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Disposition and association of the steric stabilizer Pluronic® F127 in lyotropic liquid crystalline nanostructured particle dispersions

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ABSTRACT

Liquid crystalline nanostructured particles, such as cubosomes and hexosomes, are most often colloidally stabilised using the tri-block co-polymer Pluronic® F127. Although the effect of F127 on the internal particle nanostructure has been well studied, the associative aspects of F127 with cubosomes and hexosomes are poorly understood. In this study the quantitative association of F127 with phytantriol-based cubosomes and hexosomes was investigated. The amount of free F127 in the dispersions was determined using pressure ultra-filtration. The percentage of F127 associated with the particles plateaued with increasing F127 concentration above the critical aggregation concentration. Hence the free concentration of F127 in the dispersion medium was proposed as a key factor governing association below the CMC, and partitioning of F127 between micelles and particles occurred above the CMC. The association of F127 with the particles was irreversible on dilution. The F127 associated with both the external and internal surfaces of the phytantriol cubosomes. The effects of lipid and F127 concentration, lipid type, dilution of the dispersions and internal nanostructure were also elucidated. A greater amount of F127 was associated with cubosomes comprised of glyceryl monooleate (GMO) than those prepared using phytantriol. Hexosomes prepared using a mixture of phytantriol and vitamin E acetate (vitEA) had a greater amount of F127 associated with them than phytantriol cubosomes. Hexosomes prepared using selachyl alcohol had less F127 associated with them than phytantriol:vitEA-based hexosomes and GMO-based cubosomes. This indicated that both the lipid from which the particles are composed and the particle internal nanostructure have an influence on the association of F127 with lyotropic liquid crystalline nanostructured particles.

1. Introduction

Lyotropic liquid crystalline nanostructured particles are submicron-sized particles composed of dispersed liquid crystalline mesophases [1]. Two types of lyotropic liquid crystalline nanostructured particles were studied in this work, cubosomes and hexosomes formed from the dispersion of inverse bicontinuous cubic (V_2) and reverse hexagonal (H_2) mesophases respectively [2,3]. These particles are the subject of a large amount of research, primarily aimed at characterising their internal nanostructure due to their potential application in the pharmaceutical [4–6], agricultural [7] and food industries [8,9].

Coarse dispersions of the V_2 and H_2 mesophases are not colloidally stable and will generally aggregate soon after dispersion. To date, non-ionic steric stabilisers have been most often employed for the stabilisation of these dispersions, as ionic stabilisers typically disrupt the internal nanostructure. A number of stabilisers have been used in attempts to create stable liquid crystalline dispersions, such as block copolymers from the Pluronic® series [10], PEG-copolymers with lipid-mimetic hydrophobic anchors [11], β-casein [12], hydrophobically modified cellulose [13], hydroxypropyl methyl cellulose acetate succinate [14], clay colloid laponite [15], vitamin E TPGS (d-α-tocopheryl polyethylene glycol 1000 succinate) [16] and stabilisers from the Myrj series [17] among others. However, the stabilisation of cubosomes and hexosomes is most commonly achieved using the tri-block copolymer Pluronic® F127 (referred to from here on as F127), first reported by Gustafsson et al. [18].

F127 has been used to stabilise different mesophase particles, including phytantriol cubosomes [10,19], glyceryl monooleate (GMO) cubosomes [10,18], phytantriol:vitamin E acetate hexosomes [16], GMO:oleic acid (OA) hexosomes [20], selachyl alcohol (SA) hexosomes [21] and chemotherapeutic amphiphile prodrug cubosomes [22]. However the relationship between F127 and cubosomes and hexosomes has not been well established. The amount of stabiliser required to produce a good quality dispersion has been studied [10,18,23], as has the effect of the stabiliser on

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particle nanostructure [10,24]. For example, Gustafsson et al. established that there was a change in nanostructure of GMO-based cubosomes in the presence of F127, with the $V_{2(Pn3m)}$ nanostructure changing to $V_{2(Im3m)}$ at sufficiently high F127 concentrations [25]. This has been suggested to be the result of F127 incorporating into the GMO bilayer, causing swelling and ultimately a transformation of nanostructure. In contrast the nanostructure of phytantriol cubosomes was unaffected by high concentrations of F127, with the $V_{2(Pn3m)}$ nanostructure observed for both the bulk matrix and dispersed particles [10,16].

Improved characterisation of the relationship between lyotropic liquid crystalline nanostructured particles and F127 is necessary. Such characterisation will lead to a greater understanding of the behaviour of lyotropic liquid crystalline nanostructured particles, such as their adsorption to surfaces [7,26,27] and the transfer of lipid between lyotropic liquid crystalline nanostructured particles and other particles such as emulsions [19,28,29] which is mediated by co-existing F127 micelles. In the latter case, *a priori* knowledge of the amount of free F127 in the dispersion is expected to improve mechanistic understanding of these processes.

Consequently in this study, the proportion of F127 associated with cubosomes and hexosomes was investigated. Pressure ultra-filtration (PUF) was used to separate a small fraction of the free solution containing F127 from the particles. The amount of F127 in the filtrate was quantitatively determined using a colorimetric assay.

It was hypothesised that the amount of F127 associated with the particles would depend on the internal lyotropic liquid crystalline nanostructure of the particles, as well as the lipid from which the particle is prepared. The quantities of F127 and lipid used to produce the particles were also hypothesised to influence the amount of F127 associated with the particles. As the association of F127 to other surfaces has been observed to be irreversible [30], the association of F127 with lyotropic liquid crystalline nanostructured particles was hypothesised to be irreversible. A number of systems of different composition were investigated to determine the influence of the above variables on the association of F127 with liquid crystalline nanostructured particles. Phytantriol was used as the primary lipid to prepare cubosomes as it has been established that the presence of F127 does not affect the nanostructure [16], therefore any changes to the quantitative association of F127 with the cubosomes would not be due to F127-induced changes in the particle nanostructure. First, the relationship between F127 and phytantriol cubosomes was investigated extensively, in particular the effect of concentrations of F127 and phytantriol. The effect of dilution of phytantriol cubosomes on F127 binding was also investigated. Hexosomes composed of phytantriol:vitamin E acetate (9:1 w/w ratio) were also investigated for comparison with cubosomes. Finally the effect of the lipid comprising the particles on association of F127 with the particles was investigated using cubosomes prepared from GMO and hexosomes from selachyl alcohol (SA).

2. Methods and materials

2.1. Materials

Phytantriol was purchased from DSM Nutritional products (Grenzach, Germany). Vitamin E acetate 96% (St. Louis, MO, USA), ammonium thiocyanate (Steinheim, Germany) and iron (III) chloride 45% solution (Steinheim, Germany) were all purchased from Sigma–Aldrich. Dichloromethane was from Merck (Kilsyth, Australia) and selachyl alcohol was from Cee Chemicals (Blacktown, Australia). Pluronic® F127 was from Sigma (St. Louis, USA). Glyceryl monooleate (GMO) (Myverol 18–99 K) was from Kerry Ingredients (Norwich, USA). Milli-Q water (0.05 μS cm^{-1} at 25 °C) was purified through a Millipore system (Sydney, Australia).

2.1.1. Preparation of lyotropic liquid crystalline nanostructured particle dispersions

Liquid crystalline nanostructured particles were prepared by adding molten lipid drop-wise to F127 solutions, creating coarse dispersions. The concentration of F127 was varied between 0.5% and 5.0% w/v and lipid concentrations between 5.0% and 20.0% w/w, depending on the experiment. These coarse dispersions were then subjected to 20 min of 1 s on, 1 s off pulsed ultrasonication at 30% amplitude with a Misonix Ultrasonic Liquid Processor (Farmingdale, USA) fitted with a microtip.

2.1.2. Particle size determination and surface area calculation of lyotropic liquid crystalline nanostructured particles

The particle size of the lyotropic liquid crystalline nanostructured particles (*Z*-average) was determined by dynamic light scattering (DLS), using a Malvern Zetasizer Nano (Nano-ZS) (Malvern, UK) instrument with Sarstedt polystyrene cuvettes (10 × 4 × 45 mm) (Nűmbrecht, Germany). The refractive index and viscosity of water were assumed.

Dynamic light scattering provides particle size based on the hydrodynamic diameter of equivalent spheres. From these diameters, the surface area of the phytantriol cubosomes was calculated under the assumption that the particles were spherical. This assumption is close to the observed geometries for cubosomes [31]. The surface area was calculated in order to compare the amount of F127 associated with particles per unit surface area when different concentrations of F127 and phytantriol were used in dispersion preparation. This approach also assumed that association of the F127 to the particles was only *via* surface adsorption. These assumptions may not lead to exact relationships being established between surface area and the amount of F127 adsorbed to the particles, however they do provide for an initial point of comparison.

2.1.3. Pressure ultra-filtration of lyotropic liquid crystalline nanostructured particle dispersions

Pressure ultra-filtration (PUF) was conducted using a 10 mL Amicon PUF device from Millipore (Bedford, USA) with Millipore regenerated cellulose membranes (NMWL 30,000, 25 mm diameter) (Billerica, USA). Regenerated cellulose membranes were chosen because F127 was observed to bind extensively to polyethersulfone membranes in preliminary calibration experiments. All filtrations were conducted using nitrogen gas at a pressure of 50 psi. The chamber of the PUF device was stirred continuously using an IKA Color Squid stirrer (Wilmington, USA). The retention of F127 by the membranes was established by filtering F127 solutions in the range of 0.01–3.00% w/v F127. The amount of F127 detected in the filtrate was then determined via a colorimetric assay (see Supplementary Information for assay details, validation data and calibration curve).

The effectiveness of the membrane for excluding lyotropic liquid crystalline nanostructured particles from passing through into the filtrate was also determined to verify that the concentration of F127 detected in the filtrate reflected the true free concentration of F127. This was accomplished using DLS (see above) to determine the derived count rate of dispersions as previously reported for liposomes by Wallace et al. [32]. The derived count rate was obtained using the DTS (Nano) software from Malvern Instruments (Malvern, UK) and is a measure of the intensity of the scattered light on an absolute scale, rather than the count rate obtained from the attenuated light source. A calibration curve was constructed using diluted cubosome dispersions. Dispersions of phytantriol and GMO containing 10.0% w/w lipid in 1.0% w/v F127 solution with Milli-Q water were diluted in 1:10 (v/v) serial dilutions in water to give dispersions containing between 1.0×10^{-5} and 1.0% w/v lipid. The derived count rate of the diluted disper-