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New nanoparticles obtained by co-assembly of amphiphilic cyclodextrins and nonlamellar single-chain lipids: Preparation and characterization

Cảnh Hưng Nguyễn[a,1], Jean-Luc Putaux[b], Gianluca Santoni[c], Sana Tfaili[d], Sophie Fourmentin[e], Jean-Baptiste Coty[a], Luc Choisnard[f], Annabelle Gèze[f], Denis Wouessidjewe[f], Gillian Barratt[a], Sylviane Lesieur[a], François-Xavier Legrand[a,*]

[a] Institut Galien Paris-Sud, CNRS UMR 8612, Univ. Paris-Sud, Université Paris-Saclay, 5 rue Jean-Baptiste Clément, F-92290 Châtenay-Malabry, France
[b] Centre de Recherches sur les Macromolécules Végétales, CNRS UPR 5301, Univ. Grenoble Alpes, BP 53, F-38401 Grenoble Cedex 9, France
[c] European Synchrotron Radiation Facility, 71 avenue des Martyrs, F-38000 Grenoble, France
[d] Lip(Sys)^2, EA 7357, Chimie Analytique Pharmaceutique, Univ. Paris-Sud, Université Paris-Saclay, 5 rue Jean-Baptiste Clément, F-92290 Châtenay-Malabry, France
[e] Unité de Chimie Environnementale et Interactions sur le Vivant, EA 4492, SFR Condorcet FR CNRS 3417, Université Littoral Côte d'Opale, 145 avenue Maurice Schumann, F-59140 Dunkerque, France
[f] Département de Pharmacochimie Moléculaire, CNRS UMR 5063, Univ. Grenoble Alpes, F-38000 Grenoble, France

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ABSTRACT

This work aimed at preparing new nanoscale assemblies based on an amphiphilic bio-esterified β-cyclodextrin (β-CD), substituted at the secondary face with *n*-decanoic fatty acid chains (β-CD-C_{10}), and monoolein (MO) as new carriers for parenteral drug delivery. Stable binary (β-CD-C_{10}/MO) and ternary (β-CD-C_{10}/MO/stabilizer) nanoscale assemblies close to 100 nm in size were successfully prepared in water by the solvent displacement method. The generated nanoparticles were fully characterized by dynamic light scattering, transmission electron microscopy, small-angle X-ray scattering, residual solvent analysis, complement activation and the contribution of each formulation parameter was determined by principal component analysis. The β-CD-C_{10} units were shown to self-organize into nanoparticles with a hexagonal supramolecular packing that was significantly modulated by the molar ratio of the constituents and the presence of a steric or electrostatic stabilizer (DOPE-PEG_{2000} or DOPA/POPA, respectively). Indeed, nanoparticles differing in morphology and in hexagonal lattice parameters were obtained while the co-existence of multiple mesophases was observed in some formulations, in particular for the β-CD-C_{10}/MO/DOPA and β-CD-C_{10}/MO/POPA systems. The mixed β-CD-C_{10}/MO/DOPE-PEG_{2000} nanoparticles (49:49:2 in mol%) appeared to be the most suitable for use as a drug delivery system since they contained a very low amount of residual solvent and showed a low level of complement C3 activation.

1. Introduction

Non-lamellar liquid crystalline phases formed by lyotropic lipids are particularly attractive for the formulation of active pharmaceutical ingredients because of their unique structure of lipid assembly interpenetrated with nanoscale water channels that provide multiple compartments for the efficient loading and sustained release of hydrophilic, lipophilic or amphiphilic molecules (Guo et al., 2010; Kulkarni, 2012). In recent years, nano-assemblies generated from these phases, referred to as ISAsomes (Internally Self-Assembled "somes" or particles), and in particular cubosomes and hexosomes, have been increasingly investigated as

Abbreviations: β-CD-C_{10}, β-cyclodextrin *n*-decanoic ester; MO, monoolein; DOPE-PEG_{2000}, ammonium salt of 1,2-dioleoyl-*sn*-*glycero*-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000]; DOPA, sodium salt of 1,2-dioleoyl-*sn*-*glycero*-3-phosphate; POPA, sodium salt of 1-palmitoyl-2-oleoyl-*sn*-*glycero*-3-phosphate.

* Corresponding author.
E-mail address: francois-xavier.legrand@u-psud.fr (F.-X. Legrand).
[1] Present address: Department of Pharmaceutics, Hanoi University of Pharmacy, Hanoi, Vietnam.

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promising alternatives to other lipid-based drug nanocarriers such as liposomes or lipid nanoparticles (Azmi et al., 2015; Ganem-Quintanar et al., 2000; Guo et al., 2010; Milak and Zimmer, 2015; Mulet et al., 2013; Shah et al., 2001). The majority of these studies have chosen 1-oleylglycerol or monoolein as the main component. Monoolein is indeed a versatile lyotropic lipid since it forms different mesophases in water, the topology of which can vary as a function of hydration and temperature to include lamellar, bicontinuous cubic and hexagonal molecular packings whose lattice parameters can be adapted to incorporate co-lipids or other guest molecules. As a result of this reverse bicontinuous cubic phases consisting of a highly twisted lipid bilayer delimiting two non-intersecting water channels, these nano-assemblies can accommodate a wide range of therapeutic substances varying in water solubility and molecular mass, from traditional active molecules to biological macromolecules, notably peptides and proteins (Boyd et al., 2007; Chen et al., 2012; Lee et al., 2009; Peng et al., 2010; Rizwan et al., 2010; Wyatt and Dorschel, 1992; Zhen et al., 2012). In the same way, inverse hexagonal phases based on monoolein organized as juxtaposed cylindrical aqueous cores delimited by lipid monolayer shells have been attracting increasing attention for the delivery of drugs (Duttagupta et al., 2016; Guo et al., 2010; Meli et al., 2017). The particular interest of these liquid crystalline mesophases lies in the fact that their hierarchical nanostructure can determine the release kinetics and bioavailability of drugs while protecting them and reducing their toxicity. Effective sustained release and improvement of oral or topical absorption of incorporated hydrophilic model drugs have been clearly demonstrated in vitro and in vivo (Boyd et al., 2007; Guo et al., 2010; Lopes et al., 2006; Meli et al., 2017; Nazaruk et al., 2015; Peng et al., 2010; Rizwan et al., 2010; Wyatt and Dorschel, 1992; Zhen et al., 2012).

Most studies conducted to date have focused on the use of non-lamellar lipid mesophases for oral or topical administration rather than for parenteral routes, due to the rather high viscosity of the formulations (Milak and Zimmer, 2015; Shah et al., 2001). As far as intravenous administration is concerned, some modifications have to be made to the formulation. Most importantly, the mesophases must be dispersed as particles with a size in the submicron range and furthermore be stabilized so as to have long-circulating properties in the cardiovascular system. Nanoscale cubosome or hexosome formulations have been tested over the last twenty years and special efforts have been made to ensure that they retain a stable colloidal state in physiological fluids (Guo et al., 2010; Guo et al., 2010; Mulet at al., 2013). Steric stabilization by hydrophilic polymer coating has been preferred to electrostatic stabilization since rapid elimination of charged nanoparticles from the systemic circulation as a result of complement activation has been reported (Fraser et al., 2013; Han et al., 2010; Hartnett et al., 2014; Lindell et al., 1998). However, the introduction of polymer stabilizers such as amphiphilic triblock copolymers based on ethylene oxide or poly(ethylene glycol)-grafted lipids can induce structural changes that can modify the encapsulation yields and release processes of drugs (Cervin et al., 2009; Johnsson et al., 2006; Zeng et al., 2012).

Furthermore, the reduced size of the mesophase dispersions significantly increases the surface area of the particles in direct contact with the external dispersion medium, increasing the risk of both reduced drug loading efficiency and loss of sustained release action. To address these issues, the addition of components able to reinforce the nanoporosity of ISAsomes while preserving their original liquid crystalline state can be envisaged. An attractive possibility is to use hollow deep vase-like building blocks based on cyclodextrins (CDs): cyclic oligosaccharides able to form host-guest inclusion complexes with a wide range of hydrophobic compounds (Kfoury et al., 2015; Uzqueda et al., 2009). CDs are already in use for some pharmaceutical applications, mainly to enhance bioavailability and stability of drugs and to reduce some of their undesirable properties such as volatility and taste. Nanoscale CD assemblies for multi-drug delivery have been developed from amphiphilic derivatives that have been synthesized by enzymatic transesterification with long-chain fatty acids on hydroxyl groups at one or both faces of the native CDs (Choisnard et al., 2011). These bio-esterified CD molecules can self-assemble or be incorporated into lipid-based reverse cubic mesophases, playing the role of structural stabilizer while their cavity enhances the incorporation yield of hydrophobic substances, as demonstrated by a recent study (Zerkoune et al., 2016). More precisely, nanodispersions composed of monoolein, polysorbate-80 and a bio-transesterified CD with C10 chains grafted mostly on the secondary face (referred to as β-CD-C_{10}) with a total degree of substitution (TDS) of 7.3 were formed by hydration of a three-component film in excess water followed by sonication. Small-angle X-ray scattering (SAXS) characterization revealed that, in the absence of the amphiphilic CD, no inner ordered structure was observed while, in contrast, the presence of 4 mol% β-CD-C_{10} induced reverse cubic molecular packing. Moreover, it was shown that the CD-containing nanostructures could entrap up to 94% of the hydrophobic Oil Red model molecule (Zerkoune et al., 2016).

Following on from these promising results, the present work focuses on the preparation, and characterization of mixed β-CD-C_{10}/monoolein nanoscale assemblies, and their evaluation as drug carriers for parenteral administration. For this purpose, nanodispersions with or without steric stabilizers were prepared by the well-known solvent displacement method, while accurately monitoring the amount of residual solvent using appropriate techniques. Systematic investigations were undertaken to determine the influence of the composition on the feasibility of forming mesoporous nano-objects in isotonic media with a well-defined size distribution as well as on the structural variability of their inner molecular organization. Furthermore, the interaction of the multicompartment nanoscale systems prepared by this method with blood components was examined in detail to predict their systemic behavior.

2. Material and methods

2.1. Materials

The β-CD-C_{10} amphiphilic cyclodextrin (MM = 2293.7 g mol^{-1}; TDS = 7.5) was synthesized by an enzymatically-assisted pathway using thermolysin as catalyzer and decanoic vinyl esters (C_{10}) as acyl donors (Choisnard et al., 2006, 2007). It was obtained as a white powder which was analyzed by matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) to determine the mean molecular mass and the mean substitution degree of the batch (Choisnard et al., 2011). Monoolein (MO) was obtained from Sigma-Aldrich at its highest purity and was used without any recrystallization. The ammonium salt of 1,2-dioleoyl-*sn-glycero*-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DOPE-PEG_{2000}) and sodium salts of 1,2-dioleoyl-*sn-glycero*-3-phosphate (DOPA) or of 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phosphate (POPA) were purchased from Avanti Polar Lipids. Anhydrous organic solvents (HPLC grade) were purchased from Carlo-Erba and used without further purification. Ultrapure water (γ = 72.2 mN. m^{-1} at 22 °C, resistivity 18.2 MΩ·cm) was produced by a Millipore Milli-Q Direct 8 water purification system and sterile isotonic 5% glucose aqueous solution was purchased from C.D.M. Lavoisier.

2.2. Nanoparticle preparation

The nanoparticle suspensions were prepared using the solvent displacement technique. Five milligrams of amphiphilic