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Note

Inclusion of carvone enantiomers in cyclomaltoheptaose (β-cyclodextrin): thermal behaviour and H → D and D → H exchange

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Abstract

The inclusion compounds of carvone enantiomers in cylcomaltoheptaose (β-cyclodextrin, βCD) are studied at defined temperatures above room temperature and in relation to H → D and D → H exchanges. Loss of water molecules and release of carvone molecules from the βCD cavity are caused by increase of temperature above room temperature and are measured by the integrated intensities of the O–H and C–H Raman stretching bands, respectively. In turn, H → D and D → H exchanges are monitored by the integrated intensities of the O–H and O–D Raman stretching bands, respectively. All of these processes were followed in real time with a Raman spectrometer equipped with CCD detection. The results indicate that distinct carvone enantiomers lead to the formation of different βCD inclusion hydrates that have different water content and hydration structures. In particular, the results suggest that *S*Carv–βCD has a greater water content, dehydrates strongly for temperatures above room temperature, and exchanges protons faster than the *R*Carv–βCD complex. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: β-Cyclodextrin; Carvone enantiomers; Inclusion compounds; Thermal dehydration; Thermal guest release; H → D/D → H exchange

1. Introduction

β-Cyclodextrin (cyclomaltoheptaose, βCD) has attracted over the years considerable attention, mostly for its ability to include in its cavity, guest molecules of suitable size.[1] Recently, this feature has been considered in relation with the possibility of chiral discrimination, in aqueous solution, against carvone (5-isopropenyl-2-methylcyclohex-2-enone, Carv) enantiomers.[2] It was found that the apparent association constant at room temperature for the 1:1 inclusion of *S*-(+)-carvone in βCD is double of that for *R*-(−)-carvone, whereas, at 45 °C, both enantiomers have association constants two orders of magnitude smaller, with the *S*-(+)-carvone inclusion being then slightly weaker than that of the *R*-(−) enantiomer.[2]

On the other hand, crystalline hydrates of βCD equilibrate their water content rapidly with the atmosphere, despite the fact that their crystal lattices do not have permanent channels, an indication that fast diffusion of water molecules is due to transient fluctuations in the lattices.[3] In addition, exchange experiments carried out with water marked either with D or ^{18}O showed that the H → D exchange is complete, extending to sterically unaccessible OH groups.[4] Moreover, the long-range transport of hydrogen takes place by diffusion of intact water molecules.[4] The dynamics of hydration and dehydration processes in βCD,[5] as well as the H → D and D → H exchange dynamics,[6] could be followed in real time by measuring the integrated intensities of the O–H and O–D stretching Raman bands, respectively, using a Raman spectrometer equipped with CCD detection. For all of their peculiar features, crystalline βCD hydrates have been considered as good models for studying hydration processes in biomolecular systems.

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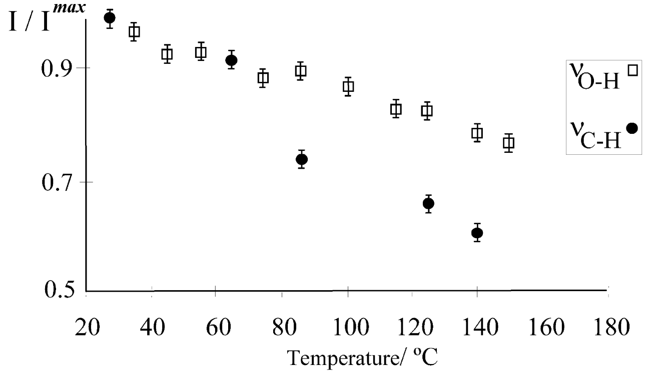
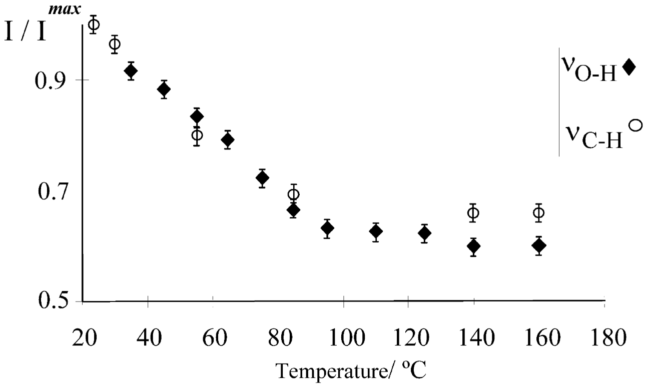
In this work, the inclusion compounds of carvone enantiomers in β-cyclodextrin are studied at defined temperatures above room temperature and in relation with H → D and D → H exchanges. Loss of water molecules and release of carvone molecules from the βCD cavity are caused by increase of temperature above room temperature, and are measured by the integrated intensities of the O–H and C–H Raman stretching bands, respectively. In turn, H → D and D → H exchanges are monitored by the integrated intensities of the O–H and O–D Raman stretching bands, respectively. As it was mentioned above, these Raman measurements allow for real-time monitoring of the corresponding processes, since CCD detection is used in the Raman spectroscopic measurements.

2. Results and discussion

Thermal behaviour.—The thermal behaviour of the inclusion compounds of carvone enantiomers in βCD was studied by monitoring the integrated intensities of the O–H and C–H Raman stretching bands for defined temperatures from room temperature up to ca. 160 °C (three independent experiments, $cv = 3.2\%$). The chosen ν_{CH} Raman band occurs at 3047 cm^{-1} and is assigned to the $C_{sp^2}H$ stretching vibrations in carvone.



a)*R*Carv–βCD



b)*S*Carv–βCD

Fig. 1. Integrated intensities of the Raman O–H and C–H stretching bands (I_{OH}/I_{OH}^{max} and I_{CH}/I_{CH}^{max}), for defined temperature values ($cv = 3.2\%$): (a) *R*Carv–βCD; (b) *S*Carv–βCD.

Fig. 1 shows the integrated intensity of the Raman O–H and C–H stretching bands (I_{OH}/I_{OH}^{max} and I_{CH}/I_{CH}^{max}) as a function of temperature for *R*Carv–βCD (Fig. 1a) and *S*Carv–βCD (Fig. 1b). The I_{OH}/I_{OH}^{max} and I_{CH}/I_{CH}^{max} ratios decrease as temperature increases, due to loss of water molecules and delivery of carvone molecules from the βCD cavity, respectively.

As can be seen for *R*Carv–βCD (Fig. 1a), both measured quantities (I_{OH}/I_{OH}^{max} and I_{CH}/I_{CH}^{max}) decrease in an approximately linear fashion, with a larger slope for I_{CH}/I_{CH}^{max}. In particular, at 140 °C, I_{CH}/I_{CH}^{max} has been reduced to ca. 60% of the initial value, whereas I_{OH}/I_{OH}^{max} has decreased only to ca. 75% of its initial value, thus suggesting that the water molecules are more tightly bound to the crystal lattice in *R*Carv–βCD than the carvone molecules are bound to the βCD cavity. This observation suggests a far greater importance for the hydrogen-bonding interactions between water and βCD than between *R*Carv and βCD, a conclusion previously corroborated by molecular mechanics and quantum mechanical calculations.[2]

For *S*Carv–βCD (Fig. 1b), I_{OH}/I_{OH}^{max} and I_{CH}/I_{CH}^{max} follow the same pattern of variation, albeit different from the previous case. In fact, these quantities present linear and pratically coincident reductions, from room temperature to ca. 95 °C, and remain approximately constant after this temperature with values around ca. 65%.

As a first and general conclusion, these results suggest that the inclusion compounds of the carvone enantiomers are hydrates with different water content, distinct hydration structures and carvone enantiomers bound differently to βCD. This behaviour has also been previously observed in other thermal transition analysis performed by differential scanning calorimetry (DSC).[7,8]

If it is assumed that the hydration water has been completely lost at 140 °C in both inclusion compounds, then the above-mentioned results point to ca. seven removable water molecules per βCD unit for *R*Carv–βCD, and ca. 11 water molecules in the case of *S*Carv–βCD. As concluded above, the seven water molecules of *R*Carv–βCD are more strongly bound to βCD than the 11 water molecules of *S*Carv–βCD.

In order to get additional insight into the water structures in *R*Carv–βCD and *S*Carv–βCD, H → D and D → H exchange experiments, with CCD Raman spectroscopic monitoring of the O–H and O–D integrated intensities, were performed. These results are presented and discussed in the next subsection.

H → D/D → H exchange experiments.—In these experiments, the relevant kinetic variable, θ, plotted

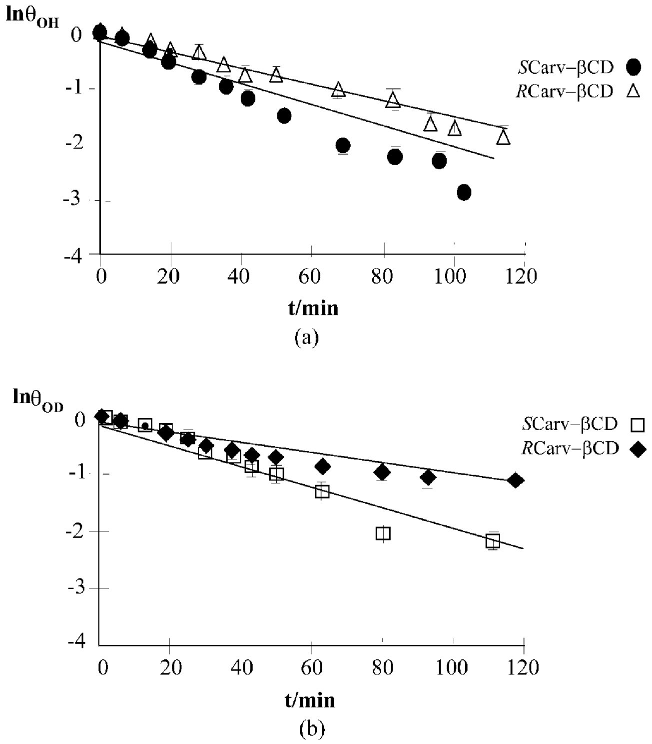


Fig. 2. Ln θ_{OH} and ln θ_{OD} versus time, for H → D (a) and D → H (b) exchange experiments of *R*Carv–βCD and SCarv–βCD, respectively ($cv = 3.2\%$; see text).

Table 1
Results for H → D and D → H exchange experiments in inclusion compounds *R*Carv–βCD and *S*Carv–βCD[a]

	k (10^{-2} min^{-1})	$t_{1/2}$ (min)
*R*Carv–βCD		
H → D	1.3	53
D → H	1.1	64
*S*Carv–βCD		
H → D	2.5	28
D → H	1.8	38

[a] Sample quantity is ca. 20 mg, k is the effective rate constant, and $t_{1/2}$ the time for 50% exchange.

against time, is the fraction of exchangeable protons or deuterons linked to O-atoms, as defined by $\theta_{OH} = (I_{OH} - I_{OH}^{min})/(I_{OH}^{max} - I_{OH}^{min})$ or by $\theta_{OD} = (I_{OD} - I_{OD}^{min})/(I_{OD}^{max} - I_{OD}^{min})$, respectively. These quantities, θ_{OH} and θ_{OD}, both vary from 0 to 1, and apply to the H → D and D → H exchange experiments, respectively. As D_2O diffuses into, and H_2O diffuses out of the sample, and H → D exchange processes at *R*Carv–βCD or *S*Carv–βCD hydroxyl groups take place, θ_{OH} continuously decreases, and in parallel, θ_{OD} increases. The sum $I_{OH}/I_{OH}^{max} + I_{OD}/I_{OD}^{max}$ is ca. 1.0 at all times (deviations from unity were $< 5\%$), i.e., the total number of O–H and O–D bonds is conserved during the exchange experiments. In the conserved summation of intensity ratios, separate normalization of each term is required because the O–H and O–D stretching vibrations have different Raman activities.

The results obtained from a complete cycle of experiments, H → D/D → H, are presented in Fig. 2 (ln θ versus time) for the inclusion compounds *R*Carv–βCD and *S*Carv–βCD (three independent experiments). As it can be seen, these exchange processes follow first-order kinetics, since ln θ is linear in time ($cv = 3.2\%$). This expected behaviour has also been previously observed for the βCD crystalline hydrates.[4,6]

The corresponding rate constants and half-life times are listed in Table 1. For *R*Carv–βCD, 50% of the H → D exchange occurs in 53 min, whereas 50% of the reverse exchange takes 64 min. A slower D → H exchange is a manifestation of the zero-point vibrational energy, since the (O)D → (O)H activation energy is greater than for the activation energy for the inverse process (the O – D oscillator has a zero-point vibrational energy lower than O – H). In turn, 50% of the H → D exchange for *S*Carv–CD is appreciably faster than for *R*Carv–βCD ($t_{1/2} = 28$ min), whereas the inverse 50% exchange takes 38 min.

Several conclusions may be drawn from the above-mentioned exchange experiments. First of all, proton-by-deuteron exchanges in the carvone–βCD inclusion hydrates have rate constants of the same order of magnitude as in βCD hydrates (although these exchange rate constants are not absolute, since they are sample-size dependent). Thus samples of the same amount were used to allow for an order of magnitude comparison.[4,6] In addition, these isotopic exchanges are extremely fast, if compared with corresponding values for non-cyclic oligosaccharides like raffinose and α,α-trehalose. Finally, the exchange experiments point to different hydration structures for *R*Carv–βCD and *S*Carv–βCD, thus confirming the temperature variation in the above studies.

According to the above-mentioned results, the inclusion compound *S*Carv–βCD has a higher water content, dehydrates strongly for temperatures above room temperature, and exchanges protons faster than *R*Carv–βCD. In addition, at the same temperature above room temperature, a greater amount of *S*Carv is released from the βCD cavity than for *R*Carv. Interestingly, the above-mentioned results seem to suggest that chiral discrimination against carvone enantiomers leads to different water content and structures in the inclusion compounds that were studied.

3. Experimental

Materials.—βCD was kindly offered by Wacker Chemie, Munich, Germany, and the carvone (5-isopropenyl-2-methylcyclohex-2-enone) enantiomers were purchased from Aldrich Chemical Co. βCD was recrystallized and obtained as the hydrate by cooling a concentrated aqueous solution from ca. 80 °C to room temperature in a Dewar flask.

Preparation of inclusion compounds.—βCD hydrate (5 g, 4.41 mmol) was dissolved in boiling distilled water (100 mL) to form a clear solution. The carvone enantiomers (0.52 g, 3.5 mmol) were well dispersed in distilled water (10 mL) before adding them dropwise it to the βCD solution. The mixture was then vigorously stirred overnight on a magnetic stirrer at room temperature. White precipitates were filtered and dried for 1–2 days under an ambient atmosphere. Filtration allowed separation of the excess βCD. Any non-included guest compound was easily lost by evaporation. The βCD–carvone inclusion compounds were characterized by ^{1}H NMR spectroscopy. The stoichiometry and the apparent inclusion constants were determined by 'Job plots' and linear regression methods, respectively.[2,9] The apparent association constant at room temperature for the 1:1 inclusion comnplex of S-(+)-carvone in β-cyclodextrin is 1330 mol dm^{-3} ($cv = 10\%$), double of that for R-(−)-carvone (680 mol dm^{-3}, $cv = 10\%$), whereas, at 45 °C, both enantiomers have association constants two orders of magnitude smaller, with the encapsulation of the S-(+) enantiomer being slightly weaker than that of the R-(−) enantiomer.[2]

Thermal behaviour experiments.—A sample of each of the inclusion compounds was inserted in a Kimax capillary tube which, in turn, was put into direct thermal contact with the metal base of a Jovin–Yvon furnace, designed for Raman applications, at a temperature range from ambient up to 400 °C, controlled with a Eurotherm controller. After thermal equilibration at a particular temperature, the Raman spectra were recorded in the 2800–3800 cm^{-1} region. These spectra were acquired ca. 5 min after setting the oven temperature at a particular value. This time delay was determined to be sufficient to restore thermal equilibrium between the sample and the heat source, as it was previously confirmed by determination of the melting point (180–182 °C) of methyl-βCD (cat. no. 33261-5), obtained from Aldrich Chemical Co.

H → D exchange experiments.—A powdered sample of inclusion compound was placed in a closed cell over a reservoir of D_2O,[5] and the O–H and O–D stretching Raman bands were recorded at short time intervals using CCD detection. After equilibration, the D_2O reservoir of the cell was quickly replaced by an H_2O reservoir, so that the reverse exchange process, D → H, could be monitored for the same sample.

All the Raman spectra were recorded on a T64000 Jobin–Yvon spectrometer, working in the subtractive configuration (i.e., double pre-monochromator stage in subtractive configuration, plus third stage spectrograph), with relevant slit widths set to 320 μm and the intermediate slit between the pre-monochromator and spectrograph wide open (14 mm). The detecting device was a CCD detector, and an integration time of 15 s was used. Spectral data for the 2800–3800 cm^{-1} overall region were collected in 10 subregions, hence corresponding to a total acquisition time of 150 s. 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 instrument's software.

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References

1. Connors, K. A. *Chem. Rev.* **1997**, *97*, 1325–1354.
2. Moreira da Silva, A. M.; Empis, J.; Teixeira-Dias, J. J. C. *J. Inclusion Phenom.* **1999**, *33*, 81–97.
3. Steiner, Th.; Koeller, G. *J. Am. Chem. Soc.* **1994**, *116*, 5122–5134.
4. Steiner, Th.; Moreira da Silva, A. M.; Teixeira-Dias, J. J. C.; Muller, J.; Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1452–1453.
5. Moreira da Silva, A. M.; Steiner, Th.; Saenger, W.; Empis, J.; Teixeira-Dias, J. J. C. *Chem. Commun. (Cambridge)* **1996**, 1871–1872.
6. Moreira da Silva, A. M.; Steiner, Th.; Saenger, W.; Empis, J.; Teixeira-Dias, J. J. C. *Chem. Commun. (Cambridge)* **1997**, 465–466.
7. Chang, Y.; Reineccius, G. A. *J. Food Sci.* **1990**, *55*, 1686–1695.
8. Giordano, F.; Novak, C.; Moyano, J. R. *Thermochim. Acta* **2001**, *380*, 123–151.
9. Job, P. *Ann. Chim.* **1928**, *9*, 113–134.