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Characterization of a new methylated β-cyclodextrin with a low degree of substitution by matrix-assisted laser desorption/ionization mass spectrometry and liquid chromatography using evaporative light scattering detection

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Abstract

A new methylated β-cyclodextrin with a low degree of substitution was characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and high-performance liquid chromatography (HPLC) with evaporative light scattering detection. Using α-cyano-4-hydroxycinnamic acid as the matrix and the thin layer method as the deposition procedure, MALDI-TOF-MS revealed that the mixture was composed of CDs bearing from 2 to 8 methyl groups with an average degree of substitution (DS) of 0.7 (i.e. 0.7 methyl groups per glucopyranose unit). Using a Purospher Star RP-18 endcapped column with acetonitrile–water mobile phase in gradient elution mode, HPLC was employed at analytical scale to obtain a chromatographic fingerprint of the crude mixture and at semi-preparative scale to fractionate it. MALDI-TOF-MS of these fractions revealed that the overall retention of the different derivatives, which depicts their polarity, was mainly driven by the DS and increased with the number of methyl groups on the CD moiety.

Keywords: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; High-performance liquid chromatography; Evaporative light scattering detection; Methylated β-cyclodextrins; Degree of substitution

1. Introduction

Cyclodextrins (CDs) are torus-shaped cyclic oligosaccharides composed of six, seven and eight α-1,4-linked D-glucopyranose units for the α-, β- and γ-CD, respectively [1]. These hydrophilic molecules present hydrophobic cavities allowing the formation of complexes with many guest molecules of appropriate size. Moreover, they have the ability to discriminate between structurally related compounds such as isomers or enantiomers. Thanks to these properties, CDs and their derivatives are used in a wide variety of industrial and pharmaceutical applications [2] as well as in analytical chemistry where they are employed as separating agents in several techniques [3,4].

Some of the most widely used derivatives are the methylated β-CDs (Me-β-CDs). Indeed their properties (low toxicity, high aqueous solubility and binding capacity, reasonable price) allow their use in the pharmaceutical industry as drug carriers (solubilization, stabilization) [5] and in analytical chemistry as chiral selectors in techniques such as capillary electrophoresis [6].

However, selective methylation of the native β-CD is not easy because the hydroxyl groups present at positions 2, 3 and 6 of each glucopyranose unit are in competition during the reaction [7]. Most of the commercially available Me-β-CDs are therefore mixtures of CDs bearing different numbers

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of methyl groups at different positions. In other words, these mixtures contain several degrees of substitution (DS, defined as the number of methyl groups per glucopyranose unit) that each have a large number of positional and regional isomers. The heterogeneity of such mixtures makes their characterization essential.

Several methods have already been reported to accurately analyze mixtures of intact Me-β-CDs. Mass spectrometry has been extensively used for the determination of their substitution pattern and average DS. Several ionization techniques have been employed such as fast atom bombardment [8,9], plasma desorption [10], matrix-assisted laser desorption/ionization [11–13] and electrospray [13–16]. In addition, separative methods such as liquid chromatography [8,9,17–19] and subcritical fluid chromatography [16,19] have often been used to obtain a fingerprint of mixtures and identify their major components. In the same goal, capillary electrophoresis has been employed with indirect detection [20]. Another way to gain deeper insight into the composition of Me-β-CDs is to analyze the glucose derivatives obtained after chemical hydrolysis [8,9,21]. This method allows the determination of the average DS and provides information on the position of methyl substituents on the glucopyranose units.

All these methods were mainly designed for the analysis of dimethyl-β-CDs (DM-β-CDs, DS = 2) that possess an average of 14 methyl groups. These derivatives are of special interest since, compared with that of native β-CD, the aqueous solubility of Me-β-CDs increases as the number of methyl groups reaches around 13–14 and then decreases with additional substituents [5].

However, to our knowledge the analysis of Me-β-CDs with a low degree of substitution (DS < 1) is poorly described. Yet their properties are different from those of DM-β-CDs especially polarity (or hydrophobicity) and solubility, as mentioned earlier, and they may therefore require specific analytical conditions.

The present study reports the characterization of a new Me-β-CD having an average DS in the range 0.4–0.7. This product was designed by the manufacturer as a pharmaceutical grade CD suitable for encapsulation of active products [22] and present a good water solubility (which increases with temperature unlike DM-β-CDs), an enhanced ability to form inclusion compounds and initial results indicate a good potential for biological tolerance.

The crude commercial mixture was analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to determine its substitution pattern and its average DS and by high-performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) to obtain a chromatographic fingerprint. Then, in order to better understand the chromatographic behaviour and the substitution of the different derivatives, fractions obtained by semi-preparative chromatography were analyzed by MALDI-TOF-MS.

2. Experimental

2.1. Reagents

HPLC-grade acetonitrile (MeCN) and methanol (MeOH) were purchased from SDS (Peypin, France) and deuterium oxide (D_2O) from Aldrich (Milwaukee, WI, USA). 18 MΩ deionized water was produced by an Elgastat UHQ II system (Elga, Antony, France).

α-Cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) were purchased from Aldrich (Milwaukee, WI, USA), 1-hydroxy-isochinolin (HIC) and 3-hydroxypicolinic acid (HPA) from Fluka (Buchs, Switzerland) and sodium chloride (NaCl) from Prolabo (Paris, France).

β-CD and Me-β-CD (Kleptose® Crysmeb Exp, Lab 3487) were obtained from Roquette (Lestrem, France). The Kleptose® Crysmeb is described as a new generation of Me-β-CD produced through selective methylation of β-CD using a technology patented by Roquette and characterized by an average DS in the range 0.4–0.7 [22].

2.2. Nuclear magnetic resonance

The ^{1}H NMR experiments were carried out at 25 °C on a DPX 250 MHz Advance apparatus (Bruker Biospin, Rheinstetten, Germany). Calibration was made with the D_2O signal.

Samples of β-CD and Me-β-CD were prepared in D_2O at a concentration of 5 mg in 0.75 mL. The solutions were evaporated to dryness under nitrogen stream and reconstituted in 0.75 mL of D_2O. This operation was repeated two times in order to ensure a complete exchange of the hydroxyl protons.

The ^{1}H NMR spectrum of the Me-β-CD was identical to that obtained by the supplier [22] and the DS calculated from the integration was found to be 0.68 which is in the expected range of 0.4–0.7.

2.3. Mass spectrometry

The mass spectrometry experiments were performed on a linear MALDI-TOF model Omniflex system (Bruker Daltonics, Bremen, Germany) in positive ionization mode using a nitrogen laser (λ = 337 nm, 2 ns pulse width). The acceleration voltage was 17.70 kV and the detector signal was digitized at a sampling rate of 500 MHz. Spectra were recorded between 0 and 5000 Da. Calibration was made with the native β-CD. The lowest possible laser power was applied to induce the desorption and ion formation.

Matrices solutions were prepared in MeOH at a concentration of 50 mM. Me-β-CD solutions were prepared in water containing 0.5 mM NaCl. The crude mixture was dissolved to a concentration of 0.5 mM and the fractions collected by semi-preparative chromatography were evaporated to dryness under nitrogen stream and reconstituted in the appropriate volume to obtain a concentration factor of 100 compared to the initial volume.