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# The impact of guest compounds on cyclodextrin aggregation behavior: A series of structurally related parabens

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ABSTRACT

Several studies have demonstrated the presence of aggregates in aqueous cyclodextrin containing solutions. The presence of guest compounds has been shown to influence this cyclodextrin aggregation process. In an attempt to gain insight into the effect of the physicochemical properties of the guest compound on 2-hydroxypropyl-β-cyclodextrin aggregation formation, a series of structurally related parabens was selected as model compounds. Using nuclear magnetic resonance spectroscopy and phase solubility studies, these parabens, differing only in side chain length, were demonstrated to form inclusion complexes with 2-hydroxypropyl-β-cyclodextrin. Additional techniques were subsequently applied to evaluate the aggregation behavior of this cyclodextrin in presence of the selected parabens. Solutions containing a broad range of 2-hydroxypropyl-β-cyclodextrin concentrations were saturated with the guest compounds and were used as test media. Results obtained from dialysis experiments, dynamic light scattering and mass spectrometry revealed a positive effect of the side chain length of the parabens on aggregate formation: in presence of heptylparaben, more and larger aggregates were observed than in presence of parabens with shorter side chains such as methyl- and butylparaben. No clear connection could be demonstrated between the cyclodextrin concentration and the extent of aggregate formation in presence of the guest compound.



## 1. Introduction

The use of cyclodextrins (CDs) has become indispensable in pharmaceutical industry today: their unique structural properties, together with their favorable safety profile, explain the popularity of CDs as solubilizing excipients (Loftsson and Brewster, 2010). The hydrophobic internal cavity of CDs enables inclusion of lipophilic compounds, while the inclusion complex retains a hydrophilic character due to its outer surface. The considerable number of marketed products containing CDs underscores the value of these cyclic oligosaccharides for drug development (Loftsson and Brewster, 2010). Although the solubility enhancing effect of CDs is traditionally regarded as the result of the formation of inclusion complexes, an increasing amount of evidence is being generated on the possible contribution of non-inclusion complexes (De et al., 2008; Loftsson et al., 2004, 2002; Malanga et al., 2016). Indeed, CDs and CD complexes exhibit a tendency to aggregate and have been shown to form a variety of structures including rods, discs, spheroids, spheres, bilayers, vesicles, or reversible micelles (Ryzhakov et al., 2016). While originally CD aggregation was postulated based on random observations and deviations from theoretical equations, analytical techniques are currently being optimized to specifically detect and characterize the aggregates present in CD containing solutions. He et al. reviewed the abundance of microscopic data demonstrating aggregation of native and modified CDs and their inclusion complexes (He et al., 2008). Other techniques such as dynamic light scattering (DLS), nuclear magnetic resonance spectroscopy (NMR) and dialysis membrane permeation methods were shown to be very useful in characterizing CD aggregation behavior (Bonini et al., 2006; Duan et al., 2005; Jansook et al., 2010). Unfortunately, the poor stability of the CD aggregates makes them hard to study. Often forces inherent to a specific analysis technique may be disruptive to the aggregates. Nevertheless, factors that influence aggregation behavior could be defined. For instance, increasing the

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temperature of the medium, applying strong agitation forces or adding apolar solvents to the medium results in disassembly of the aggregates (Messner et al., 2011b). Ryzhakov et al. recently published a comprehensive review discussing the self-assembly of CDs in more detail (Ryzhakov et al., 2016).

Although aggregates have been observed in simple aqueous solutions containing solely CDs, the presence of guest molecules has been shown to affect this aggregation behavior (Ghosh et al., 2011; Lucio et al., 2017; Messner et al., 2011a). Given the fact that, for most clinical applications, CDs are used in combination with a guest compound, knowledge of the influence of drug inclusion on CD aggregation behavior could prove to be highly valuable to understand and predict this phenomenon. The value of gaining mechanistic insight into this aggregation process relies on the one hand on being able to control or prevent aggregation, for example in formulations intended for intravenous administration, for which the presence of (sub)visible particles may lead to batch rejection. On the other hand, the formation of these nanoparticulate aggregates can also be exploited as a novel formulation strategy. In oral drug delivery for instance, the use of nanosuspensions has led to improved oral bioavailability of a number of poorly soluble compounds, mostly caused by improved dissolution characteristics (Kesisoglou and Mitra, 2012). Moreover, Calleja et al. demonstrated the mucus penetrating qualities of CD containing nanoparticles which resulted in a superior oral bioavailability of paclitaxel as compared to the commercially available Taxol® (Calleja et al., 2014).

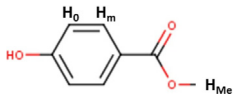
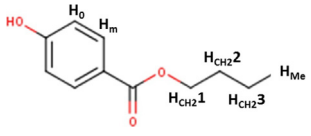
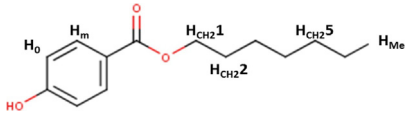
This study aims to contribute to unraveling the influence of the physicochemical properties of guest compounds on the self-assembly of CD complexes. With this objective in mind, a series of parabens, only differing in side chain length was selected to specifically study the effect of an increasing side chain length on the aggregation behavior of the CD complexes. The chemical structures of the selected parabens are depicted in Table 1. The interaction between HP-β-CD and methyl-, butyl- or heptylparaben was characterized using various analytical techniques including phase solubility studies, DLS, mass spectrometry (MS), NMR and dialysis membrane permeation. This multidisciplinary approach allows uncovering factors driving CD aggregation, which is one of the main goals of the overarching IWT project CYAGGTECH.

## 2. Materials and methods

### 2.1. Chemicals

Methylparaben, butylparaben and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) were kindly provided by Johnson & Johnson (Beerse, Belgium). The degree of substitution of HP-β-CD was 0.63. Heptylparaben was purchased from Alfa Aesar (Karlsruhe, Germany). Sodium acetate trihydrate and methanol were supplied by VWR International (Leuven, Belgium). Deuterium oxide was purchased from Cambridge Isotope laboratories, Inc. (MA, USA). Purified water for analytical purposes was obtained using a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

**Table 1**
Chemical structures of the selected parabens.

| | |
|---|---|
| methylparaben |  |
| butylparaben |  |
| heptylparaben |  |

### 2.2. Bi-dimensional ROESY $^1H$ NMR studies

Samples for $^1H$ NMR measurements were prepared by adding an excess amount of methyl-, butyl- or heptylparaben to vials containing 20% of HP-β-CD. Vials were rotated/shaken at room temperature for 48 h. Subsequently, all solutions were filtered through 0.45 μm PTFE filters (Macherey-Nagel). $^1H$ NMR spectra were recorded on a Bruker 500 MHz spectrometer, in deuterated water ($D_2O$). The probe temperature was set at 298 K. The NMR data were processed with the Bruker TOPSPIN 2.0 software. The chemical shifts (δ) were reported as ppm and the residual solvent signal (4.77 ppm) was used as the internal reference.

### 2.3. Solubility experiments

The apparent solubility of methylparaben, butylparaben and heptylparaben was determined in water containing various concentrations of HP-β-CD (0%, 5%, 15% and 25% w/v). All solubility experiments were performed in triplicate. An excess of paraben powder was added to microcentrifuge tubes containing 0.5 ml of the CD solutions, which were subsequently placed in a prewarmed shaking incubator [25 °C at 175 rpm (KS 4000 i control incubator from Ika (Staufen, Germany))] for 48 h. The solid phase was separated from the dissolved part using centrifugation (15 min, 20.817*g* at 25 °C). The top layer was carefully removed by aspiration. 10 μl of the supernatant of each paraben microcentrifuge tube was diluted 1/1000 or 1/10,000 in one HPLC vial containing methanol:water (50:50 v/v) and the three parabens were quantified simultaneously using an HPLC system with UV detection. The remaining volume of the supernatant was used as donor medium for the permeation studies.

### 2.4. Permeation method

By comparing the permeation of compounds across membranes with different molecular weight cutoff (MWCO) values, it is possible to get an idea about the relative abundance of aggregates in the medium. The permeation of methylparaben, butylparaben and heptylparaben in presence of 0%, 5%, 15% or 25% w/v HP-β-CD was evaluated using the HTD 96b from HTDialysis, LLC (Gales Ferry, CT, US). The donor and acceptor compartments were separated by cellulose membrane strips with a MWCO of 3.5 kDa or 12–14 kDa (the molecular weight of HP-β-CD is 1.4 kDa). The membranes were hydrated according to the manufacturer's instructions. The acceptor compartment was filled with 150 μl of water containing an equal concentration of HP-β-CD as compared to the donor medium. The donor compartment was loaded with 150 μl of the supernatant, as described in the previous section 'solubility experiments'. Samples of the acceptor compartment (10 μl) were taken after 30 min for each of the parabens tested, diluted in one HPLC vial containing methanol:water (50:50 v/v) and the three parabens were quantified simultaneously using an HPLC system with UV detection.

### 2.5. HPLC analysis of parabens

The concentrations of parabens were determined by reversed phase HPLC with UV detection. Samples obtained from the