Novel nano-engineering phytantriol-F127-based cubosomes for antibiotic delivery

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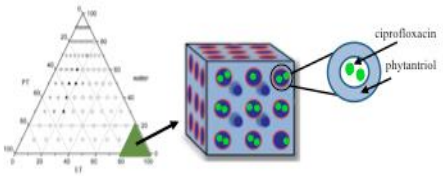
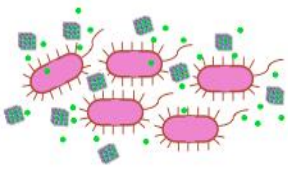


Cubosomes preparation


Antimicrobial action of ciprofloxacin-loaded cubosomes

Nanoparticles exhibited exceptional properties for drug delivery via different routes with excellent pharmacokinetics profiles using minimal dosages with almost no systemic side effects. The delivery of drug molecules for therapeutic applications usually faces problems of solubility and bioavailability that cubosomes can easily overcome. Cubosomes are cubic lipid-based nanoparticles with increased surface area, low viscosity and high heat stability. The preparation of cubosomes and drug encapsulation are still procedures requiring important improvements that have limit their use as drug delivery system. This work we developed a simple experimental procedure able to produce controlled-size cubosomes with different hydrodynamic size dependent of the phytantriol/ethanol (lipid/hydrotrope) ratio. Moreover, cubosomes were easily loaded with ciprofloxacin without structural changes. The results of this study will greatly impact on the applicability of cubosomes as drug delivery system.

Introduction

Nanotechnology is one of the most promising approaches assisting antimicrobial therapy design as it can provide novel vehicle systems to deliver antimicrobial agents. Nanoparticles exhibit exceptional properties for drug delivery via different routes with excellent pharmacokinetic profiles minimizing systemic side effects [1]. Generally, nanoparticles are entities that are usually less than 100 nm in diameter, in some cases between 100 and 500 nm, which are represented in the form of biodegradable or non-biodegradable drug carriers [1]. In clinical context, liposomes have been intensively investigated for drug delivery. Liposomes are spherical vesicles composed by bilayer membranes of amphiphilic lipids with an internal aqueous space and its relevance arises from their high biocompatibility, ability to incorporate both hydrophobic and hydrophilic drugs, safe drug delivery profiles and ease of preparation. However, liposomes still present limitations, such as storing stability, drug leakage and lack of size control and a limited loading capacity of lipophilic drugs, Hence, the use of liposomes for drug delivery is limited [2].

Nanosized dispersions of liquid crystalline phases had attracted our attention because of their interesting approach of encapsulation and release of drugs [3,4]. This liquid crystal structures are composed by amphiphilic molecules that when mixed with right solvent have the ability to spontaneously self-assemble leading to the formation of well-defined thermodynamically stable complexes, such as the cubosomes [4]. The colloidal dispersion of bicontinuous cubic liquid crystalline structures in water using suitable surfactant can result in cubosomes exhibiting 100 to 300 nm [5]. Cubosomes are attractive drug delivery systems because they can incorporate a great variety of chemical compounds in their domains and exhibit larger surface area, lower viscosity and high heat stability [6,7]. Although these unique physicochemical characteristics and the potential for controlled release through functionalization, the production of cubosomes has some challenges that need to be overcome, such as the presence of liposomes vesicles in the cubosomes dispersions and high energy input [4,6].

Therefore, the main goal of this study was to strengthen the assumption that cubosomes will be the new trend in drug delivery system improving their preparation and drug encapsulation method. We aimed to develop and optimize the production of controlled-size cubosomes to further load them with ciprofloxacin (CIP), a fluoroquinolone antibiotic that is administrated to treat infections caused by gram-negative bacteria. Through an optimized controlled-size and an ease of preparation, cubosomes applicability as drug delivery systems would be greatly enlarged.

Methods

In this work, a bottom-up approach was used to form the cubosomes through the dispersion of a mixture comprising the liquid crystal forming lipid, the polymer and a hydrotrope in excess of water with minimal energy input [6]. Based on phase diagram of the phytantriol (PT) – ethanol (ET) – water system described by *Y. Chen et al.* [8], different mixtures of phytantriol/ethanol (lipid/hydrotrope) were prepared as described in Table 1. The preparation of cubosomes also required a stabilizer in order to provide colloidal stability, prevent re-coalescence to bulk cubic phase and create stable dispersions [6,9]. In this study, we used the block copolymer Pluronic F127 (or Poloxamer 407) dissolved in water. The initial solutions of phytantriol/ethanol described in Table 1 were used to form cubosomes by solvent shifting. Briefly, phytantriol/ethanol solutions were added at once (solvent shifting) to different F127 solutions resulting in distinct PT: F127 ratios (Table 2). The resulting dispersions were stirred for 2 min and kept at room temperature. To verify whether ethanol had influence on the structure and size of formed cubosomes, dispersions were led at room temperature overnight to promote ethanol evaporation. All the final dispersions prepared were labeled as, for instance, A1, the letter to identify the initial solution (Table 1) and the number to discern the final PT: F127 ratio (Table 2).

Table 1. Composition of the initial solutions used to form cubosomes.

Sample	Composition (phytantriol (w/w)	Composition (ethanol (w/w)
A	0.01	0.99
B	0.05	0.95
C	0.1	0.9

The final dispersions were characterized in terms of particle size (hydrodynamic diameter) and polydispersity index (PDI) using a dynamic light scattering instrument (DLS) at angles of

90 º and 173 º. Measurements were performed at 25 ºC, during 4 runs at 90 s each run. The results were analyzed using MATLAB software according to the functions described by *Hassan et al.* [10] with some optimizations.

Table 2. Different final phytantriol/F127 ratio in the formed cubosomes.

Sample series	PT: F127 ratio
1	0.1
2	0.5
3	1
4	5
5	10

To encapsulate CIP into cubosomes, the same method described above was used. CIP was dissolved in the mixture of phytantriol and ethanol and adding 1 M of NaOH until the mixture was completely dissolved and clean. At the end, the final dispersions were centrifuged at 2000 rpm for 5 min to remove the non-encapsulated CIP. The supernatants containing the cubosomes loaded with CIP were collected for DLS analysis.

Results & Discussion

Cubosomes were prepared using a bottom-up approach because it needs less energy input and produces cubosomes more efficiently, with smaller size, and long-term stability. Moreover, this approach is easily scale-up to commercial and industrial batches [6]. Even though monoolein is the most common lipid used to prepare lipid nanoparticles, we used phytantriol due to its superior chemical stability, absence of the ester group and availability of commercial phytantriol with high purity [7,11].

Before DLS measurements, the dispersions were first screened visually in order to discard the dispersions containing visible phase separation. For instance, dispersions from series 5 (A5 to C5) exhibited visible aggregates indicating that the resultant cubosomes were too large and not suitable for drug delivery proposes. Therefore, only transparent and clean dispersions were analyzed by DLS.

DLS data demonstrated that our innovative experimental setup was able to produce cubosomes with diverse diameters in a controlled-size way. Using the dispersions A3, B2 and C1, we produced cubosomes with reduced size (hydrodynamic diameter), ranging from 150 to 270 nm, whereas using the dispersions B3 and C2 larger cubosomes (>300 nm) were obtained.

Table 3. Hydrodynamic diameter and polydispersity index of the cubosomes dispersions.

Sample name	Hydrodynamic diameter (nm)	Polydispersity index (PDI)
A3	152	0.19
A3*	148	0.17
B2	209	0.16
B2 + CIP	265	0.28
B3	327	0.30
B3 + CIP	344	0.52
C1	264	0.19
C2	354	0.25

* Ethanol evaporation overnight.

In terms of the polydispersity, all the dispersions showed to be monodisperse as PDI scores varied from 0.08 to 0.7 [12]. This result suggested that dispersions contained a unique and homogeneous population of cubosomes, indicating that controlled-size cubosomes were atttained. Moreover, these data reinforced the role of F127 in cubosomes stabilization without compromising the particle size.

DLS results also showed that ethanol seemed not interfere in the structure of the cubosomes. The hydrodynamic diameter of cubosomes derived from A3 dispersion with and without ethanol evaporation was similar, as well as the PDI indicating both dispersion as monodisperse.

CIP encapsulation into cubosomes demonstrated to be an easy procedure with satisfactory encapsulation efficacies. More importantly, cubosomes loaded with ciprofloxacin seemed not exhibit structural alterations. DLS data demonstrated that cubosomes derived from B2 and B3 dispersions with and without CIP had identical hydrodynamic diameter, approx. 286 nm, and controlled-sized of cubosomes were still maintained as PDI score was below 0.7.

In conclusion, our data revealed that controlled-size cubosomes with different range of diameters can be produced using a simple experimental workflow with minimal energy input. Ciprofloxacin encapsulation into cubosomes was easily performed and no structural alterations were detected. These findings will greatly impact on the applicability of cubosomes as drug delivery systems.

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