes upon hydration.

4. Functionalized Cubic Phase Liquid Crystals

It is usually assumed that the loading and release properties from cubic phase liquid crystals are solely governed by the solubilized active. Loading properties are governed by the partition of actives between existing phases. Laughlin (1996) notes that partitioning is driven by thermodynamic constraints that force the chemical potential of the active in each phase to be identical at equilibrium. In a more simplified view, higher affinity of the active for the liquid crystal leads to higher loading. Engström et al. (1999), for example, demonstrate that changing the ionization state of an active alters its solubility and consequently its loading in liquid crystalline phases: at lower pH, the active is

more hydrophobic and can be loaded to higher levels, while the opposite is also true. This is observed for a wide range of actives including lidocaine, prilocaine and clomethiazole (CMZ) and phenol butylamine. However, Chang and Bodmeier (1997a) note that amphiphilic actives such as chlorpheniramine maleate, diltiazem-HCl and propranolol-HCl bind differently to the monoglyceride, leading to further partition differences. They suggest that partition correlates better with the surface activity of the actives than solubility. At short times, Chang and Bodmeier (1997b) note that release is diffusion controlled, showing a square root dependency on time. At longer times, however, the release rates of actives such as chlorpheniramine maleate and pseudoephedrine hydrochloride follow a different rule primarily because they interact with the liquid crystal. The release rates also slow as the concentration in each phase approaches its equilibrium partition value. Further, several temperature-jump experiments suggest that propranolol hydrochloride releases faster with temperature owing to increased diffusivity through the matrix and increased drug solubility in the water. The common thread that emerges from this body of work is that the active material properties drive the loading and release properties and the utility of the cubic phase as a delivery vehicle.

The concept of functionalization is to control the loading and release properties of the active by changing the properties of the cubic phase. Functionalization is achieved by incorporating amphiphilic molecules into the liquid crystal; the hydrophobic portion of the amphiphile inserts into the bilayers of the cubic phase and the hydrophilic portions extend into the water channels. By customizing the specific properties of the hydrophilic portions, it is possible to control their interactions with the actives. As an example, a positively-charged hydrophilic portion is expected to increase the loading of a negatively-charged active. The release properties at short times should be altered because the active is ‘grabbed’ by the positively-charged amphiphile during diffusion out of the liquid crystal. The release properties at long times are also altered as the increased affinity between active and cubic phase alters the partitioning. Taken together, customizing the properties of the cubic phase is an alternative method

to changing the loading and release properties of an active, offering a greater potential for tailored release properties over a broader range of applications and conditions.

One approach to functionalization requires formulating small amphiphiles, such as surfactants, into the cubic phase. Surfactants used in this capacity are often termed 'anchors', and must be chosen with care. The addition of octyl glucoside (Angelov et al., 1999), sodium oleate (Caboi et al., 2002), cetyltrimethylammonium bromide (Gustafsson et al., 1998), and sodium cholate (Gustafsson et al., 1999), for example, all tend to convert the cubic phase to a lamellar phase, even with relatively small additions. Caboi et al. (2001) suggest these conversions reflect different 'locations' of the anchors in the cubic phase. Anchors that incorporate into the bilayer force lamellar phase formation while more polar anchors that locate at the interfacial regions tend to force reverse hexagonal phase formation. Lynch et al. (2003) present an optimal set of anchor properties that maximize functionalization without significantly altering the underlying structure of the cubic phase. Ideal anchors have low water solubility, low Krafft Temperature, an accessible hydrophilic group with which the active can interact, and critical packing parameter (ratio of head group volume and tail volume) close to unity. Such surfactants typically form vesicles in aqueous solutions. Other reports that successfully add large amounts of surfactant into the cubic phase have surfactants with these properties. Templer et al. (1992), for example, demonstrate that biological lipids with comparable properties can be formulated into the monoolein cubic phase at a relatively large weight percentage with only minor increase of the lattice parameter. Most importantly, several reports suggest that the inclusion of anchors alters the loading and release properties of actives solubilized in the cubic phase. Lindell et al. (1998) show that distearoyl phosphatidylglycerol in cubic phase retards the release of timolol maleate. In addition, Engström et al. (1999) suggest that the inclusion of dioleoyl phosphatidylcholine into cubic phase alters the loading of 4-phenylbutylamine. Lynch et al. (2003) solubilize quaternary ammonium surfactants into the cubic phase, affecting the loading and release properties of ionized ketoprofen, an anti-

inflammatory active ingredient. The anchors are added to greater than 20 % w/w without altering the bicontinuous structure of the cubic phase. The report also provides discussion of the magnitude of the interaction based on the molecular structure of the anchor hydrophilic portion.

A second approach to functionalization is to formulate large amphiphilic polymers or 'tethers' into the liquid crystal. Although this approach is less studied, certain reports indicate that it is viable. For example, Landh (1994) demonstrates through a phase diagram that Poloxamer 407 can be included to a large extent into the monoolein cubic phase. It is suggested that the polymer is included in the internal bilayers and not just on the surface of the dispersed cubosomes. In fact, Nakano et al. (2001) demonstrate that at low concentrations of Poloxamer 407, most of the polymer adheres to the surface of the particles. However, at high concentrations of the polymer, there is a conversion of the cubic phase liquid crystal from P_{n3m} to I_{m3m} symmetry suggesting that the polymer partitions into the bulk of the cubic phase matrix without destroying the structure. Further, Puvvada et al. (1993) demonstrate that the insertion of sodium alginate (water-soluble polysaccharide) into the cubic phase likewise changes the symmetry of the liquid crystal from P_{n3m} to I_{a3d} but induces only moderate changes to the phase diagram. This suggests a polymer-rich cubic phase forms under such conditions. The inclusion of the polymer also modifies the release properties of proteins by increasing the 'viscosity in the pore structure'. The addition of sodium citrate alters this behavior almost instantaneously by removing the gel properties of the polymer.

In summary, functionalization of cubic phases to control and optimize their loading, release, and partitioning of active ingredients is viable across many formulations. The application or formulation must be practical and economical. Given the relative cost of surfactant and pharmaceutical active ingredients, functionalization seems quite feasible. Finally, the approach offers greater opportunity for triggered release of actives as a result of stimuli such as change in pH, salt levels, or addition of solvent. All these tools are at the discretion of the formulator.

5. Clinical Evaluation of Skin Conditioning by Cubic Phase

One application of cubic phase liquid crystals, such as in the monoolein-water system, is their use as controlled release systems for delivery of selected water- and oil-soluble materials (Engström et al., 1992). Such applications require an understanding of the interface between the bicontinuous cubic materials and the biological epithelia which they contact; e.g., the gut, oral mucosa, and the skin. In the case of skin, the ultimate biological interface is constituted by a thin (~20 micron thick) cross linked biopolymer called the stratum corneum. Proponents of drug delivery across human skin point to the stratum corneum as the chief obstacle and impediment to successful passage of a molecule or drug into the living epidermis and/or the bloodstream. Numerous strategies have been developed, therefore, to disrupt the architecture of the stratum corneum using high energy ultrasound, laser ablation, electrophoresis, and chemical penetration enhancers in order to create momentary micropores or channels for drug passage (Barry, 2001).

Alternative approaches can be envisioned wherein a nanostructured cubic phase can be nondestructively juxtaposed with the stratum corneum for therapeutic or drug delivery purposes. This approach implies the development of a seamless interface between the cubic phase and the underlying stratum corneum, and by extension, between the stratum corneum and the underlying epidermis. Recently, a membrane folding model involving phase transitions from cubic-to-lamellar morphologies has been proposed to explain formation of the epidermal barrier (Norlen, 2001a, b). These considerations emphasize the importance of understanding biophysical interfaces at the nanostructural level. Work on the thermal, mechanical, and electrical properties of cubic phases in contact with the stratum corneum must be performed as a foundation for all specific applications.

A better understanding of the biological-biophysical interface of skin- cubic phase systems is, therefore, deemed essential for cubic phase applications involving transdermal drug delivery (with or without electrophoresis), the development of adhesive and skin protection strategies, and for electrical sensing from the skin surface. The biocompatibility of monoolein preparations or other conceivable cubic phases also needs to be established. The more seamlessly such a material intercalates with the epidermal barrier, the more likely a practical "window" can be established for specific applications.

In this section we summarize briefly the results of two clinical studies involving acute (minutes to hours) and chronic (several weeks) exposure of human stratum corneum to monoolein-water cubic phases. Both studies are conducted on normal adult human female volunteers with consent and institutional review board approval.

The first study contrasts the effects of two cubic phase formulations with two standard barrier creams containing petrolatum as the primary lipid base. The water vapor permeability of the contact materials is assessed using an *in vitro* gravimetric system as described in the legend of Figure 6. The results indicate that the cubic phases are highly permeable to water and fail to exhibit a clear dose response as a function of film thickness. The formulations in the clinical study include: 1) 75% monoolein, 25% water, 2) 75% monoolein, 20% water, 5% glycerin, 3) petrolatum (long chain hydrocarbon mixture, 0% water), and Eucerin cream® (petrolatum, lanolin, 17% water). Treatments are applied to one of six skin sites measuring 2.5 cm x 5 cm on the volar forearm at levels of 2.5 mg/cm^2 to simulate typical concentrations of emollients in the skin literature. Ten subjects are enrolled and evaluated. The key focus of this study is to characterize water related properties of the skin surface in contact with the standard emollients and the cubic phases.

Figure 7 shows the clinical effects of the treatments on transepidermal water loss (TEWL) as measured *in vivo* with the Dermalab evaporimeter. The Eucerin water-in-oil emulsion showed increased TEWL at 30 minutes post application presumably as a function of water loss from the formula itself. In

contrast, the cubic phases showed no such behavior and TEWL is indistinguishable from control sites at this time. Petrolatum, as expected for an occlusive film, reduces TEWL. By 2 hours post application, there are no clear effects of any ointment to affect TEWL (data not shown).

The effects of the various treatments on water handling properties are evaluated by means of the sorption-desorption test (Agache et al., 2001). In this standardized test, surface electrical capacitance readings are measured prior to and at measured intervals following topical application and removal of exogenous water (Visscher et al., 2002). The area under the desorption curve indicates the degree to which the skin surface and adsorbed materials will bind exogenous water (Visscher et al., 2001). In this experiment, the cubic gels are demonstrated to increase water binding to the skin surface as indicated by an increased area under the sorption-desorption curve (Figure 8).

In the second experiment, the efficacy of a cubic phase test formulation consisting of 75% monolein and 25% water is tested in a standardized model of xerotic (dry) skin. In this study 20 healthy adult female volunteers free of dermatitis but with a visual skin dryness grade $\geq$ 2 are evaluated. The cubosome test formulation is compared to a glycerine-containing moisturizing lotion (Vaseline® Intensive Care Dry Skin Cream) versus a no treatment control site. Following a one week washout period with no lotion treatment, subjects applied 1 mg/cm^2 of product to the lower legs over a 10 cm x 10 cm treatment area twice a day for 14 days.

The skin condition is evaluated by a combination of visual and objective biophysical methods (Li et al., 2001; Loden, 1995). Visual grading is assigned by expert skin graders using a 0-3 scale for dryness and a 0-4 scale for erythema. Table 1 summarizes the results of this preliminary study. The cubic phase does little to ameliorate the overall skin dryness and, in fact, seems, in some cases, to exacerbate the condition. The possibility, thus, arose that free oleic acid generated by endogenous epidermal metabolism may contribute to this effect (Jiang and Zhou, 2003). In addition, one of the obvious findings of this study is the difficulty of applying the monoolein-water admixture to the skin surface while

in the cubic phase. In the study, the cubic phase was supplied to subjects in small dispensible syringes and a measured aliquot was topically rubbed into the site until a uniform film was achieved. It is also possible that the application process itself results in increased friction and/or trauma to the xerotic skin site.

These preliminary studies allow the following general conclusions:

1. Bulk cubic phases are difficult to handle and difficult to apply to human skin. In contrast, the relatively anhydrous lamellar phase of the monoolein-water admixture is relatively fluid and easy to apply. The application of relatively dehydrated, starch-encapsulated cubosome particles offers another potential method for forming cubic phase nanoarchitectures on human skin (Spicer et al., 2002a).
2. The paradoxical addition of exogenous water to the lamellar phase of a topically applied monoolein-water admixture results in formation of the more viscous cubic architecture. Thus, the simple addition or removal of exogenous water provides a means of controlling the phase behavior and, thus, the physical nature of the topical gel.
3. The cubic phase is highly vapor permeable when measured over a hydrated support structure such as Gore-Tex (Figure 6) as well as over human skin (Figure 7). In the experiment shown in Figure 6, there is no effect of film thickness to decrease the water vapor gradient. Does this unusual property reflect the bicontinuous nature of the water phase in the cubic material? Vapor permeability combined with a physical barrier is a desirable characteristic in wound healing applications (Visscher et al., 2001). High viscosity and high vapor permeability are two physical properties distinguishing monoolein-water cubic phases from occlusive skin ointments such as petrolatum.
4. The cubic phase is hygroscopic on human skin as judged by instrumental tests such as the sorption-desorption test (Figure 8). Thus, exogenous water is sequestered in the cubic gel architecture and may result in a

phase change. The amount of water can be ascertained by standardized electrical tests such as the sorption-desorption test.

5. The clinical application of a cubic phase as a therapeutic agent for xerotic skin yields conflicting results (Table 1). In the study presented, twice daily application of a cubic phase to the dry lower legs of normal adult females evoked increased erthyema, increased visual dryness, and increased transepidermal water loss compared to a standard glycerin-containing, petrolatum-based emollient. Whether this response is the result of a potential irritating effect of the cubic phase or a secondary effect due to repeated application of a very viscous and difficult to apply ointment requires further study.

In summary, we anticipate that cubic phase systems will find increasing application as drug delivery vehicles and platforms for adhesives, skin protectants, and biomonitoring devices. The utility of these binary nanostructured systems can be extended by the ability to control the physical phase of the system; e.g., the transition from lamellar to cubic phase (Lynch et al., 2003) and the use of cubosome powder precursors (Spicer et al., 2002a). Such systems will be particularly versatile if it can be demonstrated that the biological interface itself possesses a cubic architecture (Norlen, 2001a, b).

6. Conclusions

Bicontinuous cubic liquid crystalline phases, either in bulk or cubosome form, offer unique properties of particular interest to the personal care industry. Cubic phase materials can be formed by simple combination of biologically compatible lipids and water and are thus well-suited for use in treatments of skin, hair, and other body tissue. Some observations of skin irritation by bulk cubic phase point to the need for further study of alternative formulations that avoid residual oleic acid and use much lower viscosity cubosome dispersions. The ability to form cubosomes either in use, during formulation, or during manufacture offers greatly enhanced flexibility for product development efforts. Formulation of

personal care products containing cubosomes often requires additional surface-active ingredients. For this reason the capability to tailor the active ingredient loading of cubic phases and control the tolerance of cubic phases for other ingredients is a crucial tool in further development efforts.

Company Contacts

Meyer, is this at P&G or the vendors?

Supplier Lists

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Formulation 1

1. Cubosome dispersion formed by dilution of an isotropic solution

Ingredient	Wt. Percent
Monoolein	10
Ethanol	5
Water	1.8
Poloxamer 407	1
Water	82.2
Total	100%

Mixing Instructions

Weigh the Part A ingredients into a suitable vessel equipped with a mixer. The materials form a clear, low viscosity isotropic liquid. Combine the Part B ingredients into a separate vessel and stir until all polymer is dissolved. Inject Part B solution into Part A and mix only as much as needed to produce cubosomes of the desired size. A colloidally stable dispersion of cubosomes forms.

Formulation 2

2. Powder cubosome precursor

Ingredient		Wt. Percent
Monoolein		10
HICAP 100 Starch		30
Water		60
	Total	100%

Mixing Instructions

Weigh the Part A ingredients into a suitable vessel equipped with a high-shear mixer. Upon shearing the materials will form a relatively low viscosity coarse dispersion of cubosomes. Spray-dry to produce a powder with a final composition of about 4% w/w water, 72% starch, and 24% monoolein.

Formulation 3

3. Bulk cubic phase gel for skin treatment

Ingredient	Wt. Percent
Monoolein	57
Glycerin	5
Water	38
Total	100%

Mixing Instructions

Melt the Part A ingredient into a suitable vessel by heating above 40° C and stirring. In a separate vessel, mix the Part B ingredients until completely homogeneous. Add the Part B ingredients to the Part A ingredients and mix well. The mixture forms a very viscous, clear gel that needs to be mixed well to ensure uniform incorporation of all Part B ingredients.

Author Biographies

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References

Afriat, I. and Biatry, B., Use of Cubic Gel Particles as Agents against Pollutants, Especially in a Cosmetic Composition, Eur. Pat. Appl. (L'Oreal, Fr.)., Ep, (2001).

Agache, P., Mary, S., Muret, P., Matta, A. M., and Humbert, P., "Assessment of the Water Content of the Stratum Corneum Using a Sorption-Desorption Test," *Dermatology*, **202** 308-13, (2001).

Anderson, D. M. and Wennerström, H., "Self-Diffusion in Bicontinuous Cubic Phases, L3 Phases, and Microemulsions," *Journal of Physical Chemistry*, **94** 8683-94, (1990).

Andersson, S., Jacob, M., Ladin, S. and Larsson, K., "Structure of the Cubosome - a Closed Lipid Bilayer Aggregate," *Zeitschrift fur Kristallographie*, **210** 315-8, (1995).

Angelov, B., Ollivon, M. and 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*, **15** 8225-34, (1999).

Barry, B. W., "Novel Mechanisms and Devices to Enable Successful Transdermal Drug Delivery," *Eur. J. Pharm. Sci.*, **14** 101-14, (2001).

Biatry, B., Cosmetic and Dermatological Emulsion Comprising Oily and Aqueous Phase, Eur. Pat. Appl. (L'oreal, Fr.)., Ep, (2000).

Biatry, B., Use of Phytantriol as Anti-Pollution Agent in a Cosmetic Composition, Eur. Pat. Appl. (L'Oreal, Fr.)., Ep, (2001).

Briggs, J., Chung, H., and Caffrey, M., "The Temperature-Composition Phase Diagram and Mesophase Structure Characterization of the Monoolein/Water System," *J. Phys. II France*, **6** 723-51, (1996).

Boyd, B., "Characterisation of Drug Release from Cubosomes Using the Pressure Ultrafiltration Method," *International Journal of Pharmaceutics*, **submitted** (2003).

Caboi, F., Amico, G. S., Pitzalis, P., Monduzzi, M., Nylander, T., and Larsson, K., "Addition of Hydrophilic and Lipophilic Compounds of Biological Relevance to the Monoolein/Water System. I. Phase Behavior.," *Chemistry and Physics of Lipids*, **109** 47-62, (2001).

Caboi, F., Borne, J., Nylander, T., Khan, A., Svendsen, A., and Patkar, S., "Lipase Action on a Monoolein/Sodium Oleate Aqueous Cubic Liquid Crystalline Phase-a Nmr and X-Ray Diffraction Study," *Colloids and Surfaces, B: Biointerfaces*, **26** 159-71, (2002).

Chang, C.-M. and Bodmeier, R., "Binding of Drugs to Monoglyceride-Based Drug Delivery Systems," *International Journal of Pharmaceutics*, **147** 135-42, (1997a).

Chang, C.-M. and Bodmeier, R., "Swelling of and Drug Release from Monoglyceride-Based Drug Delivery Systems," *Journal of Pharmaceutical Sciences*, **86** 747-52, (1997b).

Drummond, C. J. and Fong, C., "Surfactant Self-Assembly Objects as Novel Drug Delivery Vehicles," *Current Opinion in Colloid and Interface Science*, **4** 449-56, (2000).

Engström, S., Lindman, B. and Larsson, K., Method of Preparing Controlled-Release Preparations for Biologically Active Materials and Resulting Compositions. Fluid-Carbon International AB (Malmo, SE), USA, 1992.

Engström, S., Norden, T. P. and Nyquist, H., "Cubic Phases for Studies of Drug Partition into Lipid Bilayers," *Europoean Journal of Pharmaceutical Sciences*, **8** 243-54, (1999).

Enriquez, J. and Goldberg, R., "Transforming Life, Transforming Business: The Life-Science Revolution," *Harvard Business Review*, **March-April** 96-104, (2000).

Ericsson, B., Larsson, K., and Fontell, K., "A Cubic Protein-Monoolein-Water Phase," *Biochimica et Biophysica Acta*, **729** 23-27, (1983).

Fontell, K., Mandell, L., and Ekwall, P., "Isotropic Mesophases in Systems Containing Amphiphilic Compounds," *Acta Chem. Scand.*, **22** 3209-23, (1968).

Gustafsson, J., Ljusberg-Wahren, H., Almgren, M., and Larsson, K., "Cubic Lipid-Water Phase Dispersed into Submicron Particles," *Langmuir*, **12** 4611-13, (1996).

Gustafsson, J., Ljusberg-Wahren, H., Almgren, M. and Larsson, K., "Submicron Particles of Reversed Lipid Phases in Water Stabilized by a Nonionic Amphiphilic Polymer," *Langmuir*, **13** 6964-71, (1997).

Gustafsson, J., Oraedd, G., Nyden, M., Hansson, P. and Almgren, M., "Defective Lamellar Phases and Micellar Polymorphism in Mixtures of Glycerol Monooleate and Cetyltrimethylammonium Bromide in Aqueous Solution," *Langmuir*, **14** 4987-96, (1998).

Gustafsson, J., Nylander, T., Almgren, M. and Ljusberg-Wahren, H., "Phase Behavior and Aggregate Structure in Aqueous Mixtures of Sodium Cholate and Glycerol Monooleate," *Journal of Colloid and Interface Science*, **211** 326-35, (1999).

Hyde, S. T. Andersson, S. Ericsson, B., and 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," *Zeitschrift fur Kristallographie*, **168**, 213-219, (1984).

Hyde, S., Andersson, A., Larsson, K., Blum, Z., Landh, T., Lidin, S., and Ninham, B. W.: The *Language of Shape*, Elsevier, New York, 1997.

Jiang, S. J. and Zhou, X. J., "Examination of the Mechanism of Oleic Acid-Induced Percutaneous Penetration Enhancement: An Ultrastructural Study," *Biol. Pharm. Bull.*, **26** 66-8, (2003).

Jones, J. L. and Mcleish, T. C. B., "Concentration Fluctuations in Surfactant Cubic Phases: Theory, Rheology, and Light Scattering," *Langmuir*, **15** 7495-503, (1999).

Landh, T., "Phase Behavior in the System Pine Needle Oil Monoglycerides-Poloxamer 407-Water at 20°," *Journal of Physical Chemistry*, **98** 8453-67, (1994).

Landh, T. and Larsson, K., Particles, Method of Preparing Said Particles and Uses Thereof. GS Biochem AB, USA, 1996.

Larsson, K., "Two Cubic Phases in Monooein-Water System," *Nature*, **304** 664, (1983).

Laughlin, R. G., *The Aqueous Phase Behavior of Surfactants*, Academic Press, 1996.

Li, F., Conroy, E., Visscher, M. and Wickett, R. R., "The Ability of Electrical Measurements to Predict Skin Moisturization. Ii. Correlation between One-Hour Measurements and Long-Term Results," *Journal of Cosmetic Science*, **52** 23-33, (2001).

Lindell, K., Engblom, J., Jonstroemer, M., Carlsson, A. and Engström, S., "Influence of a Charged Phospholipid on the Release Pattern of Timolol

Maleate from Cubic Liquid Crystalline Phases.," *Progress in Colloid & Polymer Science*, **108** 111-18, (1998).

Lindstrom, M., Ljusberg-Wahren, H., Larsson, K., and Borgstrom, B., "Aqueous Lipid Phases of Relevance to Intestinal Fat Digestion and Absorption," *Lipids*, **16** 749-54, (1981).

Ljusberg-Wahren, H., Nyberg, L., and Larsson, K., "Dispersion of the Cubic Liquid Crystalline Phase - Structure, Preparation, and Functionality Aspects," *Chimica Oggi*, **14** 40-43, (1996).

Loden, M., "Biophysical Methods of Providing Objective Documentation of the Effects of Moisturizing Creams," *Skin Research and Technology*, **1** 101-08, (1995).

Lutton, E. S., "Phase Behavior of Aqueous Systems of Monoglycerides," *Journal of the American Oil Chemist's Society*, **42** 1068-70, (1965).

Luzzati, V., Tardieu, A., Gulik-Krzywicki, T., Rivas, E., and Reiss-Husson, F., "Structure of the Cubic Phases of Lipid-Water Systems," *Nature*, **220** 485-88, (1968).

Lynch, M. L. and Spicer, P. T., “Cubic Liquid Crystalline Compositions and Methods fro their Preparation,” USA Patent App. (2002).

Lynch, M. L., Ofori-Boateng, A., Hippe, A., Kochvar, K. and Spicer, P. T., "Enhanced Loading of Water-Soluble Actives into Bicontinuous Cubic Phase Liquid Crystals Using Cationic Surfactants," *Journal of Colloid and Interface Science*, **in press** (2003).

Nakano, M., Sugita, A., Matsuoka, H. and Handa, T., "Small-Angle X-Ray Scattering and 13c Nmr Investigation on the Internal Structure of "Cubosomes"," *Langmuir*, **17** 3917-22, (2001).

Nielsen, L., Schubert, L. and Hansen, J., "Bioadhesive Drug Delivery Systems 1. Characterisation of Mucoadhesive Properties of Systems Based on Glyceryl Mono-Oleate and Glyceryl Monolinoleate," *European Journal of Pharmaceutical Sciences*, **6** 231-39, (1998).

Norlen, L., "Skin Barrier Formation: The Membrane Folding Model," *Journal of Investigative Dermatology*, **117** 823-29, (2001a).

Norlen, L., "Skin Barrier Structure and Function: The Single Gel Phase Model," *Journal of Investigative Dermatology*, **117** 830-36, (2001b).

Puvvada, S., Qadri, S. B., Naciri, J. and Ratna, B. R., "Ionotropic Gelation in a Bicontinuous Cubic Phase," *Journal of Physical Chemistry*, **97** 11103-7, (1993).

Ribier, A. and Biatry, B., Cosmetic Compositions Comprising a Stable Aqueous Dispersion of Phytantriol-Based Gel Particles Containing a Long-Chain Surfactant as Dispersant and Stabilizer, Eur. Pat. Appl. (Oreal S. A., Fr.)., Ep, 1995, p. 13 pp.

Ribier, A. and Biatry, B., Cosmetic or Dermatologic Oil/Water Dispersion Stabilized with Cubic Gel Particles and Method of Preparation, Eur. Pat. Appl. (Oreal S. A., Fr.)., Ep, 1996, p. 16 pp.

Ribier, A. and Biatry, B., Oily Phase in an Aqueous Phase Dispersion Stabilized by Cubic Gel Particles and Method of Making. L'Oreal (Paris, FR), USA, 1998.

Schreiber, J. and Albrecht, H., Hair Care Products with a Content of Disperse Phase Liquid Crystals Which Form Cubic Phases, Ger. Offen. (Beiersdorf AG, Germany) DE (2002a).

Schreiber, J. and Albrecht, H., Cosmetic Cleaning Products with a Content of Disperse Phase Liquid Crystals Which Form Cubic Phases, Ger. Offen. (Beiersdorf AG, Germany) DE (2002b).

Schreiber, J. and Eitrich, A., Deodorant and Antiperspirant Products with a Content of Disperse Phase Liquid Crystals Which Form Cubic Phases, Ger. Offen. (Beiersdorf AG, Germany) DE (2002).

Schreiber, J., Schwarzwaelder, C. and Cassier, T., Skin Care Products with a Content of Disperse Phase Liquid Crystals Which Form Cubic Phases, Ger. Offen. (Beiersdorf AG, Germany) DE (2002).

Scriven, L. E., "Equilibrium Bicontinuous Structure," *Nature*, **263** 123-25, (1976).

Spicer, P. T., Hayden, K. L., Lynch, M. L., Ofori-Boateng, A. and Burns, J. L., "Novel Process for Producing Cubic Liquid Crystalline Nanoparticles (Cubosomes)," *Langmuir*, **17** 5748-56, (2001).

Spicer, P. T., Small, W. B., Lynch, M. L. and Burns, J. L., "Dry Powder Precursors of 'Soft' Cubic Liquid Crystalline Nanoparticles (Cubosomes)," *Journal of Nanoparticle Research*, **4** 297-311, (2002a).

Spicer, P. T., Small, W. B. and Lynch, M. L., "Cubic Liquid Crystalline Compositions and Methods for their Preparation," USA Patent App., Procter & Gamble Co., (2002b).

Templer, R. H., Madan, K. H., Warrender, N. A. and Seddon, J. M., "Swollen Lyotropic Cubic Phases in Fully Hydrated Mixtures of Monoolein, Dioleoylphosphatidylcholine, and Dioleoylphosphatidylethanolamine," *Springer Proceedings in Physics*, **66** 262-5, (1992).

Trubiano, P. C., “Succinate and Substituted Succinate Derivatives of Starch,” in *Modified Starches: Properties and Uses*, O. B. Wurzburg, ed., CRC Press, Boca Raton, FL (1986).

Visscher, M. O., Hoath, S. B., Conroy, E. and Wickett, R. R., "Effect of Semipermeable Membranes on Skin Barrier Repair Following Tape Stripping," *Arch. Dermatol. Res.*, **293** 491-99, (2001).

Visscher, M. O., Chatterjee, R., Ebel, J. P., Laruffa, A. A. and Hoath, S. B., "Biomedical Assessment and Instrumental Evaluation of Healthy Infant Skin," *Pediatr. Dermatol.*, **19** 473-81, (2002).

List of Figure Captions

Figure 1 - Molecular structure of the lipid most commonly used to form cubosomes, monoolein. Monoolein is a commonly used monoglyceride food emulsifier.

Figure 2 – Cryo-TEM photograph of cubosomes formed in the monoolein-water system using the conventional high shear dispersion technique. Both micron-scale and sub-micron cubosomes can be formed, down to a lower limit of about 50 nm. Also visible are lamellar vesicles that are a common non-equilibrium byproduct of high shear-dispersion processes. Reproduced with permission from *Langmuir*, 2001, 17, 5748-5756. Copyright 2001 Am. Chem. Soc.

Figure 3 – Molecular structure of phytantriol, a second amphiphile that forms cubosomes in aqueous systems. Phytantriol is often used in sunscreens and other personal care formulations.

Figure 4 – Schematic of the ternary phase behavior of the ethanol-monoolein-water system. The dilution trajectories indicate the cubosome-forming processes possible in such a system. Path A forms smaller, sub-micron, cubosomes upon dilution by starting from an isotropic liquid solution. Path B starts from a macroemulsion state and forms micron-scale cubosome particles upon dilution. Reproduced with permission from *Langmuir*, 2001, 17, 5748-5756. Copyright 2001 Am. Chem. Soc.

Figure 5 – SEM photographs of spray-dried powder precursors of cubosomes. The left-hand side photo shows powders with a 3:1 starch:monoolein ratio exhibiting desirable morphology and good encapsulation of the sticky lipid. The right-hand side photo shows powders with a 2:1 starch:monoolein ratio, exhibiting poorer encapsulation of the monoolein and much larger particle size because of agglomeration.

Figure 6 - Water vapor transport measured *in vitro* across petrolatum and cubic phases of varying thickness. In this experiment, a Gore-Tex sheet was immobilized over a plastic weigh boat containing water. Water vapor transport was determined gravimetrically by the temporal reduction of the weight of the boat/Gore-Tex combination either alone (control) or following application of 0.5 (A), 1.0 (B), or 2.5 (C) mg/cm^2 of a cubic phase containing 75% monoolein and 25% water. Column D indicates water transport across a cubic phase containing 75% monoolein, 20% water, and 5% glycerin. The petrolatum film was 0.5 mg/cm^2. All cubic phases produced a small reduction in water vapor transport but there was no dose response effect observed. Petrolatum was highly occlusive.

Figure 7 - Transepidermal water loss measured on normal adult human skin 30 minutes after topical application of 2.5 mg/cm^2 of Eucerin cream® (petrolatum, lanolin, 17% water), Petrolatum (long chain hydrocarbon mixture, 0% water), and monoolein-based cubic phases containing 25% water (A), 25% water @ 1.0 mg/cm^2 (B), and 20% water + 5% glycerin (C). At this time point, TEWL is significantly elevated over the Eucerin sites and lower over the petrolatum sites (★) presumably reflecting slow evaporation of water from the formulation with Eucerin cream and occlusion with petrolatum. In contrast, the cubic phases are similar to controls but higher than the petrolatum sites.

Figure 8 - Water holding capacity of skin sites measured by the area under the sorption-desorption curve 4 hours after application of the same test ointments shown in the previous figure. In this study, the Eucerin site was significantly less than controls whereas all cubic phases exhibited significant increases in water holding capacity compared to the control sites (★).

TABLE 1. Summary of results of clinical study on the effect of petrolatum-based emollient versus cubic phase preparation on dry (xerotic) skin. Skin grades (dryness and erythema) were scored visually using a standardized scale.

	Skin Grades		**Transepidermal Water Loss**	**Moisture Accumulation Test**
	Dryness	**Erythema**		
Day 1 • Control • Petrolatum • Cubic phase	No differences among sites in either score		Baseline values not significantly different among groups	Baseline values not significantly different among groups
Day 4	Cubic phase significantly less dry than control site and more erythematous than control and petrolatum sites		Groups significantly different with petrolatum < controls < cubic phase sites	No significant differences among groups
Day 14	Cubic phase site drier than control and petrolatum sites; No difference in erythema among sites		Petrolatum significantly lower than control and cubic phase sites; cubic phase higher than control site	Values low indicating very dry skin in all groups; petrolatum higher than cubic phase
Day 21	Cubic phase site visibly drier than control & petrolatum sites; cubic phase more erythematous than petrolatum site		Petrolatum < control < cubic phase sites	No significant differences among groups

Transepidermal water loss and the moisture accumulation test were quantified using standardized instruments.

Monoolein

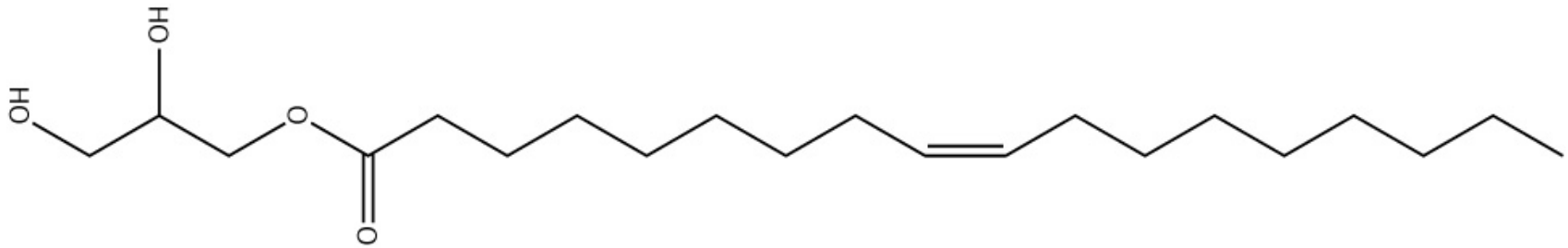

Figure 1

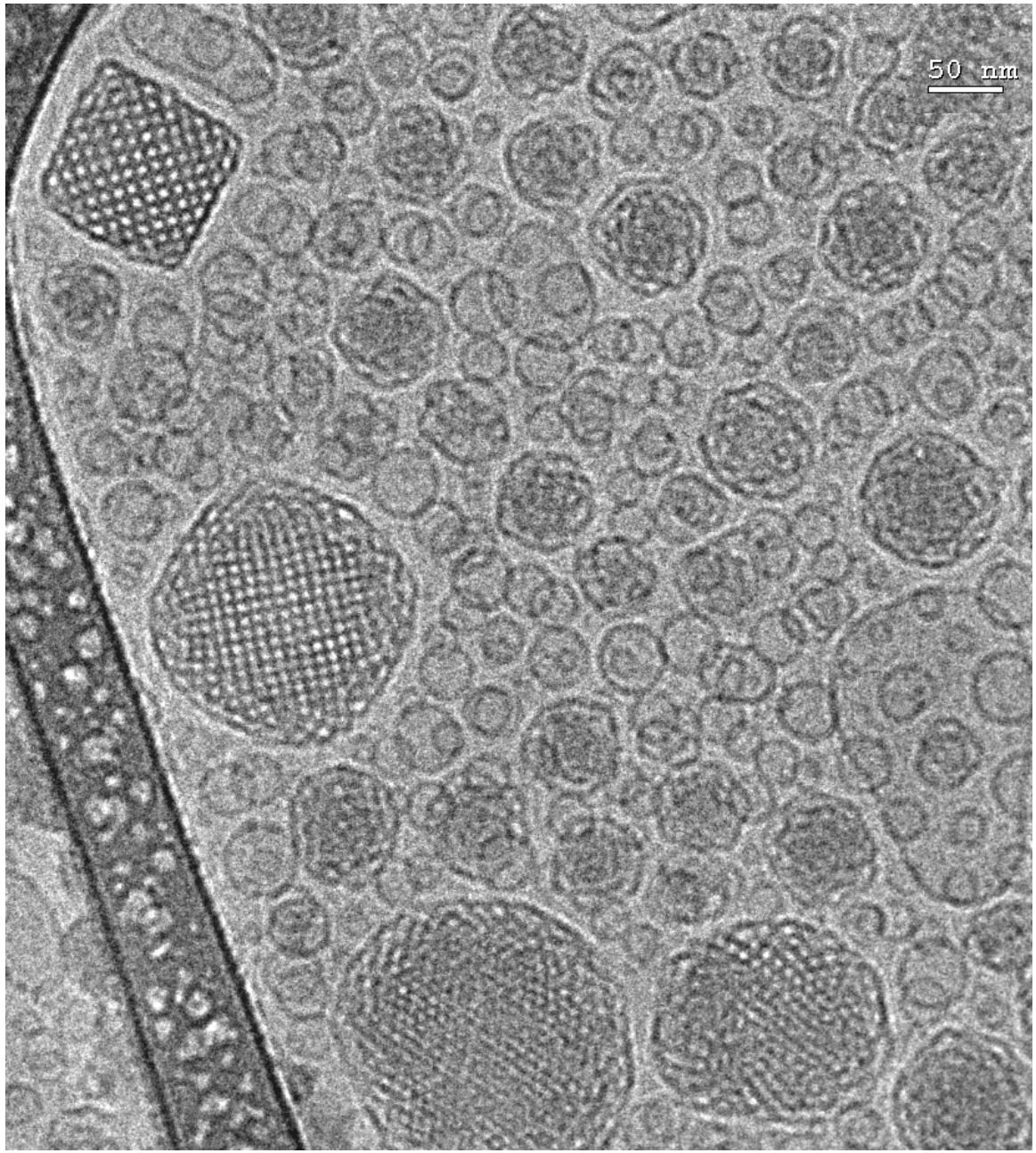

Figure 2

Phytantriol

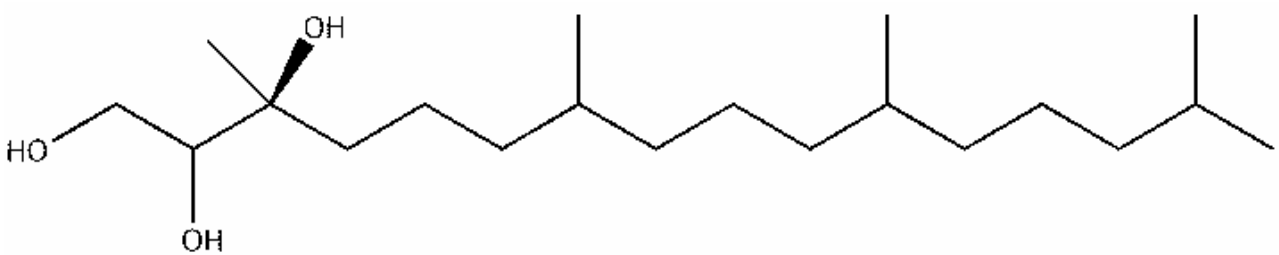

Figure 3

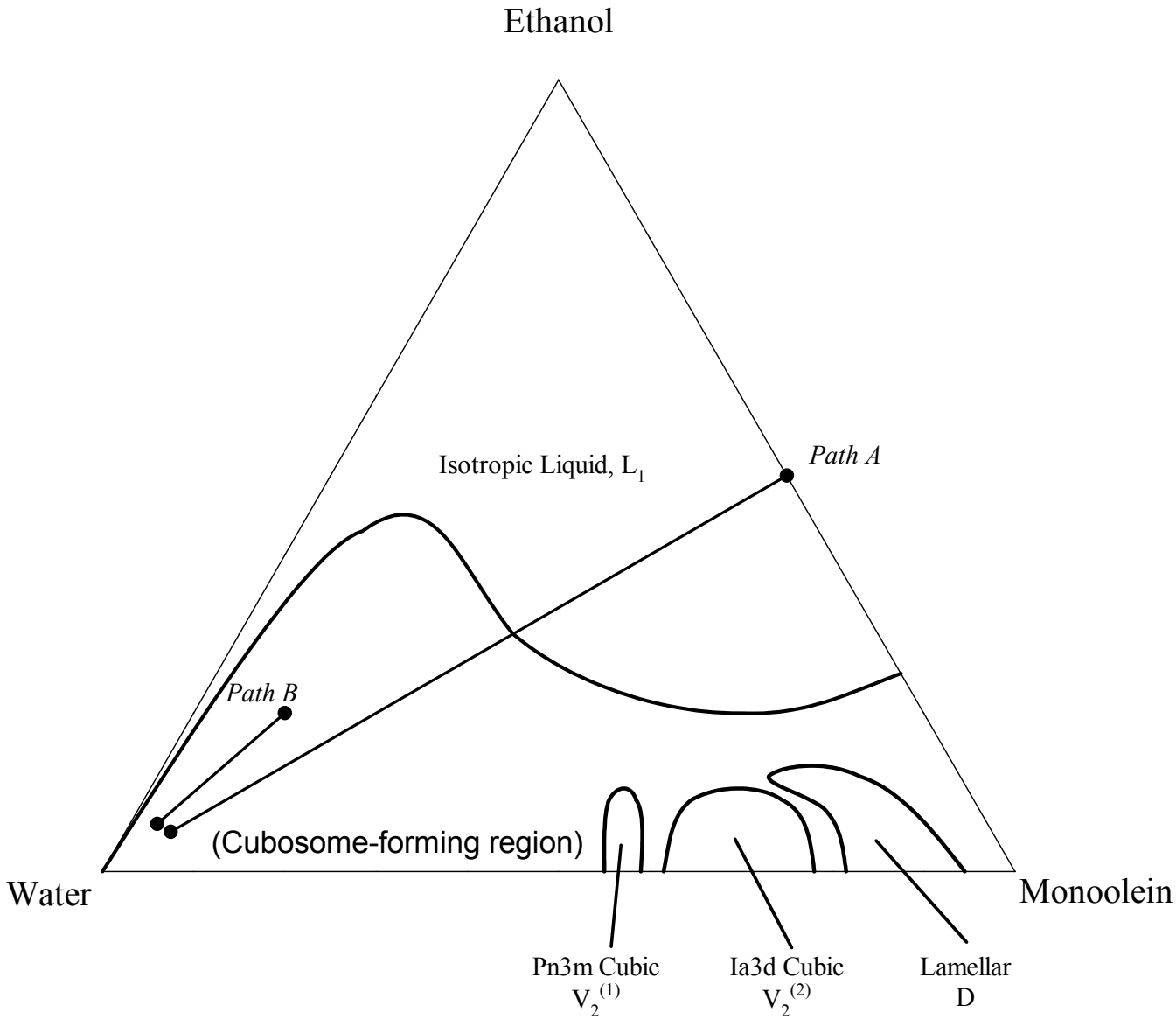
Ethanol
Isotropic Liquid, L_1
Path A
Path B
(Cubosome-forming region)
Water
Monoolein
Pn3m Cubic
$V_2^{(1)}$
Ia3d Cubic
$V_2^{(2)}$
Lamellar
D

Figure 4

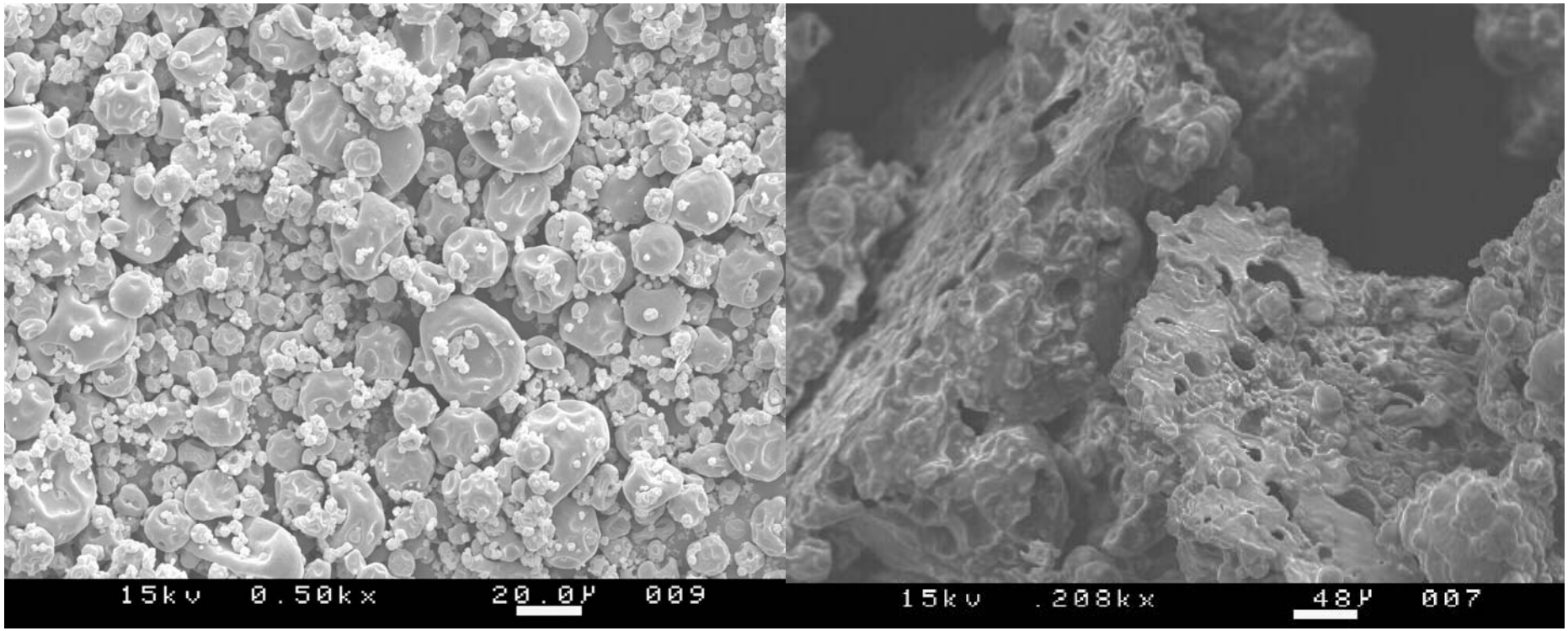

Figure 5

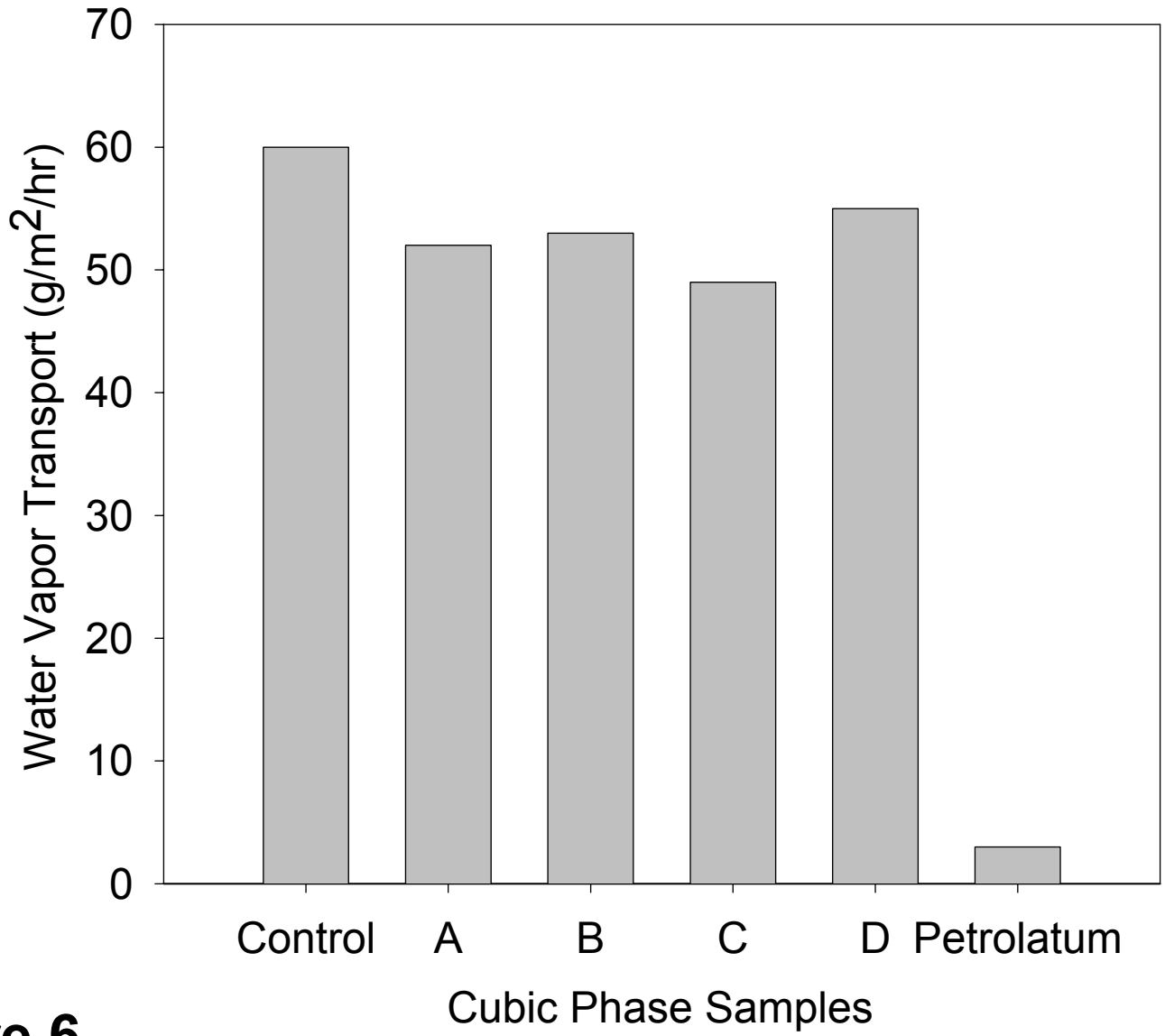
70
60
50
40
30
20
10
0
Water Vapor Transport (g/m2/hr)
Control
A
B
C
D
Petrolatum
Cubic Phase Samples

Figure 6

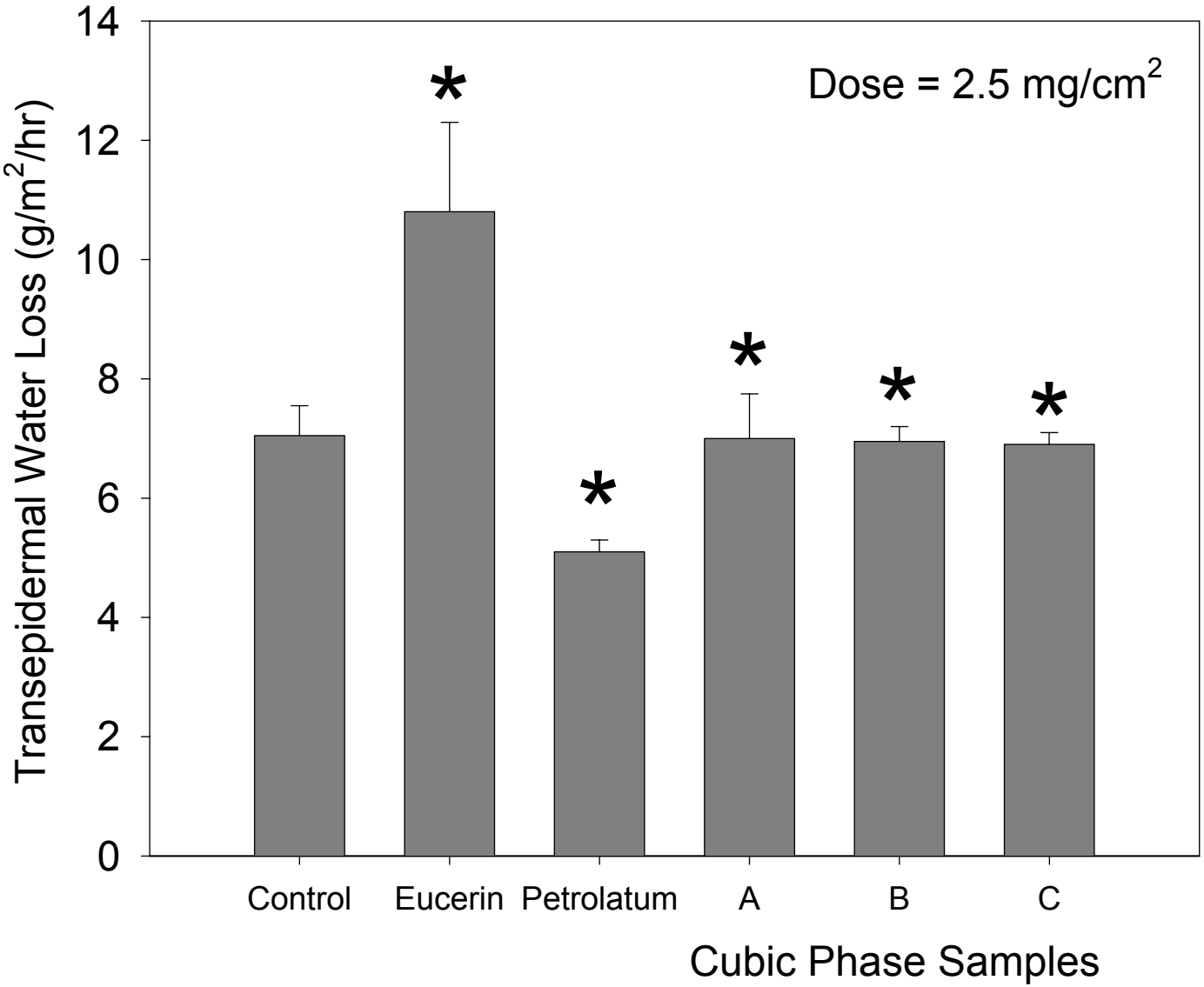
Dose = 2.5 mg/cm2
Transepidermal Water Loss (g/m2/hr)
14
12
10
8
6
4
2
0
*
*
*
*
*
Control
Eucerin
Petrolatum
A
B
C
Cubic Phase Samples

Figure 7

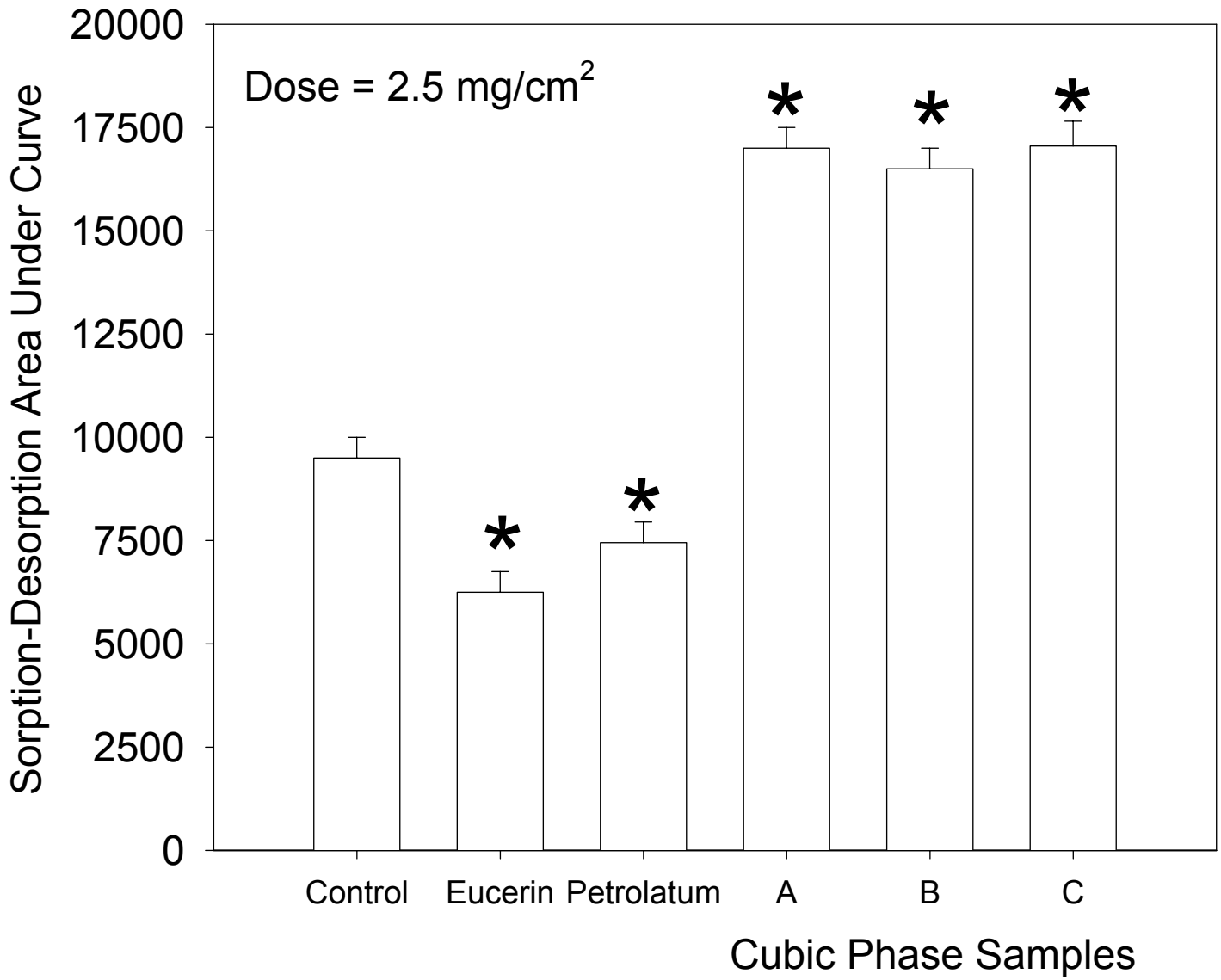
Dose = 2.5 mg/cm2
Sorption-Desorption Area Under Curve
20000
17500
15000
12500
10000
7500
5000
2500
0
*
*
*
*
Control
Eucerin
Petrolatum
A
B
C
Cubic Phase Samples

Figure 8