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Miscellaneous nanoaggregates made of β-CD esters synthesised by an enzymatic pathway

L. Choisnard [a,*], A. Gèze [a], B.G.J. Yaméogo [a], J.-L. Putaux [b], D. Wouessidjewe [a]

[a] *Université Joseph-Fourier, UFR de pharmacie ICMG FR-2607 – DPM UMR UJF/CNRS 5063, 470 rue de la Chimie, BP 53, F-38041, Grenoble cedex 9, France*
[b] *Centre de recherches sur les macromolécules végétales (CERMAV-CNRS), BP 53, F-38041 Grenoble cedex 9, France*

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Abstract

Various β-cyclodextrin (β-CD) fatty esters with different chain lengths (C4–C14) were synthesised by transesterification of β-cyclodextrin by vinyl fatty ester using thermolysin in DMSO. For each cyclodextrin derivatives, two batches of synthesis were realized. The ability of these derivatives to form nano-organized systems was investigated through the solvent displacement technique. During the formulation step, the effects of the initial concentration of β-CD fatty esters in the organic phase and that of the final volume of the aqueous non-solvent phase were studied. Except for the β-CD C4 ester, the transesterified β-CD derivatives led to measurable nanoparticles. Cryo-electron microscopy images showed a significant morphological variability. Spherical, rod-like or more irregularly-shaped nano-objects were observed with either matricial or lamellar structures. A statistical analysis by a two-way ANOVA was computed for each class of β-cyclodextrin esters in order to determine the effects of batch and formulation on the final size of nanoparticles.

Keywords: Thermolysin transesterification; β-cyclodextrin fatty esters; Nanosystems; Quasi-elastic light scattering; Cryo-transmission electron microscopy; Variance analysis

1. Introduction

α-, β- and γ-cyclodextrins are cyclic oligosaccharides made of six, seven and eight α-1.4-glycosidic-linked glycosyl residues, respectively (Biwer et al., 2002). Cyclodextrins have been widely used as pharmaceutical excipients to formulate drugs with poor bioavailability. Indeed, owing to their particular structure, which consists of a hydrophilic external surface and a more hydrophobic cavity lined with protons, cyclodextrins are capable of forming inclusion complexes with a variety of guest molecules, improving the solubility and/or stability of the guest compounds. Numerous chemical modifications have been carried out on cyclodextrins by grafting substituents to different positions (primary face, secondary face or both faces) in order to obtain amphiphilic derivatives able to form supramolecular aggregates in the form of nanoparticles considered as potential drug carriers (Davis and Brewster, 2004). Among the modifications realized, a series of molecules have been obtained by grafting fatty acids of different chain lengths on the hydroxyl groups of the secondary face. Two different synthetic ways have been investigated. The first approach involves a three-step chemical modification: (i) protection of primary hydroxyl groups, (ii) acylation of secondary face, (iii) deprotection of primary face (Zhang et al., 1991). The second approach involves the use of a thermolysin as a biocatalyser of transesterification of cyclodextrin by a vinyl fatty ester donor (Pedersen et al., 2005). Recently, we have shown that the formulation of β-CD capric ester derivative (β-CD C10) yielded hybrid structures presenting a crystal-like core surrounded by onion-like concentric bilayers, whereas β-CD caproic ester (β-CD C6) formed supramolecular assemblies presenting a homogeneous matrix (Choisnard et al., 2006).

From these results, numerous cyclodextrin derivatives have been synthesized by grafting alkyl chains of variable lengths

* Corresponding author. Tel.: +33 04 76 63 53 01; fax: +33 04 76 63 53 22.
E-mail address: luc.choisnard@ujf-grenoble.fr (L. Choisnard).

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(C4–C14) on the β-cyclodextrin. The products were tested for their ability to nano-aggregate. The morphological properties of some newly obtained cyclodextrin aggregates were investigated by cryo-transmission electron microscopy (cryo-TEM) and quasi-elastic light scattering (QELS). The results were statistically analyzed.

2. Materials and Methods

2.1. Materials

Thermolysin (EC 3.4.24.27), a protease Type X isolated from *Bacillus thermoproteolyticus rokko*, anhydrous DMSO (99%), vinyl capric ester (95%) and celite were obtained from Sigma Aldrich (Sigma Aldrich, L'Isle D'Abeau Chesnes, France). Vinyl caproic ester (99%), vinyl caprylic ester (>99%), vinyl myristic ester (>99%) and vinyl butyric ester (>97%) were purchased from TCI Europe nv (Interchim, Montluçon, France). Vinyl Lauric ester (>99%) was received from Fluka (Sigma Aldrich, L'Isle D'Abeau Chesnes, France). β-cyclodextrin (Kleptose®) was a gift of Roquette (Roquette, Lestrem, France). Anhydrous acetone was obtained from VWR (VWR International, Lyon, France). Water was freshly deionized in our laboratory.

2.2. Enzymatic reactions and products characterization

Thermolysin was immobilized on celite using a deposition technique according to the procedure previously reported (Choisnard et al., 2006). Before use, β-CD was dried under reduced pressure (1 mbar) for 48 h at 80 °C in presence of P_2O_5, the residual water content was not measured. Ten millilitres of dry β-CD (0.09 M) and vinyl fatty acid (1 M) in DMSO were placed in a capped glass vial containing 1.1 g of immobilized thermolysin. The heterogeneous medium was vigorously stirred (350 rpm) and the reaction was carried out at 45 °C for 72 h or until the vinyl fatty acid upper-phase was completely disappeared. At the end of the reaction, the immobilized thermolysin was removed from the liquid phase by centrifugation (1500 rpm; 15 min). The β-CD esters present in supernatant were precipitated by adding drop-wise a volume of 20 mL of MeOH/H_2O (35/65; v/v) and separated by centrifugation (2000 rpm; 15 min). The crude product was washed twice with 10 mL of DMSO/MeOH/H_2O (50/35/65; v/v/v) and purified by silica gel flash column chromatography using first CH_3Cl and then CH_3Cl/MeOH (10/90 or 50/50; v/v) as mobile phase. β-CD fatty ester was dried under reduced pressure and stored at room temperature. All the β-CD derivatives were analyzed by ^{1}H NMR, ^{13}C NMR and Heteronuclear Multiple-Quantum Coherence NMR (HMQC-NMR). The observations were in agreement with those previously published by Choisnard et al. (2006) and Pedersen et al. (2005). The β-CD trans-esterification occurred mainly at the C_2 position and the substitutions on C_3 and C_6 positions were considered marginal. Typical results of matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) analyze of the derivatives are reported in Table 1.

Table 1
MALDI-MS analysis summary ($m/z + Na^+$) for β-CD butyric (β-CD C4), caproic (β-CD C6), caprylic (β-CD C8), capric (β-CD C10), lauric (β-CD C12) and myristic (β-CD C14) ester

	Number of acylations per β-cyclodextrin fatty ester molecule								
	2	3	4	5	6	7	8	9
β-CD C4	×	×	×	×	m/z = 1578 (1%)	m/z = 1648 (100%)	m/z = 1718 (29%)	×
β-CD C6	×	×	×	m/z = 1647 (1%)	m/z = 1745 (18%)	m/z = 1843 (100%)	m/z = 1943 (20%)	m/z = 2041 (1%)
β-CD C8	×	×	×	m/z = 1788 (5%)	m/z = 1915 (55%)	m/z = 2041 (100%)	m/z = 2167 (4%)	×
β-CD C10	×	×	×	×	m/z = 2083 (22%)	m/z = 2237 (100%)	m/z = 2391 (22%)	×
β-CD C12	m/z = 1522 (8%)	m/z = 1704 (25%)	m/z = 1887 (54%)	m/z = 2069 (100%)	m/z = 2251 (82%)	m/z = 2434 (29%)	m/z = 2616 (1%)	×
β-CD C14	×	×	×	×	m/z = 2419 (11%)	m/z = 2630 (100%)	m/z = 2840 (8%)	×

The relative intensity of peak signals is reported between parentheses.
×: compound not detected.