Colloids and Surfaces B: Biointerfaces 151 (2017) 34–38

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Inverse hexagonal and cubic micellar lyotropic liquid crystalline phase behaviour of novel double chain sugar-based amphiphiles

George C. Feast[a], Thomas Lepitre[a], Nhiem Tran[b], Charlotte E. Conn[b], Oliver E. Hutt[a], Xavier Mulet[a], Calum J. Drummond[a,b,*], G. Paul Savage[a,*]

[a] CSIRO Manufacturing, Bag 10, Clayton South MDC, VIC, 3169, Australia
[b] School of Science, College of Science, Engineering and Health, RMIT University, GPO Box 2476, Melbourne, VIC, 3001, Australia

ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 4 November 2016
Accepted 6 December 2016
Available online 7 December 2016

Keywords:
Amphiphiles
Self-assembly
Sugars
Hexagonal phase
Fd3m
Hexosome

ABSTRACT

The lyotropic phase behaviour of a library of sugar-based amphiphiles was investigated using high-throughput small-angle X-ray scattering (SAXS). Double unsaturated-chain monosaccharide amphiphiles formed inverse hexagonal and cubic micellar (Fd3m) lyotropic phases under excess water conditions. A galactose-oleyl amphiphile from the library was subsequently formulated into hexosome nanoparticles, which have potential uses as drug delivery vehicles. The nanoparticles were shown to be stable at elevated temperatures and non-cytotoxic up to at least 200 $\mu g\,mL^{-1}$.

1. Introduction

In the area of drug-delivery, compounds that assemble into inverse cubic and hexagonal phases are of particular interest, due to the ability to maintain their self-assembled nanostructures under excess water conditions. The inverse hexagonal phase has a structure consisting of rod-like water channels, packed into a hexagonal lattice framework [1]. Upon dispersion with a suitable stabiliser the inverse hexagonal phase can form nanoparticles known as hexosomes. Encapsulated bioactive molecules, such as proteins [2–4], can be contained within either the aqueous or hydrophobic domains, dependent on the polarity of such molecule. Hexosome drug carriers [5] have been investigated for use in transmucosal [6], transdermal [7] and ocular [8] drug delivery and may exhibit slower drug release rates, particularly when compared to their inverse cubic phase analogues (cubosomes) [9].

A limited number of amphiphiles are currently available for the formulation of hexosome nanoparticles. Boyd et al. used oleyl glycerate (OG) and phytanyl glycerate (PG) hexosomes to encapsulate Irinoetecan [10], whilst Jain et al. utilised glycerol monooleate (GMO) for the delivery of Paclitaxel [11]. Hexosomes have also been generated from mixtures of similar compounds [12,13], as well as from ureas [14] and cationic amino acids [15] in a small number of examples.

We are interested in sugar-based amphiphiles for the formation of hexosomes, since it is known that many sugars bind to lectins (cell-binding proteins) and can thus be utilised for drug targeting [16,17]. Examples include hepatic endothelial cells in the liver [18] and corneal cells within the eye [19].

These sugar-based nanoparticles are able to increase the bioavailability of polar drug-like molecules, whilst also providing the opportunity for controlled and targeted release. The nanoparticle can be recognised by sugar receptors on certain cell membranes, and thus the drug payload can be released at the required site. The formation of inverse phases, including the inverse hexagonal phase, has been previously observed for Guerbet branched chain β-D-glucosides and xylosides, which are synthetic analogues of natural glycolipids [20–22].

To date, tried-and-tested amphiphiles have typically been used to obtain desired lyotropic phases for biomedical applications without much thought for strategic amphiphile design. Before the dawn of high-throughput technologies, the use of well-characterised amphiphiles was a cheap, efficient way of getting it almost-right. However the increasing use of high-throughput (HT) techniques in the amphiphile research area has allowed us to screen a wide

* Corresponding author.
E-mail address: calum.drummond@rmit.edu.au (C.J. Drummond).

http://dx.doi.org/10.1016/j.colsurfb.2016.12.004
0927-7765/

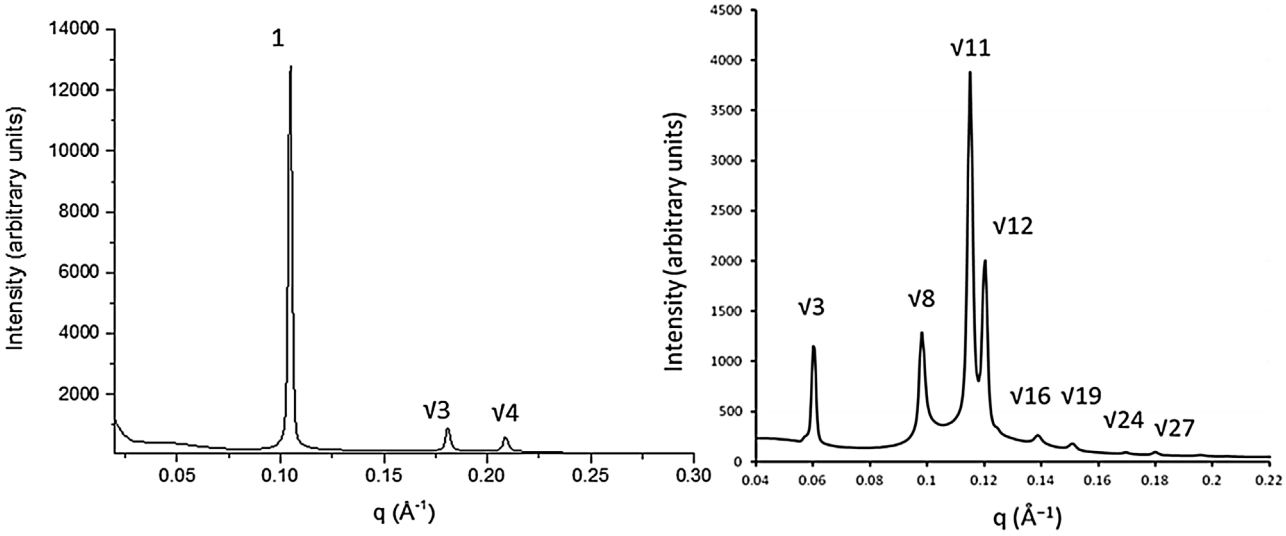

Fig. 1. HT SAXS diffraction patterns of a) Galactose-Palmitoleic amphiphile at 70 wt% water and 37 °C and b) Xylose-Oleic amphiphile at 70 wt% water and 25 °C showing the formation of a hexagonal and an Fd3 m phase respectively. The Bragg reflections are indicated.

range of conditions to design and synthesise amphiphiles specifically optimised for this task [23,24].

In previous work we synthesised a small library of single-chain glucose and galactose based amphiphiles, with systematic variations in chain length and unsaturation, and ascertained their thermo- and lyo-tropic phase behaviour using HT synchrotron small-angle X-ray scattering (SSAXS) [25]. However, the majority of these compounds formed normal phases, with no inverse phase-forming amphiphiles identified. Based on our evolving knowledge of amphiphile self-assembly design rules [26], we subsequently synthesised a large library of double and triple chain sugar-based amphiphiles more likely to adopt an inverse phase suitable for drug delivery applications [27].

The library of double chain sugar-based amphiphiles was synthesised using a high-throughput variant of the copper-catalysed azide-alkyne cycloaddition (CuAAC) [28,29] 'click' reaction [25]. The library consisted of 46 amphiphiles formed from five different sugar head groups, coupled to eleven different double-chain tails with incremental variations in chain length and unsaturation. Sugar headgroups used were glucose, galactose, mannose, xylose and lactose. The hydrocarbon chains ranged from C7 to C17 (for saturated chains), and C16:1 (palmitoleic) to C22:1 (erucic) for monounsaturated chains, as well as C18:2 (linoleic) and a branched phytanic acid derivative chains. The head and tail are connected via a triazole linkage, which is known to be stable under physiological conditions [30].

In order to determine the lyotropic phase behaviour of all 46 amphiphiles, a previously developed high-throughput small-angle X-ray scattering (SAXS) [31,32] technique was utilised. Herein we focus on the results from the monosaccharide amphiphiles with double unsaturated chains which formed inverse hexagonal and cubic micellar phases. Additionally, a selected amphiphile was dispersed to form hexosomes and cell viability assays were performed to study *in vitro* cytotoxicity of the nanoparticles.

2. Results and discussion

The SAXS phase behaviour data for the double unsaturated amphiphiles at physiological temperature is presented in Tables 1 and 2 and is discussed below. Data at 25 °C is presented in the supplementary information along with a complete description of sample preparation.

A full phase assignment at 37 °C, and associated lattice parameters for all of these materials are presented in Table 2. For glucose and galactose amphiphiles, hexagonal phases are observed for all three monounsaturated amphiphiles. As expected, the lattice parameter of the inverse hexagonal phase increases with increasing chain length in all but the structurally-similar galactose palmitoleic and oleic amphiphiles, which have almost identical lattice parameters. Furthermore, the lattice parameter of the hexagonal phase is smaller at 37 °C than at 25 °C, due to an increase in magnitude of the curvature of the polar-apolar interface within the cylinders at higher temperature. This is considered to be due to an increase in effective hydrocarbon chain volume at higher temperatures caused by increased thermal motion.

For the xylose-palmitoleic amphiphile, a hexagonal phase is observed at 37 °C. As the chain length is increased (to oleic and erucic) we observe the Fd3m phase, consisting of a cubic array of inverse micelles (Fig. 1). The formation of the highly curved micellar phase at longer chain lengths is again consistent with an increase in effective chain volume at longer chain lengths. Since xylose has a smaller head group radius than glucose and galactose, there is a larger impact on the magnitude of the interfacial curvature upon increasing chain length.

A similar pattern is observed for the two mannose amphiphiles, with the smaller palmitoleic compound giving a mixed hexagonal/Fd3m phase and the longer oleic amphiphile giving a Fd3m phase only. The mannose-erucic analogue was unavailable as discussed in previous work [27]. Due to the structural similarity of the mannose headgroup to the glucose and galactose headgroups, we might expect the mannose compounds to behave similarly to the glucose and galactose amphiphiles. However, the α- orientation at the anomeric centre reduces the volume of the head groups with respect to the side-chain, by placing the triazole perpendicular to the sugar. This is confirmed by comparison of the lattice parameters for the hexagonal phase palmitoleic compounds: glucose, 68.5 Å; galactose 69.6 Å; mannose 64.8 Å. The reduced lattice parameter associated with the mannose amphiphile reflects the increase in the magnitude of curvature of this system associated with reduced headgroup area, compared to compounds of identical chain length. This increase in curvature magnitude drives the formation of an Fd3m phase at longer effective chain length.

The HT data-set was used to select the optimum compound to progress to hexosome nanoparticle formulation. The galactose-oleic amphiphile (Fig. 2) was selected for two reasons. Firstly,