Cubosome particles of a novel Guerbet branched chain glycolipid

Malinda Salim, N. Idayu Zahid, Chia Yen Liew and Rauzah Hashim

Center of Fundamental Science of Self-Assembly, Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

ABSTRACT

Cubic liquid crystalline nanoparticles (cubosomes) of bicontinuous nature with internal networks of water channels have received great interests in nanomedicine applications, particularly as potential vehicle for loading and release of therapeutic agents. These nanoparticles have been most commonly produced using monoolein and phytantriol. In this study, we explore the use of a Guerbet branched chain glycolipid, namely 2-hexyl-decyl-β-D glucopyranoside (β-Glc−OC_{10}C_{6}), as a new and alternative material for cubosomes production. The fully hydrated glycolipid assumes a reverse bicontinuous cubic liquid crystal phase of an I_{a3d} space group with lattice parameter of ca. 74 Å, as confirmed using a small-angle X-ray scattering. Dynamic light scattering and a conventional transmission electron microscopy were used to investigate the average size and morphology of the cubosomes. The effectiveness of Poloxamer 407 (stabiliser typically used in other cubosome systems against aggregations and particle coalescence) in providing steric stabilisation of the glycolipid cubosomes was assessed through visual assessment.

CONTACT
Rauzah Hashim rauzah@um.edu.my

Supplemental data for this article can be accessed here.

© 2015 Taylor & Francis

LIQUID CRYSTALS, 2016
VOL. 43, NO. 2, 168-174
http://dx.doi.org/10.1080/02678292.2015.1085104

Guerbet glycosides are synthetic glycolipids that mimic the natural ones, such as monogalactosyl diacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). Their most important features are the presence of a hydrophobic branched-alkyl chain and hydrophilic sugar headgroup.\[1\] This family of glycosides is prepared by reacting a protected sugar with Guerbet alcohol, which was first synthesised by Marcel Guerbet in 1899. The Guerbet glycoside series is also known as Guerbet sugar, which, along with other Guerbet materials (including the alcohol, ester and acid), have been used in many industrial applications.\[2\] Due to its amphiphilicity, branched chain Guerbet glycolipids can assume various liquid crystal phases that can potentially be exploited as nano-carriers for controlled delivery of active materials. Lamellar, inverse hexagonal, inverse micellar, and inverse cubic phases have been observed in the thermotropic and lyotropic systems of different Guerbet glycosides such as glucosides and galactosides.\[3\]

Liquid crystalline nano-sized particles can therefore be formulated through the dispersion of lamellar, hexagonal, and cubic phases termed vesicles, hexosomes, and cubosomes, respectively. Despite being increasingly investigated for their bioadhesive properties,\[4\] and for skin treatment due to structure similarities in human skin,\[5\] the use of cubosomes for controlled and sustained delivery is still relatively new compared to other nanoparticles derived from liquid crystalline phases such as vesicles and micelles.\[6,7\]
Cubosomes are most commonly produced using monoolein (GMO),[8] phytantriol (PHYT),[9] and block copolymers,[10] as the self-assembly of these amphiphiles can form the triply periodic minimal surfaces (TPMS) inverse bicontinuous cubic phases (gyroid, primitive, or double diamond) that are stable in excess water. This inverse property is important for the system to not undergo phase changes during dilution in aqueous solution, such as gastrointestinal fluid,[11] while the internal water channels provide large surface area for active compound (which can be of hydrophilic, hydrophobic or amphiphilic nature) adsorption and entrapment.[6,10,12–14] Inverse cubic liquid crystalline phase of bicontinuous nature has received much attention in the recent years, particularly in the biomedical, food and cosmetic industries, due to its unique three-dimensional highly ordered periodic structure and large lipid/water interfacial area of approximately 400 m²/g of amphiphile. [15] The cubic phase of monoolein, for example, has been shown to incorporate drugs up to 5–10% of its weight without undergoing phase changes.[15] This cubic liquid crystal phase also has the advantage of being utilised as a biocompatible and a stable template for membrane protein crystallisation [16] as well as solubilisation of organic molecules.[17]

We have previously shown that 2-hexyl-decyl-β-D-glucopyranoside (β-Glc−OC₁₀C₆, see Figure 1(a)) can form inverse bicontinuous cubic liquid crystal phases in Ia₃d and/or Pn₃m space groups from the water content of 10–80 wt% at temperature <60°C.[3] Furthermore, β-Glc−OC₁₀C₆ can be regarded as a double-chain lipid (see Figure 1(a)–(b) for structural comparison with other double-chained glycolipid). [18] Derived from Guerbet alcohol, it contains two asymmetric chains that differ by two methylene units, and the two chains branched at the β-carbon position. An ether linkage connects the hydrophobic tail to the glucose at the first carbon position (C1). The ratio between the longest straight parts in the carbon chain tail to the total number of carbon, which dictates a measure of the branching, is 0.625 for β-Glc−OC₁₀C₆. This indicates a low measure of branching (smaller ratio represents higher branching, while a straight carbon chain with no branching has a ratio of 1).[19] The partial binary phase diagram of β-Glc−OC₁₀C₆ is presented in Figure S1 in the supplementary material. Evidently, Guerbet branched chain glycolipid is an alternative novel material to produce cubosomes that could potentially be utilised in pharmaceutical and theranostic applications.

Here, we investigate the formation of cubic liquid crystalline nanoparticles (cubosomes) using β-Glc−OC₁₀C₆ based on the top-down approach.[20] The glycolipid was synthesised as described previously,[21] and the anomeric purity was determined to be ca. 98% using proton nuclear magnetic resonance (¹H-NMR). Bulk cubic phase of β-Glc−OC₁₀C₆ was prepared by weighing 50 mg of anhydrous β-Glc−OC₁₀C₆ into a glass vial followed by heating to its isotropic phase at 65°C. One millilitre of water (which correspond to 50 mg/ml lipid concentration and 95% total water content) was then added with or without Poloxamer 407 (P407) stabiliser at 0–10 (P407/β-Glc−OC₁₀C₆) w/w%. Although the tested condition was beyond the range shown in the partial binary phase diagram (Figure S1), high water content was selected since concentration of lipid in dispersions was typically in the range of 2–5 wt%.[22–24] Poloxamer 407, also known as Pluronic F127, was a non-ionic amphiphilic polyoxyethylene/polyoxypropylene/polyoxyethylene (PEO/PPO/PEO) triblock copolymer that was extensively used as steric stabiliser in cubosome dispersion.[8,25] The resulting sample mixture was heated at 65°C for 1 hour, centrifuged, and equilibrated at room temperature (25°C) for a minimum of 3–5 days. Short sample equilibration time

![Figure 1. Structures of β-Glc−OC₁₀C₆ (a) and double-chained isoprenoid glucolipid (b). The ratio between the longest straight part in the carbon chain tail to the total number of carbon (a measure of branching) in (a) and (b) is 0.625 and 0.4, respectively.](image)
would result instead in the formation of hexagonal phase (hence hexosomes), as was described in our previous study.[26]

As mechanical or sonic energy is required for cubosomes formation in the top-down approach,[20] the bulk β-Glc–OC_{10}C_{6} cubic gel with 0–10 w/w% P407 was subjected to a 60-minute probe sonication (500 watt, 30% amplitude) at a pulse of 3 sec on: 15 sec off. Effectiveness of P407 as steric stabiliser was investigated by visual assessment, and the results summarised in Table 1.

Addition of P407 stabiliser yielded milky white dispersion with visible aggregates observed in all the tested concentrations, although least aggregation was obtained at 1 wt%. Meanwhile, translucent cloudy dispersion with few visible lipid aggregates was observed in sample without additive. Our observation differs from those obtained in monoolein and phytantriol systems, where the absence of stabiliser showed no dispersion formation, and enhanced cubosome stability was obtained at high (1–2%) P407 concentrations. Since a stable well-dispersed sample contains no aggregates,[25] P407 is a better steric stabiliser for phytantriol and monoolein compared to our branched glycolipid. The difference in behaviours could be related to the lipid structures. The branched hydrocarbon chain of the Guerbet glycolipid may provide a greater steric hindrance to the adsorption of propylene oxide (PO) units to the hydrophobic tails. Stability of the nanoparticles is therefore reduced, as anchoring of PO to the nanoparticle surface while extending its hydrophilic ethylene oxide units to the outer aqueous solution plays an important role in stabilisation of the cubosomes.[25,27]

Cubosome samples that contain 0 (for negative control) and 1 wt% P407 were centrifuged at 10k rpm for 5 minutes to remove large particulates prior to size analysis. Visual inspection of the resulting samples shows a translucent cloudy solution in 0% P407, and milky white dispersion in 1% P407 with minimal aggregates. Average hydrodynamic size (z-average) and zeta potential of the freshly prepared cubosomes, and the size stability over a course of 1 month room-temperature storage is reported in Table 2. Measurements were carried out using dynamic light scattering (Zetasizer Nano-ZS, Malvern). It was found that the z-average of the freshly prepared 0% P407 doubled from ca. 162 nm to ca. 365 nm after 2 weeks of incubation at room temperature, indicating particle aggregation. At 1% P407, the average diameter of the nanoparticles increased after 1 day incubation from ca. 184 to 223 nm, and stayed relatively stable for 1 month. However, the PDI was found to increase with storage time in both 0% and 1% P407, that is, sample became more heterogeneous in size. The mean particle size of our prepared cubosomes was comparable to those reported in the literatures for monoolein and phytantriol with size range of 100–500 nm.[28,29]

Zeta potential measurements of the dispersed cubic phase revealed net negative charge particles in water solution (ca.–33.8 mV) due to presence of hydroxyl groups in the sugar head (see Table 2). Phytantriol and monoolein cubosomes have been reported to possess net negative charge of ca. −35 and −29 mV, respectively.[23,28] Significant decrease in overall charge of the cubosome nanoparticles from ca.–33.8 to −13.5 mV was observed on inclusion of poloxamer additives, which may be caused by shielding of EO polymeric units on the negatively charged cubosome surface.

Internal order of non-dispersed bulk phase formed by β-Glc–OC_{10}C_{6} in 95 wt% excess water, dispersed β-Glc–OC_{10}C_{6}, and the effects of P407 addition on structure changes were characterised using small-angle X-ray scattering (SAXS) on a SAXSess (Anton Paar, Austria). The system was equipped with an X-ray generator producing Cu Kα radiation (λ = 1.542 Å) operating at 40 kV and 50 mA, and a Mythen CCD detector. Measurements were carried out at 25°C with acquisition time of 4 and 15 hours for bulk and dispersed samples, respectively.

Diffraction pattern of bulk β-Glc–OC_{10}C_{6} in 95 wt% excess water confirms a set of five Bragg peaks that are characteristic of a gyroid bicontinuous cubic phase with an Ia3d space group. The relative Bragg peak position ratios are √6, √8, √14, √16 and √29, which correspond to cubic hkl lattice planes of 211, 220, 321, 400 and 420 (see Figure 2). In an Ia3d reversed bicontinuous cubic phase, the lipids were arranged such that two aqueous channel networks (connected co-planarly 3 × 3) were interwoven yet unconnected.[30] Figure 2 demonstrates that the Ia3d

### Table 2. Average hydrodynamic size and zeta potential of the dispersed nanoparticles.

| %P407 | Fresh | 1 day | 2 weeks | 1 month | ζ (mV) |
|-------|-------|-------|---------|---------|--------|
| 0     | 162 ± 3 | 176 ± 2 | 365 ± 5 | 402 ± 16 | −33.8 ± 4 |
| 1     | 184 ± 2 | 223 ± 6 | 254 ± 2  | 255 ± 4  | −13.5 ± 2  |

### Table 1. Visual assessment of the stability of β-Glc–OC_{10}C_{6} dispersion on Poloxamer 407 addition.

| %P407 | 0% | 1% | 5% | 10% |
|-------|----|----|----|-----|
| Visual assessment | − | ++ | + | + |

Note: Key: − translucent cloudy, where lipid is dispersed in solution with few visible aggregates; + milky sample with visible aggregates; ++ milky sample with few visible aggregates.
cubic structure was retained on addition of 1% P407 stabiliser. Incorporation of P407 at even higher concentrations of 5% and 10% showed no structural changes (data not shown). Therefore, we postulated the stabiliser was only adsorbed to the surface of the glycolipid and was not internalised, as was observed in the case of phytantriol cubic system.\[25,31]\n
Lattice parameters (a) of the cubic phase are important to infer changes in the aqueous channels.\[8\] An increase in the lattice parameter, for example, is commonly attributed to the swelling or enlargement of the water channels that may be caused by insertion of stabiliser molecules into the lipid packing.\[8,31\] Comparison of the lattice parameters in Table 3 demonstrates insignificant changes in the \(Ia3d\) water channels upon addition of P407 stabilisers. The lattice parameter observed in our branched chain glycolipid system in absence of stabiliser was comparable to phytantriol, but is significantly lower than monoolein (see Table 3).\[31\] A noteworthy feature of \(\beta\)-Glc–OC\(_{10}\)C\(_6\) is the formation of higher curved compact cubic \(Ia3d\) phase in excess water,\[3\] although this space group is commonly obtained at lower hydration levels in other lipids such as monoolein and phytantriol.\[8,9\]

Water channel radii of the cubic phase was determined based on minimal surfaces using the following Equation 1,\[32\]

\[
R_w = \left[\frac{(\sigma - 2\pi \chi)}{2\pi a^2}\right] - L
\]

where \(L\) is the lipid length value or monolayer thickness, \(a\) is the lattice parameter of the cubic phase, \(\sigma\) is the dimensionless constant that represents the surface area per unit cell with a lattice parameter of unity (3.091 for \(Ia3d\)), and \(\chi\) is the Euler characteristic of the infinite periodic minimal surface geometries (−8 for \(Ia3d\)).

Table 3. Effects of P407 on structure and lattice parameters for non-dispersed \(\beta\)-Glc–OC\(_{10}\)C\(_6\) in 95% excess water, with monoolein (GMO) and phytantriol (PHYT)/excess water systems \[31\] for comparison. Data for GMO and PHYT are adapted with permission from Dong Y-D et al. Langmuir 2006; 22:9512-9518. Copyright 2006 American Chemical Society.
Lipid chain length was calculated using Equation 2,[33] where the volume fraction of hydrocarbon chain is

\[
\Phi_{ch} = 2\sigma \left( \frac{L}{a} \right) + \frac{4}{3} \pi \chi \left( \frac{L}{a} \right)^3
\]  

(2)

in which

\[
\Phi_{ch} = \frac{n_L \nu_{hc}}{n_L(\nu_{hc} + \nu_{head}) + n_w \nu_w}
\]

(3)

\( n_L \) and \( n_w \) are the number of moles of lipid and water, respectively, \( \nu_{hc} \) and \( \nu_{head} \) are the respective molar volume of hydrocarbon chain (479 Å\(^3\) for \( \beta \)-Glc–OC\(_{10}\)C\(_6\) and lipid headgroup (193.2 Å\(^3\) for \( \beta \)-Glc–OC\(_{10}\)C\(_6\)). These values were calculated based on the molecular volumes of \( \nu(CH) = 20 \) Å\(^3\), \( \nu(CH_2) = 27 \) Å\(^3\), and \( \nu(CH_3) = 27 \) Å\(^3\). [3] \( \nu_w \) is the molar volume of water (29.9 Å\(^3\)). Hence, the average radius of the \( 1a3d \) space group water channel is estimated to be ca. 15 Å.

Size of the water channels of the \( 1a3d \) cubic phase was also determined using the Garstecki and Holyst (GH) model by analysing the SAXS pattern.[34–37] Diameter of the water channel \( (D_w = 0.707a - L) \), where \( a \) is the lattice parameter and \( L \) is the lipid bilayer thickness) was calculated by determining the dimensionless lipid bilayer thickness value \( (L' = L/a) \), from Equation (4) based on nonlinear least square fitting method [34,35]. The model scattering intensities \( I_{hkl}(L) \) were fitted to the experimental intensities throughout all the \( hkl \) indices by varying the \( L' \) value, where the best fitting was obtained by minimising the sum of the squared intensity differences.

\[
I_{hkl}(L) = M_{hkl} \left[ \frac{F_{hkl}^S}{a_{hkl}2\pi(h^2 + P^2 + P^2)^{0.5}} \right]^2
\]

(4)

The values for \( M_{hkl} \) (multiplicity factor), \( F_{hkl}^S \) (dimensionless structure factor), and \( a_{hkl} \) (correction parameters for particular cubic lattices) are taken from Table 4 in reference [36] for cubic gyroid structure. Our results show only slight differences in the water channel radius, where ca. 15 Å (from methods in [33]) and 17 Å were obtained based on minimal surface characteristics [32] and GH model, respectively. The hydrophilic water channels in glycolipid are therefore smaller than those in monoolein/water systems (about 40 Å diameter).[38] Due to the small water channel size, entrapment of many large biomolecules, especially proteins in the glycolipid water channels is therefore challenging, and inclusion of a third component additive such as charged lipid [13,38,39] or single-chain sugar surfactant [35] may be necessary to tune the diameter of the water channels and decrease the lipid membrane curvature.

Meanwhile, scattering patterns of dispersed cubic phase at 1% P407 show absence of well-defined Bragg peaks with low signal-to-noise ratio after 15 hours acquisition time (Figure S2 in ESI). This may possibly be attributed to low electron density contrasts between lipid and water, low glycolipid concentrations and/or small cubosome sizes.[28,40] Poor scattering signals of dispersed cubosomes have previously been reported,[40,41] even in higher photon flux rotating-anode systems. Presence of cubosomes in the dispersion was nevertheless confirmed through negative staining-transmission electron microscopy (TEM, JEOL, JEM-2100F operating at 200 kV). Figure 3(a)–(d) shows cubosomes with cubic morphology, whereas Figure 3(e) shows a more spherical structure. Aggregation of P407 on the cubosomes was also evident. Although similar cubosome shapes have been reported,[22] no internal structural features could be clearly seen using conventional TEM visualisation. In addition, small spherical vesicles less than 100 nm size range was observed (Figure 3(f)), which was typically induced by sonication of bulk cubic phase.[29]

**Conclusions**

We demonstrated formation of novel cubic liquid crystalline nanoparticles based on Guerbet branched-chain glycolipid (2-hexyl-decyl-\( \beta \)-D-glucopyranoside, and studied the effects of Poloxamer P407 additives on the cubic structure and stability of the dispersed nanoparticles. Milky cubosome dispersion with few visible aggregates was observed in 1% P407 content, with an averaged hydrodynamic size of ca. 233 nm. In addition, we showed no effects of P407 additives on the internal cubic structure of \( \beta \)-Glc–OC\(_{10}\)C\(_6\) based on SAXS analysis.

**Acknowledgement**

The authors would like to thank Dr Hock Seng Nguan for valuable insight and discussion.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

The authors would like to thank the University of Malaya and the Ministry of Higher Education High Impact Research Grant [UM.C/625/1/HIR/MOHE/05] for financial support.
References

[1] Hashim R, Sugimura A, Minamikawa H, et al. Nature-like synthetic alkyl branched-chain glycolipids: a review on chemical structure and self-assembly properties. Liq Cryst. 2012;39:1–17. DOI:10.1080/02678292.2011.614017.

[2] O’Lenick AJ. Guerbet chemistry. J Surf Deterg. 2001;4:311–315. DOI:10.1007/s11743-001-0185-1.

[3] Zahid NI, Conn CE, Brooks NJ, et al. Investigation of the effect of sugar stereochemistry on biologically relevant lyotropic phases from branched-chain synthetic glycolipids by small-angle X-ray scattering. Langmuir. 2013;29:15794–15804. DOI:10.1021/la4040134.

[4] Nielsen LS, Schubert L, Hansen J. Bioadhesive drug delivery systems: I. Characterisation of mucoadhesive properties of systems based on glyceryl mono-oleate and glyceryl monolinooleate. Eur J Pharm Sci. 1998;6:231–239. DOI:10.1016/S0928-0987(97)10004-5.

[5] Hoath S, Norlen L. Cubic phases and human skin: theory and practice. In: Lynch ML, Spicer PT, editors. Boca Raton, FL: CRC Press; 2005.

[6] Angelova A, Angelov B, Drechsler M, et al. Neurotrophin delivery using nanotechnology. Drug Discov Today. 2013;18:1263–1271. DOI:10.1016/j.drudis.2013.07.010.

[7] Angelov B, Angelova A, Filippov SK, et al. Topology and internal structure of PEGylated lipid nanocarriers for neuronal transfection: synchrotron radiation SANS and cryo-TEM studies. Soft Matter. 2011;7:9714–9720. DOI:10.1039/c1sm06447a.

[8] Kulkarni CV, Wachter W, Iglesias-Salto G, et al. Monoolein: a magic lipid? Phys Chem Chem Phys. 2011;13:3004–3021. DOI:10.1039/C0CP01539C.

[9] Barauskas J, Landh T. Phase behavior of the phytantriol/water system. Langmuir. 2003;19:9562–9565. DOI:10.1021/la0350812.

[10] La Y, Park C, Shin TJ, et al. Colloidal inverse bicontinuous cubic membranes of block copolymers with tunable surface functional groups. Nat Chem. 2014;6:534–541. DOI:10.1038/nchem.1946.

[11] Nguyen T-H, Hanley T, Porter CJH, et al. Phytantriol and glyceryl monoooleate cubic liquid crystalline phases as sustained-release oral drug delivery systems for...
poorly water-soluble drugs II. In-vivo evaluation. J Pharm Pharmacol. 2010;62:856–865.

[12] Angelova A, Angelov B, Drehcls M, et al. Protein entrapment in PEGylated lipid nanoparticles. Int J Pharm. 2013;454:625–632. DOI: 10.1016/j.ijpharm.2013.06.006.

[13] Angelov B, Angelova A, Filipovsk S, et al. DNA/fusogenic lipid nanocarrier assembly: millisecond structural dynamics. J Phys Chem Lett. 2013;4:1959–1964. DOI: 10.1021/jz400857z.

[14] Angelov B, Angelova A, Papahadjopoulos-Sternberg B, et al. Protein-containing PEGylated cubosomic particles: freeze-fracture electron microscopy and synchrotron radiation circular dichroism study. J Phys Chem B. 2012;116:7676–7686. DOI: 10.1021/jp303863q.

[15] Ericsson B, Eriksson PO, Löffro JE, et al. Cubic phases as delivery systems for peptide drugs. Polymeric drugs and drug delivery systems. ACS Symposium Series. 469. Am Chem Soc. 1991;469:251–265.

[16] Landau EM, Rosenbusch JP. Lipidic cubic phases: A novel concept for the crystallization of membrane proteins. Proc Natl Acad Sci. 1996;93:14532–14535. DOI: 10.1073/pnas.93.25.14532.

[17] Efrat R, Kesselman E, Aserin A, et al. Solubilization of hydrophobic guest molecules in the monoolein discontinuous QL cubic mesophase and its soft nanoparticles. Langmuir. 2009;25:1316–1326. DOI: 10.1021/la8016084.

[18] Kaagaard T, Drummond CJ. Ordered 2-D and 3-D nanostructured amphiphile self-assembly materials stable in excess solvent. Phys Chem Chem Phys. 2006;8:4957–4975. DOI: 10.1039/b609510k.

[19] Claesson P, Stubenauch C, Krastev R, et al. Thin film and foam properties of sugar-based surfactants. In: Ruiz CC, editor. Sugar-based surfactants: fundamentals and applications. Boca Ratón, FL: CRC Press; 2009. p. 144.

[20] Ljusberg-Wahren H, Nyberg L, Larsson K. Dispersion of the liquid crystalline phase – structure, preparation, and functionality aspects. Chimica Oggi. 1996;14:40–43.

[21] Hashim R, Hashim HHA, Rodzi NZM, et al. Branched chain glycolides: enhanced diversity for phase behavior of easily accessible synthetic glycolitols. Thin Solid Films. 2006;509:27–35. DOI: 10.1016/j.tsf.2005.09.009.

[22] Rizwan SB, Dong Y-D, Boyd BJ, et al. Characterisation of bicontinuous cubic liquid crystalline systems of phytantriol and water using cryo field emission scanning electron microscopy (cryo FESEM). Micron. 2007;38:478–485. DOI: 10.1016/j.micron.2006.08.003.

[23] Rizwan SB, Assmus D, Boenhke A, et al. Preparation of phytantriol cubosomes by solvent precursor dilution for the delivery of protein vaccines. Eur J Pharm Biopharm. 2011;79:15–22. DOI: 10.1016/j.ejpb.2010.12.034.

[24] Muller F, Salonen A, Glatter O. Monoglyceride-based cubosomes stabilized by laponite: separating the effects of stabilizer, pH and temperature. Coll Surf A: Physicochem Eng Asp. 2010;358:50–56. DOI: 10.1016/j.colsurfa.2010.01.021.

[25] Chong JYT, Mulet X, Waddington LJ, et al. Steric stabilisation of self-assembled cubic lyotropic liquid crystalline nanoparticles: high throughput evaluation of triblock polyethylene oxide-polypropylene oxide-polyethylene oxide copolymers. Soft Matter. 2011;7:4768–4777. DOI: 10.1039/c1sm05181d.

[26] Ahmad N, Ramsch R, Esquena J, et al. Physicochemical characterization of natural-like branched-chain glycosides toward formation of hexosomes and vesicles. Langmuir. 2012;28:2395–2403. DOI: 10.1021/la203736b.

[27] Tilley AJ, Drummond CJ, Boyd BJ. Disposition and association of the steric stabilizer Pluronic® F127 in lyotropic liquid crystalline nanostructured particle dispersions. J Coll Interf Sci. 2013;392:288–296. DOI: 10.1016/j.jcis.2012.09.051.

[28] Caltagirone C, Falchi AM, Lamps S, et al. Cancer-cell-targeted theranostic cubosomes. Langmuir. 2014;30:6228–6236. DOI: 10.1021/la501332u.

[29] Barauskas J, Johnsson M, Joabsson F, et al. Cubic phase nanoparticles (cubosomet): principles for controlling size, structure, and stability. Langmuir. 2005;21:2569–2577. DOI: 10.1021/la047590p.

[30] Luzzi V, Spegt PA. Polymorphism of lipids. Nature. 1967;215:701–704. DOI: 10.1038/215701a0.

[31] Dong Y-D, Larson I, Hanley T, et al. Bulk and dispersed aqueous phase behavior of phytantriol: effect of vitamin E acetate and F127 polymer on liquid crystal nanostructure. Langmuir. 2006;22:9512–9518. DOI: 10.1021/la061706v.

[32] Anderson DM, Groner SM, Leibler S. Geometrical aspects of the frustration in the cubic phases of lyotropic liquid crystals. Proc Natl Acad Sci. 1988;85:5364–5368. DOI: 10.1073/pnas.85.15.5364.

[33] Turner DC, Wang Z-G, Groner SM, et al. Structural study of the inverted cubic phases of di-dodecyl alkyl-?-D-glucopyranosyl-rac-glycerol. J Phys II France. 1992;2:2039–2063. DOI: 10.1051/jp2:1992250.

[34] Angelov B, Angelova A, Mutalchivha R, et al. SAXS investigation of a cubic to a sponge (L3) phase transition in self-assembled lipid nanocarriers. Phys Chem Chem Phys. 2011;13:3073–3081. DOI: 10.1039/C0CP01029D.

[35] Angelov B, Angelova A, Ollivon M, et al. Diamond-type cubic lipid phase with large water channels. J Am Chem Soc. 2003;125:7188–7189. DOI: 10.1021/ja034578v.

[36] Garstecki P, Holyst R. Scattering patterns of self-assembled cubic phases. 1. The model. Langmuir. 2002;18:2519–2528. DOI: 10.1021/la011298p.

[37] Garstecki P, Holyst R. Scattering patterns of self-assembled cubic phases. 2. Analysis of the experimental spectra. Langmuir. 2002;18:2529–2537. DOI: 10.1021/la011299h.

[38] Tyler AI, Barriga HMG, Parsons ES, et al. Electrostatic swelling of bicontinuous cubic lipid phases. Soft Matter. 2015;11:3279–3286. DOI: 10.1039/C5SM03311C.

[39] Angelov B, Angelova A, Drehcls M, et al. Identification of large channels in cationic PEGylated cubosome nanoparticles by synchrotron radiation SAXS and Cryo-TEM imaging. Soft Matter. 2015;11:3686–3692. DOI: 10.1039/C5SM00169B.

[40] Hartnett TE, Ladewig K, O’Connor AJ, et al. Physicochemical and cytotoxicity analysis of glycerol monoolein-based nanoparticles. RSC Adv. 2015;5:26543–26549. DOI: 10.1039/C4RA13890B.

[41] Deshpande S, Venugopal E, Ramagiri S, et al. Enhancing cubosome functionality by coating with a single layer of poly-ε-lysine. ACS Appl Mat Interf. 2014;6:17126–17133. DOI: 10.1021/am5047872.