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Tailoring liquid crystalline lipid nanomaterials for controlled release of macromolecules

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ABSTRACT

Lipid-based liquid crystalline materials are being developed as drug delivery systems. However, the use of these materials for delivery of large macromolecules is currently hindered by the small size of the water channels in these structures limiting control over diffusion behaviour. The addition of the hydration-modulating agent, sucrose stearate, to phytantriol cubic phase under excess water conditions incrementally increased the size of these water channels. Inclusion of oleic acid enabled further control of swelling and de-swelling of the matrix *via* a pH triggerable system where at low pH the hexagonal phase is present and at higher pH the cubic phase is present. Fine control over the release of various sized model macromolecules is demonstrated, indicating future application to controlled loading and release of large macromolecules such as antibodies.

1. Introduction

Compared to small molecule therapeutics, the large size and complex structure of biological drugs such as antibodies and proteins present challenges in developing controlled release systems. Achieving longer duration release is also often more critical for these drugs due to their typical short half-lives and need for parenteral administration. Their size in particular can be problematic in achieving fine control over diffusion controlled release as tailoring pores to an appropriate dimension in polymeric materials requires a kinetically controlled process, and changes to the pore size during release can complicate release kinetics.

Lipid-based liquid crystalline materials are receiving increased attention as controlled release delivery systems for macromolecular drugs. Liquid crystal structures are formed by the self-assembly of certain amphiphilic lipids in an excess aqueous environment (Boyd et al., 2009, 2007; Fong et al., 2009; Kaasgaard and Drummond, 2006). Commonly used lipids are phytantriol (PHYT) and glycerol monooleate (GMO). The nanostructure that is formed is determined by the geometric packing of the lipid, defined by the critical packing parameter, expressed in Eq. (1) (Israelachvili, 1994).

$$\mathrm{CPP} = \frac{v}{a_o l} \quad (1)$$

where v is the hydrophobic chain volume, a_o is the surface area occupied by the hydrophilic head group area and l is the hydrocarbon chain length (Israelachvili, 1994).

The effective critical packing parameter is also influenced by factors such as the presence of additives, pH, temperature and pressure.

The liquid crystalline structures of typical interest for drug delivery purposes are the reverse bicontinuous cubic phase (V_2) and reverse hexagonal phase (H_2) (Yaghmur and Glatter, 2009). Hexagonal phase consists of infinite water rods arranged in a two-dimensional hexagonal lattice, separated by the lipid domain. The reverse bicontinuous cubic phase consists of two continuous water channels separated by a lipid bilayer (Boyd et al., 2007; Yaghmur and Glatter, 2009). Three commonly formed variants of the reverse bicontinuous cubic phase are the gyroid (Ia3d), diamond (Pn3m) and primitive (Im3m). The different reverse cubic and hexagonal structures are summarised schematically in Fig. 1 (Mulet et al., 2013).

When small molecules such as drugs are incorporated into these materials they have been shown to exhibit a diffusion controlled release profile where incorporated drug diffuses through the aqueous channels in order to escape to the continuous

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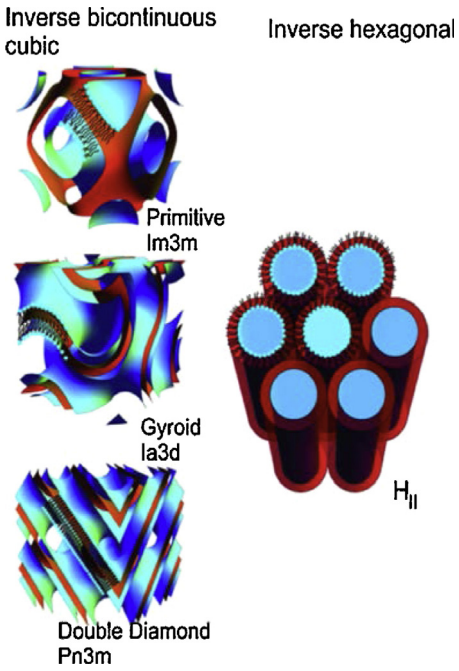

Fig. 1. Schematic representation of inverse bicontinuous cubic phase (V_2) of different space groups and reverse hexagonal phase (H_2 or H_{II}). Reproduced with permission from Mulet et al. (2013).

aqueous medium (Zabara and Mezzenga, 2014). Phan et al. showed that the release of small molecule drugs from the cubic phase is at a considerably faster rate than the other mesophases (Phan et al., 2011). The difference in release rates allows for an on-off on-demand release behaviour that would be particularly useful for pulsatile release of large macromolecules with short plasma half-lives. However, as a consequence of the limiting water channel size, release of large macromolecules is very slow (Lee et al., 2009). A means of loading larger hydrophilic molecules and facilitating triggered release would be advantageous.

There are strategies to increase the channel size in cubic phases, to make them more amenable to host and release macromolecules. The addition of sucrose stearate (ST), a sugar amphiphile with a very large hydrophilic headgroup, increases the average area of the head group at the lipid-water interface (a_o) decreasing the CPP value, thereby inducing a change in spacegroup from Pn3m to Im3m. The Im3m phase possesses a less negative curvature and a greater water intake capacity, increasing the size of the water channels. The addition of ST induced a concentration-dependent 4-fold increase in the size of water channels in the monolinolein-based cubic phase (Negrini and Mezzenga, 2012). It has also been reported that for GMO-water systems, oleic acid (OA) played a significant role in altering the phase behaviour. OA affects the molecular packing of the lipid, such that when the head group is deprotonated, electrostatic repulsion induces the formation of larger water channels (Du et al., 2014; Salentinig et al., 2010).

It is hypothesized in this study that OA has the potential to work synergistically with ST as additives to create a cubic phase with even larger water channels. Additionally, OA as an ionizable compound allows dynamic switching between H_2 and V_2 phases using pH (Du et al., 2014) and hence, has the potential to provide a mechanism to control drug release on demand. pH changes arise naturally within the human body, which makes this parameter an ideal stimulus (Kost and Langer, 2012; Langer, 1990; Negrini and Mezzenga, 2011). Specifically, at low pH, such as in the gastric compartment or in a precursor formulation the macromolecule would be trapped in the hexagonal phase when the water channels are narrow, inhibiting or preventing release, and then upon a change to typical physiological conditions in the intestine or subcutaneous space at neutral pH there would be a phase transition to a highly swollen cubic phase that triggers the release of the macromolecule in a controlled manner. Reversing the pH should induce a switch back to the previous structure and consequent drug release behaviour. Therefore, in order to test these hypotheses the impact of ST and OA on the phase behaviour of phytantriol in excess water was determined and the pH-dependent release of model macromolecular drugs of increasing size was determined to map the formulation space for on demand triggered release systems.

2. Materials and methods

2.1. Materials

Phytantriol was purchased from DSM (Singapore). Oleic acid was obtained from Croda International (Yorkshire, UK). Sucrose stearate was purchased from Mitsubishi-Kaguku Foods Corporation, Tokyo, Japan. These ingredients were used without further purification. Fluorescein isothiocyanate-dextrans (FITC-dextrans) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) with different molecular weights (4000 Da, 7100 Da, and 21,000 Da). Disodium hydrogen orthophosphate anhydrous, was purchased from APS Ajax Finechem (Auburn, NSW, Australia). Potassium dihydrogen orthophosphate, sodium chloride, citric monohydrate and trisodium citrate dihydrate were from BDH AnalaR, Merck Pty., Ltd. (Kilsyth, Victoria, Australia). The water used was obtained from a Millipore water purification system using a QuantumTM EX Ultrapure Organex cartridge (Millipore, Australia).

2.2. Preparation of bulk phase liquid crystals

The bulk liquid crystalline systems were prepared by combining phytantriol, oleic acid, sucrose stearate and FITC-dextran at different proportions, to 500 mg, in excess ethanol in a 20 mL glass scintillation vial. The mixture was vortex mixed and kept under vacuum at 40 °C for over 24 h to remove the ethanol. Phosphate buffered saline (pH 7.4) or citrate (pH 5.5) buffer at 1× concentration (10 mL) were then added to the vial and left to equilibrate at room temperature for 5–7 days. The pH of the buffer was monitored daily and adjusted using 1 M NaOH and HCl when required to maintain the pH.

2.3. Small angle X-ray scattering (SAXS)

The internal structure of the liquid crystalline systems was characterized using small angle X-ray scattering (SAXS) (Kirby et al., 2013). The equilibrated samples were loaded into a 96 well plate for high throughput structure determination. A custom-designed plate holder was used to mount the plate directly onto the SAXS/WAXS beamline. Scans were automated using a pre-loaded set of position variables based on the well positions within the plate.

Measurements were performed on the SAXS/WAXS beamline at the Australian Synchrotron (Kirby et al., 2013). An X-ray beam with a wavelength of 1.1271 Å (11 keV) was selected. The 2D SAXS patterns were collected using a Pilatus 1 M detector (active area $169 \times 179\,mm^2$ with a pixel size of 172 μm) which was located 966 mm from the sample position. The total q range for the instrument configuration outlined above was $0.016 < q < 1.06\,Å^{-1}$. Computer software, Scatterbrain, was used to acquire and reduce 2D patterns to 1D intensity *vs.* q profiles. Phase structures were identified by indexing Bragg peaks to known relative spacing ratios and lattice dimensions, a, calculated using known relationships (Hyde, 2012).