Dynamics of hydration and dehydration processes of β-cyclodextrin monitored in real time by Raman spectroscopy

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The integrated intensity of the O–H Raman stretching band of crystalline β-cyclodextrin hydrate exhibits a linear response to variations of ambient humidity for humidities > 15% and varies with temperature in a similar way as the sample thermogram; it is used to monitor, in real time, the hydration and dehydration processes which follow approximately first-order kinetics.

Crystalline hydrates of β-cyclodextrin (cyclomaltoheptaose, β-CD) are good models to study hydration processes in biomolecular systems.[1–6] The water content of crystalline β-CD hydrate is in fast equilibrium with atmospheric humidities. In the range of relative humidities 15–100%, the number of water molecules per β-CD varies continuously from about 9.4 to 12.3. The overall crystal structure is conserved, though with 2.3% reduction of the cell volume with respect to the 100% humidity level.[1] In fully hydrated β-CD, a cluster of 7 water molecules occupies the cavity of the macrocyclic β-CD molecule, and 5.4 water molecules occupy interstitial spaces.[1] At low humidities, a distinct phase II with unknown structure is formed.[3]

Although the crystal lattice at humidities > 15% does not have permanent channels, fast diffusion of water molecules occurs due to transient fluctuations in the lattice.[1] Exchange experiments carried out with water marked either with D or with ^{18}O showed that the H/D exchange is complete, hence extending also to sterically unaccessible O–H groups, and that the long-range transport of hydrogen takes place by diffusion of intact water molecules (as indicated by $^{18}O/^{16}O$ exchange).[2] However, the dynamics of water sorption in crystalline β-CD are not well understood. In this work, a Raman spectroscopic method is presented that allows the monitoring of hydration and dehydration of crystalline β-CD in real time.

Microcrystalline β-CD hydrate[†] was used for three series of experiments. The first aimed at correlating the water content in equilibrium with atmospheric humidity. β-CD hydrate was exposed to defined humidities of the atmosphere over suitable saturated salt solutions.[1] After equilibration, five samples were sealed in Kimax capillary tubes (inner diameter 0.8 mm; sample quantity *ca.* 10 mg), and Raman spectra were recorded.[‡] The Raman OH stretching band of crystalline hydrated β-CD is moderately intense and very broad, centred around 3350 cm^{-1}, and corresponds to the 21 β-CD hydroxy groups and the water molecules of hydration. The peak intensity of the ν(CH) bands occurring at a Raman shift of 2908 cm^{-1} is invariant with humidity and was taken as internal reference for measurement of the relative ν(OH) Raman band intensity.

Fig. 1 shows the Raman spectra of five samples of crystalline β-CD hydrate in equilibrium with ambient humidities of 0, 58, 79, 88 and 98%. The inset shows the relative integrated intensity under the ν(OH) band, I_{OH}, *vs.* ambient humidity. Except for the lowest humidity which should correspond to β-CD in a different phase (phase II, mostly dehydrated form),[3] all points fall on a straight line ($I_{OH} = 24h + 52$, $R = 0.99$). The constant term in this relationship corresponds to the 21 hydroxy groups in the β-CD molecules and to water molecules which are non-removable in the high humidity phase. These data show that in the humidity range 15–100%, the water content of crystalline β-CD hydrate exhibits a linear response to variations of ambient humidity. This is consistent with crystallographic data.[1]

In the second experiment, I_{OH} was measured at defined temperatures, increasing from room temp. to 200 °C. Fully hydrated microcrystalline β-CD was inserted in a Kimax capillary tube which was put into direct thermal contact with the metal base of a furnace.[§] After thermal equilibration at a particular temperature,[¶] the Raman spectrum was recorded in the 2800–3800 cm^{-1} region.

Fig. 2 shows I_{OH} as a function of temperature. After staying approximately constant between room temp. and *ca.* 40 °C, I_{OH} decreases with a varying slope between *ca.* 50 and 140 °C reflecting different states of partial dehydration. At around 140 °C, all water molecules are removed and I_{OH} remains constant as the temperature is further increased. Qualitatively, this behaviour is as observed by thermogravimetry.[8]

In the third set of experiments (hydration–dehydration sequence), I_{OH} was recorded as a function of time during hydration and dehydration of β-CD. Assuming that all OH oscillators yield approximately the same intrinsic Raman

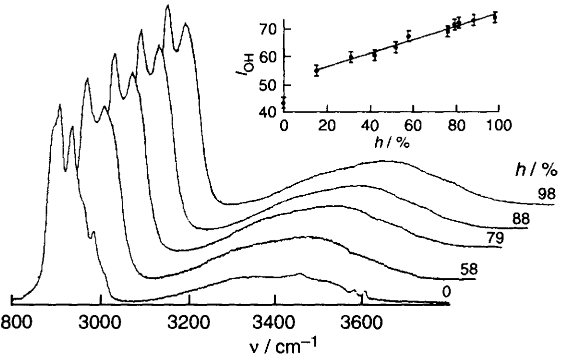

Fig. 1 Raman spectra of crystalline β-CD hydrate for different ambient humidities; the inset shows I_{OH} *vs.* ambient humidity (*h*)

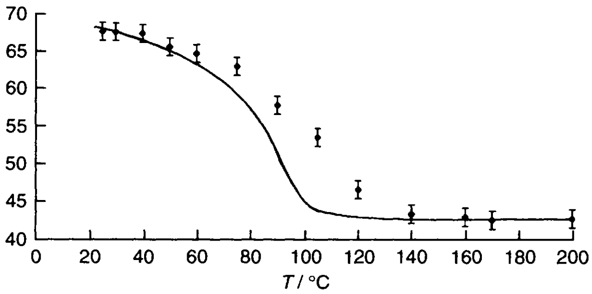

Fig. 2 The integrated Raman intensity of the OH stretching band of crystalline β-CD hydrate, I_{OH}, as a function of temperature. The continuous line is the sample thermogram.

intensity, the crystal water content per mole of β-CD, *i.e.*, $n(H_2O)/\beta$-CD, is roughly linear with I_{OH}. This is a somewhat simplistic assumption, since I_{OH} is influenced by hydrogen-bonding effects and accounts for both the antisymmetric and the symmetric intensities of water molecules. I_{OH} contains a constant term $I_{OH(min)}$ which includes the contributions from the 21 hydroxy groups and of possibly present non-removable water molecules. The relevant kinetic variable during dehydration is the *occupied fraction* of *removable* water sites, $\theta = [n - n_{(min)}]/[n_{(max)} - n_{(min)}]$. This quantity, which ranges from 0 to 1, is experimentally measured by $I_{OH} = [I_{OH} - I_{OH(min)}]/[I_{OH(max)} - I_{OH(min)}]$. For hydration, the unoccupied fraction of removable water sites $(1 - \theta)$ should be considered instead. Observation of θ and $1 - \theta$ in their variations *vs.* time allows a comparison of hydration and dehydration on an equal footing.

Fig. 3 shows I_{OH} for a hydration experiment.[||] After *ca.* 40 min, I_{OH} reaches saturation, *i.e.*, hydration is complete. The inset shows $\ln(1 - \theta)$ in the relevant time period, and the linear correlation ($R = 0.99$, $t_{1/2} \approx 9$ min for this sample) suggests first-order kinetics for the hydration process. A complementary dehydration experiment was performed starting with the previously fully hydrated sample, Fig. 4. The decay of lnθ is roughly linear, but equilibration is slower than in the hydration experiment ($R = 0.96$, $t_{1/2} \approx 35$ min). Since both experiments were performed on the same sample in the same set-up, the different $t_{1/2}$ values might be taken as an indication of different time behaviours for the *occupied-to-unoccupied* and the *unoccupied-to-occupied* changes, with the latter being appreciably faster. As the microscopic mechanisms of hydration and dehydration are not yet understood, this behaviour deserves further investigation.

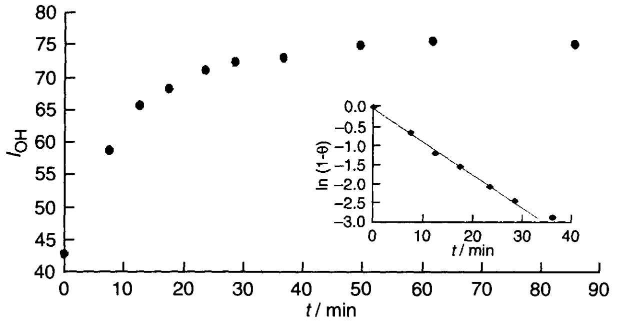

Fig. 3 I_{OH} as a function of time, for a hydration sequence of experiments; the inset shows $\ln(1 - \theta)$ *vs.* time [$(1 - \theta)$ is the unoccupied fraction of removable water sites]

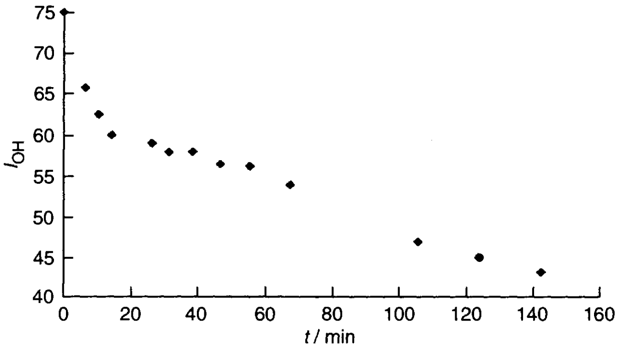

Fig. 4 I_{OH} as a function of time, for a dehydration sequence of experiments

The results presented above are not relevant for β-CD alone. It has been shown that hydration phenomena in this relatively simple bio-organic model compound are closely similar to those in much larger hydrated biological systems.[7] Therefore, we assume that the observed hydration kinetics apply in a similar way to many related systems.

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Footnotes

[†] β-CD, kindly supplied by Wacker Chemie, München, Germany, was recrystallized by cooling concentrated aqueous solutions in a Dewar flask from *ca.* 80 °C to room temp. Crystals were taken from the solvent and exposed to an atmosphere of moderate temperature and humidity (*ca.* 22 °C and 70% relative humidity). After equilibration with the atmosphere, the crystals were ground to obtain a microcrystalline powder.

[‡] T64000 Jobin Yvon spectrometer, subtractive configuration, relevant slit widths set to 300 μm, intermediate slit between premonochromator and spectrograph wide open (14 mm), CCD detecting device, integration time of 15 s. Spectral data for the 2800–3800 cm^{-1} overall region were collected in 10 subregions. An Ar ion laser (Innova 300-05 model with power track, from Coherent) provided *ca.* 100 mW at the sample position. Integrated intensities were determined using the machine software.

[§] Jobin Yvon product, designed for Raman applications in a temperature range from ambient up to 400 °C, with a temperature controller manufactured by Eurotherm.

[¶] These spectra were acquired *ca.* 5 min after setting the oven temperature at a particular value. This time delay was sufficient to restore thermal equilibrium between the sample and the heat source, as confirmed by determining the melting point (180–182 °C) of methyl-β-CD obtained from Aldrich, Madrid.

[||] Dehydrated β-CD powder was introduced in a cell with its base filled with pure water. When saturation was attained, water in the cell was replaced by dry silica gel and a dehydration set of experiments was then initiated.

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