IS IT POSSIBLE TO PREPARE TRIACETYL-β-CYCLODEXTRIN/DRUG INCLUSION COMPLEXES IN $SCCO_2$?

T. Casimiro,* E. R. Vão, M. Temtem, A. Aguiar-Ricardo, G. Ivanova, E. J. Cabrita

REQUIMTE/CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

*teresa.casimiro@dq.fct.unl.pt, Tel/Fax: +351 21 2948385

In this work, information on the molecular structure and dynamics of triacetyl-β-cyclodextrin (TA-β-CD) in supercritical carbon dioxide was obtained by performing high-pressure NMR experiments. Acetylated cyclodextrins are promising candidates to the development of prolonged drug release devices in $scCO_2$. They present high miscibility and melting point reduction in dense CO_2 which is attributed to the high accessibility of the acetyl groups to acid:base Lewis interactions. The influence of $scCO_2$ on a number of NMR spectral parameters such as chemical shifts, spin-spin coupling constants, Nuclear Overhauser Effect (NOE), spin-lattice relaxation (T1) and diffusion coefficient was studied. Structural changes of the TA-β-CD were detected which may possibly affect the inclusion capability of this cyclodextrin derivative in $scCO_2$.

INTRODUCTION

β-Cyclodextrins are cyclic oligosaccharides composed by seven glucose units, with bucket-shaped molecular structure. The central cavity provides a microenvironment where a drug molecule can enter and form an inclusion complex. Natural CDs (α-,β-, γ-cyclodextrins) and some derivatives (dimethyl-, hydroxypropyl-, etc) are water soluble. They are able to improve the bioavailability of poorly soluble drugs when inclusion complexes are formed. Supercritical CO_2 is a very attractive medium for CDs inclusion complexes preparation as alternative to aqueous and organic solvents. Several examples of the preparation of inclusion and interaction complexes of hydrophilic cyclodextrins with drugs in $scCO_2$ can be found in literature. As they are insoluble in this medium, the complexes are often prepared by mixing the CD and the guest molecule in batch or in continuous by placing both compounds in separated reactors, and passing a stream of $scCO_2$ saturated with the drug trough the CD matrix [1,2].

In recent years there has been an increase in research of CD derivatives, especially in the preparation of delayed release devices [3]. In particular TA-β-CD is a strong candidate to use in CD formulations since its hydrophobicity decreases the solubility of the guest molecule providing a longer and controlled release of the drug. Recently it has been reported that acetylated sugars derivatives exhibit high solubility in liquid and supercritical carbon dioxide [4,5,6]. The high miscibility and melting point decrease of these acetylated carbohydrates in dense CO_2 can be explained by the high accessibility of the acetyl groups to favourable acid:base Lewis interactions, thus making $scCO_2$ an interesting medium for processing a range of per-acetylated carbohydrates, from monosaccharides to cyclodextrins [7,8]. A few papers report the use of this CD derivative to form inclusion complexes. However significant conformational distortions induced by the acetylation of β-CD have also been detected in solid state and in solution leading to self-closure of the molecular cavity from both sides of its conical structure by the acetyl chains [9,10].

In this work a high-pressure apparatus especially designed for high-pressure NMR studies [11] was used to obtain information on the molecular structure and dynamics of TA-β-CD dissolved

in supercritical carbon dioxide and thus allow to discuss the feasibility of preparing host-guest inclusion complexes of this CD derivative in $scCO_2$.

MATERIALS AND METHODS

Materials

TA-β-CD and deuterated-TMS both from Aldrich were used as received. Carbon dioxide was supplied by Air Liquide with purity better than 99.998%.

Experimental procedure

The experiments were carried out in a high-pressure apparatus as already described elsewhere [11]. A known amount of cyclodextrin (4 mg) was placed directly inside the NMR tube. The system was then purged under vacuum. The high-pressure NMR tube was then loaded with a solution of CO_2+TMS (3.3 mM), previously prepared in a 10 mL high-pressure cell using a HIP manual compressor. The pressure of the system was measured using a Wika transducer. The NMR tube was kept for one day in a thermostatted ultrasound bath at 40ºC, prior to the HP-NMR experiments. The tube was then transferred to the NMR apparatus using a protective polycarbonate structure. High-pressure high resolution ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Bruker AvanceII 400 spectrometer equipped with pulse gradient and temperature control units equipment with a temperature control unit, operating at a frequency of 400 MHz.

RESULTS AND DISCUSSION

The glucosidic units of the β-cyclodextrins form a truncated cone stabilized by a network of intramolecular hydrogen bonds. Modifications of the hydroxyl groups of the CDs, as in the case of triacetylated β-cyclodextrin, can lead to significant conformational distortions and affect their physical properties and inclusion complex formation. Structural alterations have been observed both in solid state [12] and in solution [13]. Solid state structural analysis of per-acylated and per-methylated β-CDs, based on X-ray diffraction, has shown not only changes of the overall shape of the macrocycles but also conformational changes of individual pyranose rings. The orientation of the acyl chains and their engagement in intramolecular hydrogen bonding across the cavity result in a closure of the molecular cavities from the both sides of the conical structures as it has been found in the solid state studies. These structural distortions can affect the ability of per-acetylated β-CD to form the classical inclusion complexes. Inclusion of the guest molecules in the matrix between the acyl chains and not in the central cavity in solid state has been suggested. Recently published results have indicated that per-O-acetylation produces significant conformational deformation of β-CD in chloroform and methanol solutions [9]. The stereochemical distortions in solution have been attributed to the repulsive steric interaction between the acyl substituents and the absence of the strong hydrogen bond network between the secondary hydroxyl groups observed for the unsubstituted β-CDs. The truncated cone shape perturbation and expected alteration in the polarity difference between the outside and inside of the cavity has been suggested to strongly affect not only of the solubility of the cyclodextrin derivatives but also their ability to form complexes.

Despite the strong evidences for structural deformation and self-closure of the molecular cavity induced by the per-acetylation of CD, the preparation of inclusion complexes with randomly and per-acetylated β-CDs have been reported. Fernandes et al. have reported the inclusion of nicardipin hydrochlorid in per-acetylated β-CD in a water:ethanol mixture [14]. Recently, the complexation ability of TA-β-CD with respect to some arylphosphines has been studied in $scCO_2$ and the formation of inclusion complexes has been suggested [15,16,17].

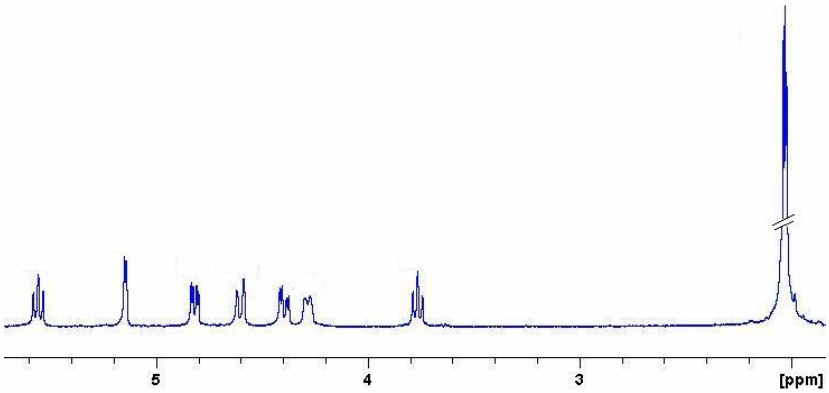

Figure 1: High-pressure 400 MHz ^{1}H NMR spectrum of triacetyl -β-cyclodextrin at 200bar and 40ºC.

In this work high resolution NMR spectroscopy was employed for structural elucidation of TA-β-CD in $scCO_2$. Typical ^{1}H NMR spectrum in $scCO_2$ is presented in Figure 1. The stereochemical and conformational structute of TA-β-CD in $scCO_2$ solution was estimated by means of the nuclear Overhauser effect (NOE). Two-dimensional NOESY (Nuclear Overhauser Effect SpectroscopY) and ROESY (Rotating-frame Overhauser Effect SpectroscopY) experiments were performed and the results analyzed. The results show NOE interactions between protons belonging to the same glucose ring but also protons belonging to adjacent rings. In $scCO_2$ solution, strong intramolecular, inter-glucose H1/H4′ dipolar interaction, characteristic for syn orientation of the $\alpha(1\rightarrow 4)$-linked glucose residues, were detected. From the integral intensity of the traces in the NOESY spectrum, it is possible to conclude that H1 produce stronger inter-glucose NOE (between H1 and H4') then intra-glucose NOE (between H1/H2), which can serve as an indication for conformation changes induced by rotation around the glycoside linkage. Also dipolar interactions between H3 and protons H2 and H4 were detected, which can be related to conformational alterations of some glucose residues.

CONCLUSIONS

These preliminary results show that TA-β-CD presents structural distortions in $scCO_2$ at 40ºC and 200 bar that could compromise the inclusion of a guest molecule into the cavity at those conditions. Further HP NMR studies are being conducted to clarify this hypothesis.

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