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Impact of preparation method and variables on the internal structure, morphology, and presence of liposomes in phytantriol-Pluronic® F127 cubosomes

Seyedeh Parinaz Akhlaghi[a], Iris Renata Ribeiro[a], Ben J. Boyd[b], Watson Loh[a,*]

[a] Chemistry Institute, University of Campinas, PO Box 6154, Campinas, SP 13083-970, Brazil
[b] Drug Delivery, Disposition and Dynamics and ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia

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ABSTRACT

The formation of significant proportions of liposomes during the preparation of dispersed cubic phase particles presents a problem in trying to understanding cubosome behavior with a view to use in applications such as drug delivery. In this study, the variables impacting on liposome formation during cubosome production were interrogated. Bottom-up (BU) and top-down (TD) approaches were employed to prepare submicron sized liquid crystalline dispersions (cubosomes) of phytantriol in water with varying amounts of Pluronic®F127 (F127) as a stabilizer. In the BU approach, ethanol was used as a hydrotrope and was later removed using a rotary evaporator, whereas in the TD approach the bulk liquid gel was dispersed using ultrasonication. We aimed at finding the optimum ratio of phytantriol-to-F127 resulting in stable, liposome-free dispersions, whether this depends on the preparation method and the resulting morphology of the particles. The average particle size and zeta potential of the samples were measured using dynamic light scattering (DLS). Cryogenic transmission electron microscopy (Cryo-TEM) images showed a substantial number of liposomes in addition to cubosomes in the dispersion containing 4–1 (w/w) phytantriol-to-F127 prepared by the BU approach compared to very low liposome content with the TD approach. The effects of the amount of F127 in both approaches, amount of ethanol on the BU method and temperature on the TD method were investigated using small-angle X-ray scattering (SAXS). The cubosomes displayed cubic double-diamond (*Pn3m*) internal structure with a lattice parameter of approximately 6 nm. In summary using the TD approach, with 4:1 phytantriol:F127 provided stable cubosome dispersion with minimal liposome co-existence.

1. Introduction

Liquid crystalline phases (mesophases) are comprised of amphiphilic molecules, which are organized spontaneously in water, and have great structural versatility. Much interest has been drawn on the inverse bicontinuous cubic phases (BCP), which contain two three-dimensional, interpenetrating but non-communicating water channels divided by a lipid bilayer [1]. Cubosomes are individual nanoparticles formed by the dispersion of the BCP in water [2]. They display low viscosity, high heat stability, large surface area, exist over a useful temperature range (4–40 °C) and can be found in almost any dilution level [3], as opposed to more hydrophilic liquid crystalline systems that will transform into micelles at higher dilutions. Larsson et al. first applied cubosomes as controlled delivery systems [4,5] and since then they have been used to deliver a variety of active compounds [6]. They are ideal candidates for delivery of active compounds, due to their inexpensive raw materials, enhanced payload and their potential for releasing their cargo in a sustained release manner [7].

Cubosome preparation can be achieved through two approaches: top-down (TD) and bottom-up (BU) techniques. In the TD route the bulk cubic phase is dispersed into an aqueous solution using high energy which can be created by either sonication, shearing or high-pressure homogenization [8]. The TD method has been reported by Ljusberg-Wahren [9] and is the most commonly used technique for the preparation of cubosomes [10]. It is a rapid method which forms cubosomes with a size below 200 nm and low polydispersity [11]. However, a drawback of this method is that it is only feasible in a small scale [12]. Furthermore, this approach requires high thermal/mechanical energies which in

* Corresponding author.
E-mail address: wloh@iqm.unicamp.br (W. Loh).

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turn can cause problems in the incorporation of labile compounds such as proteins and peptides [13].

In the BU method also referred to as the solvent dilution, liquid precursor or hydrotope method, a single-phase solution is diluted into a two phase system consisting of cubosomes and an aqueous phase [11]. This is a more recently developed technique first introduced by Spicer and coworkers [2]. It requires low energy and takes advantage of the miscibility of an auxiliary solvent (e.g., ethanol) in a continuous aqueous phase. Upon the addition of excess water, lipid droplet nucleation occurs and the mixture spontaneously phase separates and the cubosomes precipitate [12]. The main advantage of this method compared to the TD technique is that less energy is required to form the dispersions, which allows for large scale preparation of cubosomes [14] and is particularly preferred in case of incorporation of sensitive compounds [15]. However, the main disadvantages of the BU method are the difficulties in controlling particle size and the possible presence of solvent in the final sample, which is often incompatible with solvent-sensitive compounds incorporated in the cubosomes such as proteins and may be unacceptable for medical applications [15].

The most frequently investigated liquid crystal structures for biomedical applications are prepared by cubic mesophases of unsaturated monoglycerides (GMO) and phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) that are capable of forming viscous reverse phases (hexagonal and bicontinuous cubic) in excess water under physiological conditions and temperature. Phytantriol has gained more recent interest in the biomedical field compared to monoglycerides due to several reasons, such as superior chemical stability compared to monoglycerides due to the absence of the ester group [16], and availability of commercial phytantriol with 95% purity, whereas monoglycerides are produced from various sources which have significantly different purities [17]. Cubosomes require the addition of another surfactant to kinetically stabilize these colloidal dispersions against aggregation. Pluronic®F127 is a nonionic linear triblock polymer that contains a polypropylene oxide block (PPO) between two polyethylene oxide blocks (PEO) [18] and acts as a steric stabilizer via the adsorption and incorporation of its hydrophobic PPO blocks onto the surface of the nanoparticles while also maintaining the inner cubic phase structure [19].

In a number of studies, researchers have investigated the preparation of cubosomes via the TD and BU methods and aimed at optimizing the preparation procedure for producing cubosomes with consistent size and structure. Rizwan et al. first reported the preparation of phytantriol cubosomes using the BU method and compared them to cubosomes made by TD method. They investigated the effect of the hydrotrope concentration and type as well as the lipid-to-stablizer ratio on the cubosome formation [12]. Recently, Kim and coworkers used the BU approach to prepare cubosomes of phytantriol, F127, and vitamin E. An ethanolic solution was used to dissolve phytantriol, the stabilizer and vitamin E. Upon the addition of deionized water, bilayer vesicles were formed. After the removal of ethanol from the aqueous mixture, vesicles were converted to cubosomes with well-defined internal structures studied by cryo-TEM and SAXS [13].

There have been several reports on the coexistence of liposomes in the cubosome preparations which can make it difficult to interpret experimental results aimed at understanding cubosomes behavior [20–23]. In order to evaluate whether cubosomes have true benefit over other particles such as liposomes it is crucial to understand and, where possible, to eliminate the production of coexisting liposomes in cubosome dispersions.

Therefore, in this study, we aim to determine the critical compositional and processing determinants in avoiding coexisting vesicle production in the preparation of phytantriol-F127 cubosomes. For this purpose, we applied BU (using ethanol in a solvent displacement method) and TD (using ultrasonication) approaches while varying the F127 concentration to find the optimum procedure. We also investigated the effects of ethanol added in the BU method and temperature increase by ultrasonication in the TD method on the internal structure of the dispersions. Such information can assist in making decisions in choosing the approach to prepare cubosomes for various applications.

2. Experimental

2.1. Materials

Phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) was purchased from SARFAM (São Paulo, Brazil) and used as received. Pluronic®F127 (PEO_{98}-PPO_{67}-PEO_{98}) with an average molar mass of 12,600 Da was acquired from Sigma-Aldrich (St. Louis, USA) (Fig. 1). Deionized water (Milli-Q, Millipore Corp., Bedford, MA) was used to prepare all aqueous samples.

2.2. Methods

2.2.1. Cubosome preparation

2.2.1.1. Top-down method. Phytantriol (200 mg) was weighed into a glass vial and heated at 40 °C until free flowing. Aqueous solution (10 mL) containing different concentrations of F127, prepared from a 2 wt% stock solution of F127, were added to the vial containing phytantriol (Table 1). Subsequently, this mixture was homogenized by ultrasonication (Hielscher, Teltow, Germany) at 40 °C, amplitude

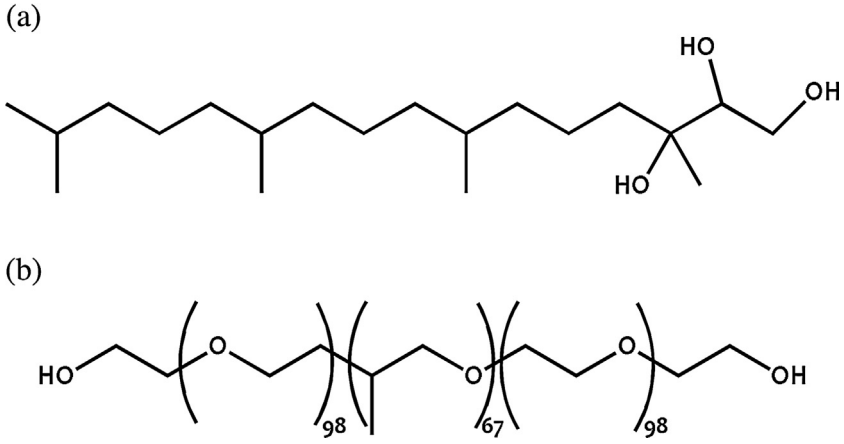

Fig. 1. Chemical structure of (a) phytantriol and (b) Pluronic®F-127.