THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

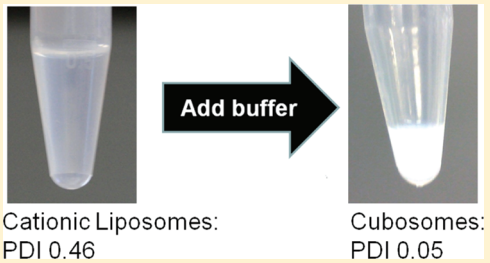
pubs.acs.org/JPCB

Salt Induced Lamellar to Bicontinuous Cubic Phase Transitions in Cationic Nanoparticles

Benjamin W. Muir,* Guoliang Zhen,* Pathiraja Gunatillake, and Patrick G. Hartley

CSIRO Materials Science and Engineering, Bayview Avenue, Clayton, VIC 3168, Australia

ABSTRACT: The development of improved methods to allow the low energy production of cubic phase forming nanoparticles (cubosomes) is highly desired. The lamellar to hexagonal and cubic phase change of these lipid nanoparticles has previously been induced via the lowering of pH and the addition of calcium ions to anionic lipid nanoparticles. We have developed a method to produce low polydispersity cubosomes without the requirement of high energy input such as shear, sonication or homogenization under physiological conditions. We have found that the simple addition of phosphate buffered saline solution to aqueous dispersions of cationic liposome vesicles made with phytantriol results in the spontaneous formation of cubosomes after vortex mixing. This finding demonstrates the potential of utilizing this technique to incorporate shear and temperature sensitive compounds into cubosomes under extremely mild conditions for biomedical and nanotechnological applications.



INTRODUCTION

The self-assembly of amphiphilic molecules into complex phases such as the bicontinuous cubic phase is a broad area of research.[1] Of specific interest is the use of lipids to generate these complex structures.[2] They are particularly attractive as therapeutic agents for cosmetic, drug delivery and medical imaging applications.[2a,3] In the cubic phase, lipid bilayers are arranged so as to produce periodic three-dimensional networks by contorting the bilayers into the shape of infinite periodic minimal surfaces.[4] The lipids which form this phase include monoolein and phytantriol and result in gyroid I_{a3d} (low hydration) and double diamond P_{n3m} (high hydration, inverse cubic) phases. These lipids result in the generation of gels containing bicontinuous water and oil domains with high surface areas, temperature stability and high viscosity that are optically isotropic. Because of their amphiphilic nature both hydrophobic and hydrophilic compounds can be incorporated into their structure.[5] Upon addition of a polymer stabilizer such as the Pluronic F127, viscous gels can be colloidally dispersed after application of mechanical or ultrasonic energy.

A limitation of these systems has been the fact that in general, high energy techniques such as sonication, high shear and high pressure homogenization must be applied to produce stable lipid nanoparticles or cubosomes. This limits the types of compounds that may be incorporated into cubosomes and also poses problems for the industrial scale up of such systems.[6] As such there is an interest to find low energy techniques to produce colloidally stable cubic phase nanoparticles (cubosomes). Spicer et al.[6] have previously employed the use of a hydrotope (5−10% (v/v) ethanol) to spontaneously form cubosomes with monoolein. This method has been used by Rizwan et al.[7] to produce phytantriol cubosomes with ovalbumin encapsulated in their structure. Recently it has also been shown that the modulation of electrostatic interactions via addition of Ca^{2+}, Mn^{2+} and positively charged peptides on negatively charged vesicle surfaces can induce a lamellar to inverse bicontinuous cubic phase change in a dioleoylphosphatidylglycerol/monoolein system.[8] Salt induced lamellar to cubic phase transitions have also been reported in biological lipid membranes.[3b,9] Yamazaki et al.[9a,b] have observed this phase change in mixtures of monoolein with acidic lipids such as oleic acid and dioleoylphosphatidic acid after the addition of saline. They found that when there are a greater amount of electrostatic interactions due to surface charges on the lipid membranes (in water), lamellar structures are favored. The spontaneous curvature of the membranes increased upon addition of NaCl resulted in the formation of cubic phases.

In most systems studied this phase change from lamellar to cubic appears to be dependent on the formation of an intermediate, short-lived hexagonal phase. A lamellar, to hexagonal then cubic phase change has also been observed in a dioleoylphosphatidylserine/monoolein system by reducing the pH to less than 3.[10] The short-lived intermediate hexagonal phase was found to form rapidly (within 12 s) and within half an hour the hexagonal to cubic phase transition was complete. Conn et al.[9c] have reported the dynamics of a lamellar to cubic phase transition in monoeladin in bulk gels. They proposed that the fluid lamellar phase consists of closely packed onion-like vesicles which confine a highly swollen cubic phase that acts as a seed for the formation of ordered P_{n3m} phases via the formation of stalks and then fusion pores. Similar short-lived nonequilibrium intermediate structures have been observed in a cryo-TEM study of the lamellar−cubic transition in a nucleolipid, 5′-deoxy-5-fluoro-*N*4-(cis-9-octadecenyloxycarbonyl) cytidine by Mulet et al.[9b] A study by Yaghmur et al.[11] has shown that upon heating vesicles of monoelaidin stabilized with

Received: January 8, 2012
Revised: February 13, 2012
Published: February 23, 2012

dx.doi.org/10.1021/jp300239g | *J. Phys. Chem. B* 2012, 116, 3551−3556

Pluronic F127, a liposome to cubosome transition occurs at 65 °C.

In this article we report the first use of a binary cationic/cubic phase forming lipid system, didodecyldimethylammonium bromide (DDAB)/phytantriol (Phyt) that when mixed with phosphate buffered saline (PBS) results in the spontaneous formation of cubosomes. The cubosomes produced exhibit a narrow particle size range with uniform shape and minimal by-products such as lipid vesicles commonly found during the synthesis of cubosome solutions using conventional high energy processes are observed.[12] The cubosome production technique can be performed with liquid precursors under physiological conditions making it suitable for the potential incorporation of sensitive biomolecules into the cubosome dispersions. Another benefit over existing low energy production methods includes the fact that the method does not require the subsequent removal of hydrotrope from solution.

EXPERIMENTAL METHODS

Materials. Phytantriol (98.0%) was obtained from DSM Nutritional Products. Pluronic F127 (poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide), didodecyldimethylammonium bromide (DDAB), and phosphate buffered saline (100 mM PBS) tablets were purchased from Sigma and used as received (Figure 1).

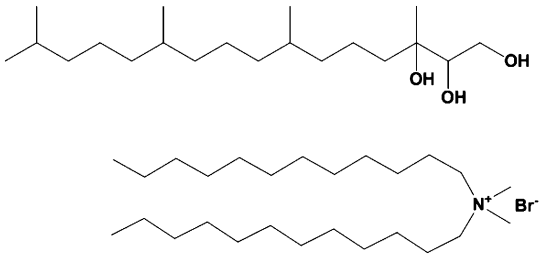


Figure 1. Chemical structure of phytantriol (phyt) amphiphile (top structure) and didodecyldimethylammonium bromide (DDAB) amphiphile (bottom structure) used to produce vesicles that spontaneously form cubosomes after addition of buffer.

Synthesis of Nanoparticles. Phytantriol and DDAB vesicle dispersions were prepared by vortex mixing 600 mg of phytantriol with 90 mg of DDAB and 60 mg of Pluronic F127 in a glass vial at 70 °C. The glass vial was then cooled to 60 °C, and 10 mL of Milli-Q water was added prior to dispersion via ultrasonication (MisonixXL2000, Misonix Incorporated) for 3 min in pulse mode (15 s pulses interrupted by 5 s breaks) at 70% of maximum power. This resulted in a clear dispersion being produced which was stored at 25 °C for 48 h prior to further experimentation. The dispersions were combined with PBS buffer (100 mM) at a volume ratio of 1 to 2 (v/v = 1:2). Typically 50 μL of the DDAB/Phyt vesicle dispersion was mixed with 100 μL of PBS buffer then vortex mixed in a 1.5 mL Eppendorf tube for 10 s. This results in a final PBS concentration of 0.66 mM. The resultant solution immediately turned milky upon addition of buffer. The cubosome solutions were dialyzed against Milli Q water for 24 h using a Spectra/Por membrane with a molecular weight cut off at 6000−8000 g/mol. The cubosome solution turned from milky to transparent and increased viscosity upon dialysis.

Particle Size Measurements. Particle size measurements were performed in PBS buffer (100 mM) using a Zetasizer-Nano instrument (Malvern, U.K.). Measurements were conducted at 25 °C, using automated settings in standard disposable cuvettes.

Cryo-TEM Measurements. Samples for cryo-TEM studies were examined using a Gatan 626 cryoholder (Gatan, Pleasanton, CA, USA) and Tecnai 12 Transmission Electron Microscope (FEI, Eindhoven, The Netherlands) at an operating voltage of 120 kV. Images were recorded using a Megaview III CCD camera and AnalySIS camera control software (Olympus) using magnifications in the range 60 000−110 000 × 200-mesh copper grids coated with perforated carbon film (Lacey carbon film: ProSciTech, Qld, Australia) were used to prepare all samples using a method which has been reported previously.[13]

SAXS Analysis. Small-angle X-ray scattering (SAXS) experiments were performed on the SAXS/WAXS beamline at the Australian Synchrotron. Glass capillaries (1.5 mm) containing sample solutions were placed in a temperature controlled sample holder maintained at 37 °C. Samples were exposed to the 12 keV X-ray beam of dimensions 2500 μm × 130 μm and a typical flux of 5×10^{12} photons/s and diffraction patterns were recorded using a Pilatus 1 M detector (Dectris, Switzerland). A silver behenate standard was used to calibrate the reciprocal space vector for analysis. Data reduction (calibration and integration) was performed using AXcess, a custom-written SAXS analysis program written by Dr. Andrew

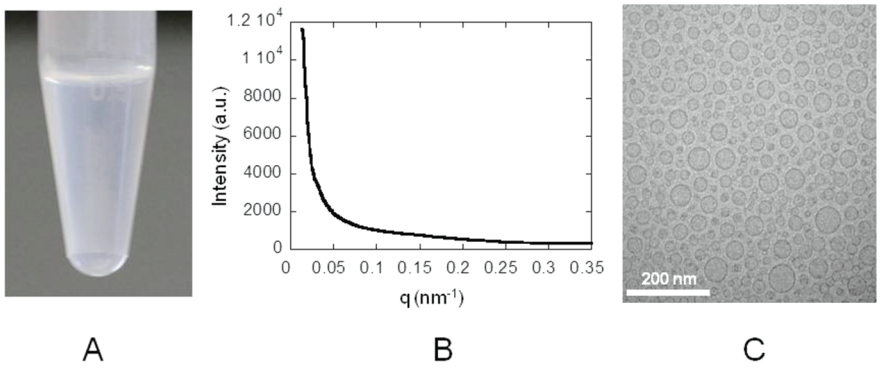


Figure 2. Characterization of the dispersion prepared by ultrasonication of phytantriol (phyt) and 15 wt % (w/w) didodecyldimethylammonium bromide (DDAB) in water which is stabilized by the Pluronic F127. (A) Visual observation (optical image) in water shows a transparent liquid. (B) Small angle X-ray scattering (SAXS) shows monotonically decreasing scattering at low Q and a lack of Bragg peaks, indicating a dispersion of nonliquid crystalline particles. (C) Cryo-TEM shows spherical aggregates in the particle size range 10−60 nm, consistent with unilamellar vesicles.

dx.doi.org/10.1021/jp300239g | J. Phys. Chem. B 2012, 116, 3551−3556

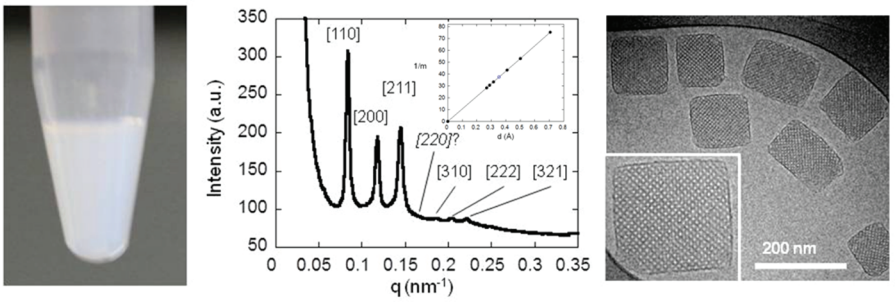


Figure 3. Characterization of the dispersion resulting from the addition of phosphate buffered saline (PBS) (final concentration of 66.6 mM) to the vesicle dispersion of phytantriol (phyt) and 15 wt % (w/w) didodecyldimethylammonium bromide (DDAB) vesicles stabilized by the Pluronic F127 shown in Figure 2. (A) Visual observation shows a turbid liquid. (B) small angle X-ray scattering (SAXS) shows the appearance of Bragg peaks consistent with an *Im*3*m* cubic space group lyotropic liquid crystalline system. Inset displays the Miller index plot obtained. The empty circle shows the expected position of the [2,2,0] reflection, which was not unambiguously detected. The slope of the plot gives the lattice parameter for the *Im*3*m* cubic phase: 106.3 Å. $m = \sqrt{(h^2 + k^2 + l^2)}$; $d = 2\pi/Q$ for Q values of the measured Bragg peaks. (C) Cryo-TEM shows well dispersed discrete uniform colloidal cubic phase particles around 140 nm in size. (inset picture shows a cubosome 140 nm in diameter with a periodic internal structure typical of cubic phase materials).

Heron from Imperial College, London.[14] The bicontinuous cubic phases with *Im*3*m* symmetry observed in this study were identified from the positions of diffracted peaks at $\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{10}$, $\sqrt{12}$, $\sqrt{14}$. All samples were measured via SAXS and cryo-TEM within 24 h of their synthesis.

RESULTS AND DISCUSSION

The process we have developed involves the simple vortex mixing of two aqueous solutions, one containing liposomes of DDAB/Phyt and the other containing PBS. The key to this process is the screening of charge on the cationic lipid in the bulk phytantriol vesicles which allows the now well documented phase transition[8a,b,9b,10,11] (lamellar to cubic) to occur at neutral pH and room temperature. This eliminates the need to use high energy processes, hydrotopes such as ethanol, or highly acidic environments to produce uniform cubosome dispersions. Phytantriol was chosen in this study as its phase behavior is well documented and it is able to tolerate significant amounts of additives (up to 10 wt %) and retain its cubic phase structure when dispersed into colloidally stable nanoparticles. At additive concentrations over 15 wt % it is generally unable to retain its cubic phase structure however. The structures of DDAB, Phyt, and the Pluronic used in this work can be seen in Figure 1. An optical image of the vesicle dispersion produced after sonication of the DDAB/Phyt gel in the presence of Pluronic F 127 and cryo-TEM images of these polydisperse vesicles can be seen in Figure 2, parts A and C, respectively. The corresponding SAXS spectra can be seen in Figure 2B displaying monotonically decreasing scattering at low Q and a lack of Bragg peaks, indicating a dispersion of nonliquid crystalline particles. Broad correlation peaks due to vesicle scattering were not detected, presumably due to the weak scattering in this dilute system. It is likely that any liposome correlation peaks in our dilute system were below the sensitivity of the SAXS measurement. Therefore, the formation of multilamellar vesicles in this study cannot be confirmed or ruled out.

Immediately after addition of PBS to this dispersion after vortexing, the solution turns milky (Figure 3A). Cryo-TEM of the milky solution (Figure 3 C) reveals a dispersion of cubosomes that are extremely uniform in size and shape displaying typical discrete colloidal nanoparticles approximately 140 nm in size which is consistent with dynamic light scattering (DLS) measurements (Table 1). SAXS analysis confirmed the

Table 1. Dynamic Light Scattering Analysis Data of Lipid Nanoparticle Size Changes (Intensity vs Size (nm)) and Measured Polydispersity Index (PDI) of Didodecyldimethylammonium Bromide (DDAB)/ Phytantriol Nanoparticle Dispersions during Addition and Removal of Phosphate-Buffered Saline (PBS) Solution

	size (nm)	PDI
vesicle dispersion in water	106	0.46
after addition of PBS (cubosome dispersion)	126	0.03
after dialysis in water (vesicle dispersion)	156	0.21
after addition of PBS (cubosome dispersion)	122	0.05

lamellar to cubic phase change in the lyotropic liquid crystalline nanoparticles after mixing with PBS (Figure 3B). The Bragg peaks measured in this SAXS experiment index to the expected spacings for an *Im*3*m* space group, with the ratios of peak positions following the progression $\sqrt{2}$, $\sqrt{4}$, $\sqrt{6}$, $\sqrt{8}$, $\sqrt{10}$, $\sqrt{12}$ and corresponding to diffraction planes with Miller indices [*h*,*k*,*l*] which are shown in the figure. We note that the expected $\sqrt{8}$ = [2,2,0] reflection could not be unambiguously identified, presumably due to it being masked by the strong $\sqrt{6}$ = [2,1,1] peak. Further analysis of the SAXS data using the Miller index plot (inset of Figure 3B) yields a lattice parameter of 106.3 Å. This is consistent with earlier observations of charged lipid containing cubosome formulations, which also showed Im3m symmetry and lattice parameters in a similar range.[15]

The cubosomes produced with this technique have a very narrow particle size range and polydispersity index which is atypical of such systems. A remarkable finding of this procedure is the decrease in polydispersity in the cubosome solution after addition of PBS buffer when compared to the significantly more polydisperse lipid vesicles (Table 1). Upon dialysis of the cubosome dispersion and removal of buffer, the nanoparticles reverted to a transparent vesicle dispersion, presumably comprising uni- or multilamellar vesicles. Upon further addition of buffer the dispersion once again converted to a turbid

dx.doi.org/10.1021/jp300239g | J. Phys. Chem. B 2012, 116, 3551−3556

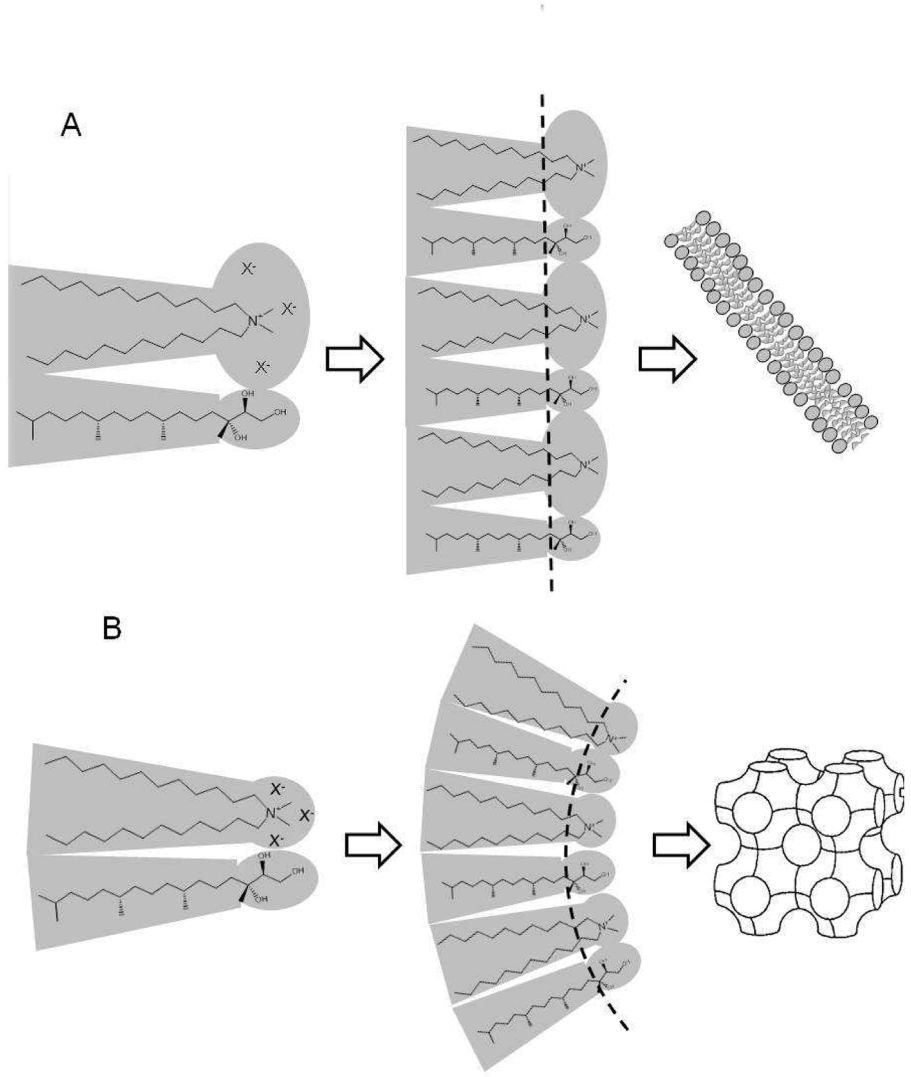


Figure 4. Schematic representation of the effect of increasing salt concentration on surfactant interactions, packing, and lyotropic liquid crystalline behavior. (A) At low salt concentration, counterion screening of the positive charge of the DDAB headgroup is minimal, resulting in a large effective headgroup area at the surfactant−water interface. The resulting surfactant packing constraints yield a planar interface, and lamellar bilayer structures (the basis for vesicle dispersions), are favored. (B) At higher salt concentrations (i.e., after buffer addition), increased charge screening results in a smaller effective headgroup area at the surfactant−water interface, and more negatively curved interfaces, (such as that found in inverse cubic lyotropic liquid crystals.

cubosome suspension (as confirmed by CryoTEM, data not shown) indicating the robustness of this technique (Table 1). It is clear that multiple lipid vesicles must fuse in order to produce a single cubosome. Cubosomes have extremely high surface areas, on the order of 400 m^2/g of cubic phase compared to their spherical counterparts, liposomes.[16] The fusion process during lamellar-cubic phase transitions of previously studied cubic phase forming lipids is understood to first result in the formation of a hexagonal phase followed by the cubic phase.[8a,b,9b,10,11] One would expect the DDAB/Phyt system to behave in a similar manner, however due to our experimental methodology in this work, no intermediate hexagonal phase material was observed.

We propose that the lamellar to final cubic phase change occurs as a result of charge shielding of the cationic DDAB lipid head groups from anions in the PBS buffers electrical double layer resulting in a change to the lipid bilayer curvature induced from closer packing of the cationic lipid headgroups. We have shown this process schematically in Figure 4. It is highly likely that an intermediate hexagonal phase is formed during this process but at this early stage we have no experimental evidence of this. Regardless of the mechanism of the lamellar to cubic phase change, the reason as to why the polydispersity index (PDI) of the cubosome dispersion should drop so dramatically compared to the vesicle dispersions PDI during this process is unclear at this stage. Polydispersity indices as low as 0.03 for cubosome dispersions are rarely observed and more typical values lie in the range of 0.05−0.2.[17]

■ CONCLUSION

In conclusion, we have reported on a new binary lipid system (DDAB/Phyt) that can be used to produce homogeneous and uniform cubosome dispersions without using high energy processes traditionally employed such as high pressure homogenization. The PBS buffer mixing process we have developed may allow for the scale-up of cubosome processing and allow sensitive bioactive molecules such as proteins, peptides, oligonucleotides and drugs to be formulated into the

buffer solution prior to mixing with the cationic vesicles. This would enable the production of colloidally stable dispersions with incorporated bioactive molecules for controlled release applications and bioassays.[18] The technique could also be used to incorporate drugs for delivery applications and contrast agents for medical imaging applications resulting in nanoparticles with small polydispersities. Future work will involve investigating other lipid additives which upon application of buffer or kosmotropic/chaotrope solutions result in the spontaneous formation of cubosomes from vesicle dispersions and the incorporation of sensitive bioactive compounds into the cubosomes using this technique.

AUTHOR INFORMATION

Corresponding Author

*E-mail: (B.W.M.) Ben.Muir@csiro.au; (G.Z.) Guoliang.Zhen@gmail.com.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Lynne Waddington for cryo-TEM analysis and Durga Acharya, Nigel Kirby, and Adrian Hawley for help with SAXS analysis. This research was undertaken on the SAXS/WAXS beamline at the Australian Synchrotron, Victoria, Australia.

REFERENCES

(1) (a) Drummond, C. J.; Fong, C. Surfactant self assembly objects as novel drug delivery vehicles. *Curr. Opin. Colloid Interface Sci.* **2000**, *4*, 449−456. (b) Engström, S. Cubic phases as drug delivery systems. *Polym Preprint* **1990**, *31*, 157−158. (c) Engström, S.; Ericsson, B.; Landh, T. A cubosome formulation for intravenous administration of somatostatin. *Proc. Int. Symp. Control Rel. Bioact. Mater.* **1996**, *23*, 89−90. (d) Luzzati, V.; Delacroix, H.; Gulik, A.; Guilk-Krzywicki, T.; Mariani, P.; Vargas, R. The cubic phases of lipids. *Current Topics in Membranes*; Academic Press: San Diego, CA, 1997; Vol. *44*. (e) Lynch, M. L.; Ofori-Boateng, A.; Hippe, A.; Kochvar, K.; Spicer, P. T. Enhanced loading of water-soluble actives into bicontinuous cubic phase liquid crystals using cationic surfactants. *J. Colloid Interface Sci.* **2003**, *260* (2), 404−413. (f) Nielsen, L. S.; Schubert, L.; Hansen, J. Bioadhesive Drug Delivery Systems I. Characterisation of mucoadhseive properties of systems based on glyceryl monooleate and glyceryl monolinoleate. *Eur. J. Pharm. Sci.* *6*, 231−239; (g) Scriven, L. E. Equilibrium bicontinuous structure. *Nature* **1976**, *263* (5573), 123−125.

(2) (a) Efrat, R.; Abramov, Z.; Aserin, A.; Garti, N. Nonionic-Anionic Mixed Surfactants Cubic Mesophases. Part I: Structural Chaotropic and Kosmotropic Effect. *J. Phys. Chem. B* **2010**, *114* (33), 10709−10716. (b) Boyd, B. J. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. *Int. J. Pharm.* **2003**, 239−247.

(3) (a) Muir, B. W.; Moffat, B. A.; Acharya, D. A.; Mulet, F.; Kennedy, D. F.; Evans, R. A.; Pereira, S.; Boyd, B. B.; Wark, K.; Nguyen, H. T.; Hinton, T.; Waddington, L.; Kirby, N.; Wright, D.; Wang, H.; Egan, G. F. Metal-free and MRI visible theranostic lyotropic liquid cyrstal nitroxide-based nanoparticles. *Biomaterials* **2012**, *33* (9), 2723−2733. (b) Masahito, Y., Chapter 7 Transformation Between Liposomes and Cubic Phases of Biological Lipid Membranes Induced by Modulation of Electrostatic Interactions. In *Advances in Planar Lipid Bilayers and Liposomes*, Liu, A. L., Ed.; Academic Press: San Diego, CA, 2009; Vol. *9*, pp 163−209.

(4) Barauskas, J.; Johnsson, M.; Joabsson, F.; Tiberg, F. Cubic phase nanoparticles (cubosome): Principles for controlling size, structure, and stability. *Langmuir* **2005**, *21* (6), 2569−2577.

(5) (a) Mulet, X.; Kennedy, D. F.; Conn, C. E.; Hawley, A.; Drummond, C. J. High throughput preparation and characterisation of amphiphilic nanostructured nanoparticulate drug delivery vehicles. *Int. J. Pharm.* **2010**, *395* (1−2), 290−297. (b) Gong, X. J.; Moghaddam, M. J.; Sagnella, S. M.; Conn, C. E.; Mulet, X.; Danon, S. J.; Waddington, L. J.; Drummond, C. J. Nanostructured self-assembly materials from neat and aqueous solutions of C18 lipid pro-drug analogues of Capecitabine-a chemotherapy agent. Focus on nanoparticulate cubosomes (TM) of the oleyl analogue. *Soft Matter* **2011**, *7* (12), 5764−5776.

(6) Spicer, P. T.; Hayden, K. L.; Lynch, M. L.; Ofori-Boateng, A.; Burns, J. L. Novel process for producing cubic liquid crystalline nanoparticles (cubosomes). *Langmuir* **2001**, *17* (19), 5748−5756.

(7) Przybylo, M.; Borowik, T.; Langner, M. Fluorescence Techniques for Determination of the Membrane Potentials in High Throughput Screening. *J. Fluoresc.* **2010**, *20* (6), 1139−1157.

(8) (a) Yaghmur, A.; Laggner, P.; Sartori, B.; Rappolt, M. Calcium Triggered L(alpha)-H(2) Phase Transition Monitored by Combined Rapid Mixing and Time-Resolved Synchrotron SAXS. *PLoS One* **2008**, *3*, 4. (b) Awad, T. S.; Okamoto, Y.; Masum, S. M.; Yamazaki, M. Formation of cubic phases from large unilamellar vesicles of dioleoylphosphatidylglycerol/monoolein membranes induced by low concentrations of Ca^{2+}. *Langmuir* **2005**, *21* (25), 11556−11561. (c) Conn, C. E.; Mulet, X.; Moghaddam, M. J.; Darmanin, C.; Waddington, L. J.; Sagnella, S. M.; Kirby, N.; Varghese, J. N.; Drummond, C. J. Enhanced uptake of an integral membrane protein, the dopamine D2L receptor, by cubic nanostructured lipid nanoparticles doped with Ni(II) chelated EDTA amphiphiles. *Soft Matter* **2011**, *7* (2), 567−578.

(9) (a) Aota-Nakano, Y.; Li, S. J.; Yamazaki, M. Effects of electrostatic interaction on the phase stability and structures of cubic phases of monoolein/oleic acid mixture membranes. *Biochim. Biophys. Acta* **1999**, *1461* (1), 96−102. (b) Li, S. J.; Yamashita, Y.; Yamazaki, M. Effect of electrostatic interactions on phase stability of cubic phases of membranes of monoolein/dioleoylphosphatidic acid mixtures. *Biophys. J.* **2001**, *81* (2), 983−993. (c) Masum, S. M.; Li, S. J.; Tamba, Y.; Yamashita, Y.; Tanaka, T.; Yamazaki, M. Effect of de novo designed peptides interacting with the lipid-membrane interface on the stability of the cubic phases of the monoolein membrane. *Langmuir* **2003**, *19* (11), 4745−4753.

(10) Alam, M. M.; Oka, T.; Ohta, N.; Yamazaki, M. Kinetics of low pH-induced lamellar to bicontinuous cubic phase transition in dioleoylphosphatidylserine/monoolein. *J. Chem. Phys.* **2011**, *134*, 14.

(11) Yaghmur, A.; Laggner, P.; Almgren, M.; Rappolt, M. Self-Assembly in Monoelaidin Aqueous Dispersions: Direct Vesicles to Cubosomes Transition. *PLoS One* **2008**, *3*, 11.

(12) Gustafsson, J.; Ljusberg-Wahren, H.; Almgren, M.; Larsson, K. Submicron particles of reversed lipid phases in water stabilised by a nonionic amphiphilic polymer. *Langmuir* **1997**, *13*, 6964−6971.

(13) Muir, B. W.; Moffat, B. A.; Harbour, P.; Coia, G.; Zhen, G. L.; Waddington, L.; Scoble, J.; Krah, D.; Thang, S. H.; Chong, Y. K.; Mulvaney, P.; Hartley, P. Combinatorial Discovery of Novel Amphiphilic Polymers for the Phase Transfer of Magnetic Nanoparticles. *J. Phys. Chem. C* **2009**, *113* (38), 16615−16624.

(14) Seddon, J. M.; Squires, A. M.; Conn, C. E.; Ces, O.; Heron, A. J.; Mulet, X.; Shearman, G. C.; Templer, R. H. Pressure-jump X-ray studies of liquid crystal transitions in lipids. *Philos. Trans. R. Soc. A: Math. Phys. Eng. Sci.* **2006**, *364* (1847), 2635−2655.

(15) Shen, H. H.; Crowston, J. G.; Huber, F.; Saubern, S.; McLean, K. M.; Hartley, P. G. The influence of dipalmitoyl phosphatidylserine on phase behaviour of and cellular response to lyotropic liquid crystalline dispersions. *Biomaterials* **2010**, *31* (36), 9473−9481.

(16) Lawrence, M. J. Surfactant systems: their use in drug delivery. *Chem. Soc. Rev.* **1994**, 417−423.

(17) Efrat, R.; Kesselman, E.; Aserin, A.; Garti, N.; Danino, D. Solubilization of Hydrophobic Guest Molecules in the Monoolein Discontinuous Q(L) Cubic Mesophase and Its Soft Nanoparticles. *Langmuir* **2009**, *25* (3), 1316−1326.

(18) Angelova, A.; Angelov, B.; Mutafchieva, R.; Lesieur, S.; Couvreur, P. Self-Assembled Multicompartment Liquid Crystalline Lipid Carriers for Protein, Peptide, and Nucleic Acid Drug Delivery. *Acc. Chem. Res.* **2011**, *44* (2), 147−156.