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# Characterization of amphiphilic β-cyclodextrin nanospheres

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**Abstract**

A new nanosphere carrier system was obtained from amphiphilic β-cyclodextrins bearing fatty acids (with a chain length of either 6 or 12 or 14 carbon atoms) grafted at the $O_2$ and $O_3$ position of the β-cyclodextrin molecule. Nanospheres, with a mean diameter of several hundred nm, were prepared by progressive dispersion.

The nanospheres were characterized by their density, surface charge, size and morphology, using several techniques, namely centrifugation, zeta potential determination, quasi-elastic light scattering and freeze-fracture electron microscopy.

*Keywords:* Amphiphilic cyclodextrins; Nanospheres; Characterization

## 1. Introduction

In recent years there has been considerable interest in the development of novel drug delivery systems in order to modify and control the pharmacokinetic behaviour of therapeutic agents. By incorporation into a carrier system, it is possible to alter both the therapeutic index and the duration of activity of drugs. One of the most studied types of carrier are cyclodextrins. Cyclodextrins have been used in pharmaceutics, cosmetics and food industries to enhance drug solubility (Duchêne and Wouessidjewe, 1990), to stabilize and protect active molecules from UV and visible light oxidation (Skiba et al., 1994), and also to physically separate incompatible drugs from one another.

The scope of the present investigation is the characterization of amphiphilic β-cyclodextrin nanospheres as a drug carrier prepared by a nanospherical crystallization technique.

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## 2. Materials and methods

### 2.1. Materials

Chemically-modified cyclodextrins were synthesized, according to the method described by Zhang et al. (1991) and reported by Skiba et al. (1995). The main reaction consists in the esterification of the $\beta$-cyclodextrin molecule at the $O_2$ and $O_3$ positions. Prior to the esterification the $O_6$ position is protected by a $ButMe_2Si$ group, using dimethyl aminopyridine as an acylation catalyst. After the esterification step, the silyl group is removed by the use of $BF_3$–$EtO_2$ to give the amphiphilic cyclodextrin. Three derivatives were synthesized: cyclodextrins with 6, 12 and 14 fatty acid carbons, which will be referred to as $\beta$CD-$C_6$, $\beta$CD-$C_{12}$ and $\beta$CD-$C_{14}$ respectively (Fig. 1). They are all water insoluble, but highly soluble in organic solvents, such as acetone and ethanol. Acetone (Prolabo, Paris, France) was of analytical grade.

### 2.2. Methods

The concept of the preparation method was based on the spherical crystallization and nanoprecipitation techniques (Kawashima et al., 1982; Fessi et al., 1992) developed by Skiba et al. (1993). The $\beta$CD-$C_m$ ($m = 6$, 12 or 14) was dissolved in acetone thermally controlled at 25°C; typically 20 ml of 20-mg/ml $\beta$CD-$C_m$ acetonic solution was slowly poured through a silicon tube fitted with a fine tip into 10 ml of distilled water and subjected to a magnetic agitation. The nanospheres were formed immediately and the colloidal suspension obtained was then evaporated to remove the solvent and concentrated under vacuum to about one-fifth of the initial volume.

The quasi-elastic light scattering (QELS) experiments were performed on suspensions at a concentration of $10^5$ cps, using a monochromatic laser ray diffusion Nanosizer N4MD (Coultertronics, Margerancy, France). The experimental conditions were the following: temperature, 20°C; reference angle, 90°; viscosity, $0.899 \times 10^{-3}$ Pa·s; refractive index, 1.330.

Small drops of a suspension, concentrated by centrifugation and containing 30% glycerol as a cryoprotectant, were placed on copper planchets and frozen in liquid propane. Freeze fracture and replication were performed using a Balzers BAF 301 apparatus (Lichenstein) equipped with an electron gun for platinum shadowing. Replicas were examined in a Philips 301 transmission electron microscope (Eindhoven, The Netherlands).

The zeta potential of nanospheres from $\beta CD_m$ ($m = 6$, 12 and 14) was measured with an ELS-800 Otsuka Electronics (G.B.X. Fr) instrument. The particles were suspended in KCl ($10^{-3}$ M), and the measurement was made at 20°C. The zeta potential values obtained are the mean of three measurements.

A suspension of nanospheres was deposited on a 0, 5, 10, 20, 30, 40 and 50% sucrose gradient; the centrifugation was performed for 4 h at 20°C at a speed of 27 000 rpm ($113\,000 \times g_{av}$) on an SW 41 TI rotor in a Beckman L7-55 ultracentrifuge. Fractions of 1 ml were collected by puncturing the bottom of the cellulose nitrate tube and measured for their absorbance at 350 nm ($OD_{350}$) in order to detect the presence of nanospheres.

For each fraction of the gradient, the index of refraction was measured at 20°C by a R401 Electronics Unit Waters type refractometer and allowed the concentration of sucrose to be

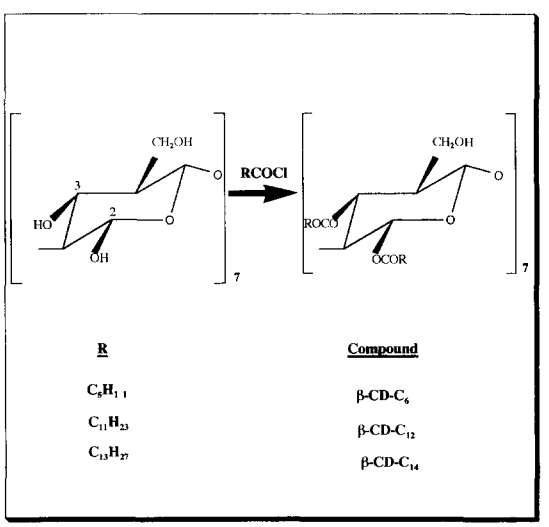


Fig. 1. Diacylation reaction of $\beta$-CD.

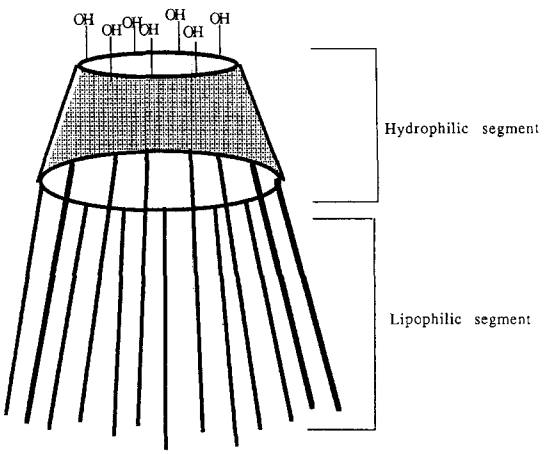


Fig. 2. Schematic representation for amphiphilic β-cyclodextrin.

estimated. The density of the fraction could then be determined by using a standard table.

## 3. Results and discussion

The acylation of the secondary hydroxyl groups of the molecule of cyclodextrin is almost complete (Zhang et al., 1991). The molecule is sketched in Fig. 2.

Immediately after mixing of an organic solution of amphiphilic cyclodextrin with water, a colloidal suspension of nanospheres is formed; this suspension is still stable upon removal of the solvent (Skiba et al., 1993).

The size of the nanospheres was measured by QELS, and depends upon several factors such as solvent/water ratio, temperature and evaporation speed (Skiba et al., 1996).

In the conditions of preparation of the present work, the estimated average diameters of nanospheres of $\beta$CD-$C_6$, $\beta$CD-$C_{12}$ and $\beta$CD-$C_{14}$ were 200, 257 and $342 \pm 40$ nm, respectively. The profile corresponded to a normal distribution and the sample could be considered monodisperse as shown by the cumulative results and polydispersity index (Table 1).

Table 1
Influence of modified β-cyclodextrin on the mean diameter size (nm) of the nanospheres ($n = 3$)

| Modified $\beta CD_m$ | Size ± S.D. (nm) | Dust | PI |
|---|---|---|---|
| $\beta$CD-$C_6$ | $204 \pm 35$ | 0 | 0.087 |
| $\beta$CD-$C_{12}$ | $257 \pm 50$ | 3 | 0.068 |
| $\beta$CD-$C_{14}$ | $342 \pm 30$ | 0 | 0 |

PI, polydispersity index.

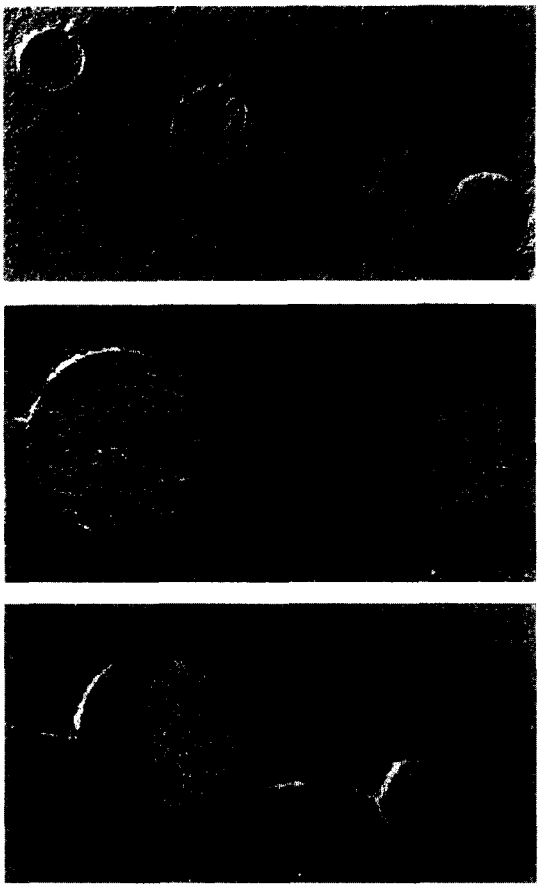

Fig. 3. Transmission electron micrographs of nanospheres after freeze-fracture. (A) $\beta$CD-$C_6$ (200 nm); (B) $\beta$CD-$C_{12}$ (250 nm); (C) $\beta$CD-$C_{14}$ (340 nm).

The density was determined from sedimentation on a sucrose gradient. The density of $\beta CD_m$ ($m = 6$, 12, 14) nanospheres were the following:

| | |
|---|---|
| $\beta$CD-$C_6$ | 1.1175 |
| $\beta$CD-$C_{12}$ | 1.0320 |
| $\beta$CD-$C_{14}$ | 1.0320 |

In the present work, the electrophoretic mobility was determined in the presence of $10^{-3}$ M KCl. The zeta potential of the nanospheres of $\beta$CD-$C_6$, $\beta$CD-$C_{12}$ and $\beta$CD-$C_{14}$ were $-26$, $-46$ and $-50$ mV, respectively. This charge probably arose from chemical interactions at the surface of the particles with the external medium and may depend on the ionic strength of the aqueous phase (Skiba et al., 1996).

The freeze-fracture micrographs show circular filled objects that correspond to the cross-fracture of spheres (Fig. 3). The interior of the nanospheres appears often as non-amorphous and shows some regular structure which will be analyzed elsewhere.

## 4. Conclusion

In the present work, the nanospheres made from amphiphilic cyclodextrin were characterized. Previous studies have shown the formation of colloidal suspensions of amphiphilic cyclodextrins, but the visualization of the particles by near-field microscopy techniques (Sommer et al., 1993; Skiba et al., 1995) provided very limited information on their morphology and no information on the type of internal nature. In the present work, the use of the freeze-fracture demonstrates that the particles are nanospheres: compact spherical filled objects. We hope to be able to describe the internal structure of these nanospheres in the near future.

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