Journal of Colloid and Interface Science 522 (2018) 126–135

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

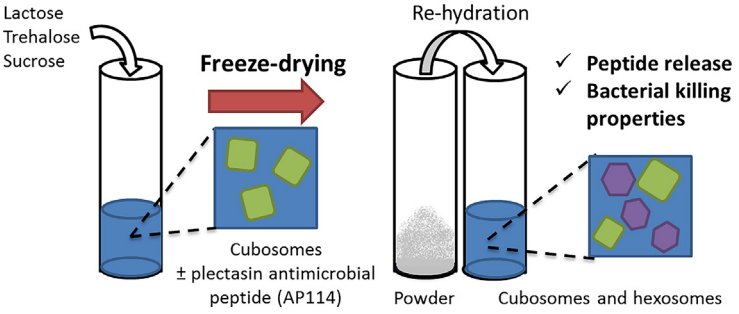
Regular Article

Freeze-dried and re-hydrated liquid crystalline nanoparticles stabilized with disaccharides for drug-delivery of the plectasin derivative AP114 antimicrobial peptide

Lukas Boge [a,d,*], Amanda Västberg [a], Anita Umerska [b], Helena Bysell [a], Jonny Eriksson [c], Katarina Edwards [c], Anna Millqvist-Fureby [a], Martin Andersson [d]

[a] RISE Research Institutes of Sweden, Drottning Kristinas väg 45, Box 5607 Stockholm SE-11486, Sweden
[b] MINT, UNIV Angers, INSERM 1066, CNRS 6021, Université Bretagne Loire, 4 rue Larrey, Angers 49933 Cedex, France
[c] Department of Chemistry – BMC, Uppsala University, Husargatan 3 Box 579, Uppsala SE-75123, Sweden
[d] Department of Chemistry and Chemical Engineering, Applied Chemistry, Chalmers University of Technology, Kemigården 4, Göteborg SE-41296, Sweden

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 January 2018
Revised 14 March 2018
Accepted 18 March 2018
Available online 20 March 2018

Keywords:
Liquid crystal
Glycerol monooleate
Cubosome
Hexosome
Freeze-drying
Plectasin
AP114
Antimicrobial peptide

ABSTRACT

Liquid crystalline nanoparticles (LCNPs), e.g. cubosomes and hexosomes, are receiving more and more attraction as drug delivery vehicles. Dry powder formulation that forms LCNPs upon hydration can be advantageous to make new routes of administration accessible. In this work, we investigate use of three disaccharides (lactose, trehalose and sucrose) as protective matrices for glycerol monooleate based LCNP forming powders produced by freeze-drying. Phase behavior, particle size and size distributions at the different preparation steps were monitored by small angle x-ray scattering (SAXS) and dynamic light scattering (DLS). Particle appearance was imaged by cryogenic transmission electron microscopy (cryo-TEM). Moreover, the therapeutic relevant antimicrobial peptide AP114 (plectasin derivative) was incorporated in the formulations. Peptide encapsulation and release as well as *in vitro* antibacterial effect were investigated. Results showed that all freeze-dried powders did form particles with liquid crystalline structure upon hydration. However, a phase transition from the bicontinuous cubic Pn3m to the reversed hexagonal was observed, as a consequence of sugar addition and the freeze-drying procedure. Data indicates that trehalose is the preferred choice of lyo-protectant in order to maintain a mono-modal particle size distribution. In addition, antimicrobial activity of AP114-containing formulations was found to be highest for the formulation containing trehalose. The release kinetics of AP114 from the nanoparticles was strongly affected by the dimensions of the hexagonal phase. Larger dimension of the hexagonal phase, significantly improved the release of AP114 and antimicrobial activity of the formulation.

* Corresponding author at: RISE Research Institutes of Sweden, Drottning Kristinas väg 45, Box 5607 Stockholm SE-11486, Sweden.
E-mail address: lukas.boge@ri.se (L. Boge).

https://doi.org/10.1016/j.jcis.2018.03.062
0021-9797/

1. Introduction

Lyotropic liquid crystalline phases of polar lipids, e.g. glycerol monooleate (GMO), has for many years been investigated for drug delivery purposes [1]. Especially the bicontinuous cubic liquid crystalline (LC) phase has gained a lot of attraction [2] since its discovery and structural determination decades ago [3–6]. Pioneers have incorporated a great variety of drugs in cubic phases, such as local anesthetics [7], proteins and peptides [8,9] as well as low molecular weight drugs [10]. A drawback with the cubic phase is the very high viscosity and sticky nature, which limits the administration routes [2]. One way to overcome the sticky nature of the cubic phase is to disperse it into LC nanoparticles (LCNPs), namely cubosomes, in presence of a stabilizer [11–13]. These LCNPs have proven to be suitable for delivery of peptide and protein drugs, with tunable release properties [14–17]. The dispersions may not be suitable for administration of drugs pulmonary or orally. Instead, powder formulations can be advantageous, both from an administrational point of view and in terms of formulation stability. A LCNP forming powder could impel for pulmonary administration that may be more difficult to achieve with the liquid dispersions. Moreover, if peptide and protein drugs are incorporated in the particles, powder formulations may be preferred from a chemical stability point of view, to avoid e.g. hydrolysis in aqueous environments [18–20].

Different techniques have been investigated for drying of lipid nanoparticles, such as freeze-drying, spray drying and spray freeze-drying [20–24]. Freeze-drying is probably the most straight-forward technique to use, due to that very small sample volumes can be handled and that it is easy to scale up. A protective matrix, e.g. starch, celluloses or sugars, is necessary to add to the formulations to prevent particle aggregation and to protect the sample from collapse, due to osmotic pressure and stress induced by the freezing and drying procedures [22]. These protective matrices are often referred to as "lyo-protectants" during freeze-drying. Sugars, especially disaccharides, have earlier shown to serve as effective lyo-protectants for biological membranes [25–27]. Disaccharides are usually better than monosaccharides in stabilizing lipid membranes, due to differences in glass transition temperature, and formation of glassy state of the sugar is important for effective stabilization of membranes [28]. Hence, they may also be suitable stabilizers for non-lamellar liquid crystalline (LC) structures. The role of the disaccharides is to form a vitrified matrix around the lipids and to replace the water in dry state around the hydrophilic head groups [29]. The size of these protective molecules is small, which makes penetration into the water channels (typically 2–10 nm diameter [14,30]) in the LC structures possible.

Only a few studies are available reporting on LC particle forming dry powder formulations. Spicer and co-workers (2002) investigated the use of spray-drying of GMO containing starch and dextran dispersions, to produce a powder precursor that formed cubosomes upon rehydration [31]. They used cryo-TEM of the hydrated powders to show the presence of cubosomes. Avachat et al. (2015) did also use dextran in combination with spray-drying to form cubosome forming powders. In similarity to Spicer et al. they also used cryo-TEM to visualize the cubosomes upon hydration [32]. Magnesium trisilicate has also been used to produce spray-dried GMO-mixtures in a study by Shah et al. (2006) [33]. Mobeus et al. (2012) investigated mannitol, polyvinyl pyrrolidone and polypropylene glycols as carrier matrices for bovine serum albumin (BSA) containing GMO-based dry powder systems [34]. Several different drying procedures (spray drying, freeze-drying and spray freeze-drying) have been used to produce the dry powder cubic precursor, resulting in powders displaying a reduced BSA release rate. In work by Nasr et al. (2016), GMO was dissolved in a methanol/chloroform mixture, which were sprayed onto sorbitol particles and dried [35]. The powder precursor formed 100 nm particles upon hydration, with a relatively narrow particle size distribution. However, no qualitative analysis for structure determination of the cubosome particles was performed. In a recent study, ovalbumin was loaded in a spray dried cubosome powder precursor, using dextran to prevent aggregation [36]. LCNPs with various LC structures formed upon hydration of the powders. The field of research is apparently lacking consistent studies that follow the LC structure, particle size and appearance as function of the different steps from the preparation of the LC gels to rehydration of the powders.

The aim of this work was to investigate how the LNCPs structure was affected by; 1) choice and concentration of disaccharide (lactose, trehalose and sucrose), 2) effect of heat-treatment, 3) effect of AMP incorporation. Phase behavior and particle size and size distributions at the different preparation steps were monitored by small angle x-ray scattering (SAXS) and dynamic light scattering (DLS), respectively. Particle appearance was imaged by cryogenic transmission electron microscopy (cryo-TEM). Moreover, the therapeutic relevant antimicrobial peptide (AMP) AP114 (also known as NZ2114, molecular weight 4411 Da, net charge +4.6 at pH 5.5) was included in some of the formulations, to investigate the formulations suitability as drug delivery vehicle. The peptide AP114 is a water soluble improved plectasin derivative, a defensin peptide found in the fungus *Pseudoplectania nigrella*, with amino acid sequence GFGCNGPWNEDDLRCHNHCKSIKGYKGGY CAKGGFVCKC [37,38]. In contrast to other AMPs, acting by disrupting bacterial membranes, AP114 inhibits the bacterial membrane biosynthesis by targeting the cellular precursor Lipid II [38]. AP114 kills Gram-positive bacteria, including *Staphylococcus aureus* and its methicillin resistant variety (MRSA), making it potentially useful for the treatment of pneumonia. Peptide encapsulation and release properties were investigated. The antibacterial effect of the peptide loaded formulations were studied *in vitro* using minimum inhibitory concentration (MIC) and time kill assay.

2. Experimental

2.1. Materials

Glycerol monoleate Capmul-90 EP/NF was obtained as a gift from Abitec Corp. (Columbus, USA) with composition 93.3 and 6.3% mono and di-glycerides, respectively, and C18:1 (oleyl content) >95%. Other chemical used were lactose monohydrate (for microbiology, Merck), trehalose dihydrate (99.0%, Sigma-Aldrich), sucrose (99.0%, Fluka Analytical) and the triblock co-polymeric stabilizer Kolliphor P407, former known as Lutrol F127, (approx. 12.500 g/mol, BASF). Antimicrobial peptide AP114 (highly water soluble, purity 99.1%) was obtained from Adenium Biotech ApS (Denmark).

2.2. Methods

2.2.1. Preparation of liquid crystalline gels

Bulk LC gels were prepared by mixing melted GMO (at 40 °C) with a water solution containing the sugars (lactose, trehalose or sucrose) with a spatula to achieve a final composition of 68.25/29.25/2.5 or 66.5/28.5/5 GMO/water/sugar, by weight. Typical mass of the gels was 2 g. The GMO:water was kept at 70:30, which without guest molecules result in a cubic (Ia3d) gel [39]. The gel was left to equilibrate overnight and homogenized by