Energetics of Water/Cyclodextrins Interactions

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Abstract

The heat capacities of solid γ-CD, 8.1 H_2O and α-CD, 6.0 H_2O have been measured between 10 and 300 K by adiabatic calorimetry. Using earlier results obtained in similar experiments with anhydrous cyclodextrins and with β-CD, 9.7 H_2O, a comparative analysis has been developed. The energetic behaviours of anhydrous and hydrated cyclodextrins (CDs) have been compared in order to investigate the role of water molecules in the stabilization of the cyclodextrin's rings and on their reactivities. Calculations, based on the additivity of thermodynamic properties, provide the energetic and entropic average contributions of water molecules in each cyclodextrin. From these results, we assumed that the water–water and water–CD interactions are rather different according to the cyclodextrin. In the (β-CD, 9.7 H_2O) structure, the water molecules seem to be better organised in a relatively independent network. Concerning hydrated α-CD and γ-CD, stronger water–CD interactions probably prevent an optimal organisation of the water–water bonds network. Differential scanning calorimetry was also used to follow the evolution of the thermal behaviour of γ-CD, nH_2O versus hydration ratio between 170 and 300 K. Our results indicate that the γ-CD ring needs at least 1.6 water molecules to be stabilized in the solid state.

Introduction

In the solid state, the cyclodextrins are described as non-defined hydrates [1, 2]. It is now recognized that the number of hydration water molecules of a solid cyclodextrin is largely dependent on the water pressure of its surrounding atmosphere [1, 3].

Despite some studies [4–7] on the evaluation of their physico-chemical properties according to the hydration rate, the interactions between the water molecules and the macrocycles are not yet well understood. The literature is rather poor concerning direct energetic approach of water-CD interactions by calorimetric measurements [1, 8–10]. These works are relative to the hydration-dehydration processes at room temperature or above. Many questions remain concerning the role of these hydration water molecules in the stabilization of the glucopyranose rings and consequently in the inclusion mechanisms in aqueous medium. In previous papers [2, 11, 12], we have described the energetic behaviour of the three native anhydrous CDs and of hydrated (β-CD at low temperature. The present study concerns the same investigations on hydrated α-CD and γ-CD. The first objective is to deepen the knowledges about their individual thermal behaviour at low temperature. The second is to compare the thermodynamic properties of the three cyclodextrins with the intention of estimating the influence of water on their relative molecular organization.

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Experimental

Materials

α-Cyclodextrin and γ-cyclodextrin powders of pharmaceutic grade were supplied by Wacker SA and used without further purification. The hydration contents were determined by thermogravimetric analysis using a Mettler TG50 thermobalance. The compounds were found to be α-CD, 6.0 H_2O (± 0.2 H_2O) and γ-CD, 8.1 H_2O (± 0.3 H_2O). The molar masses were respectively taken as 1080.9 g mol^{-1} and 1442.9 g mol^{-1} in the following calculations.

For DSC experiments, samples with various hydration content were obtained by hydrating anhydrous γ-CD and controlled as described above.

Calorimetric methods

Differential scanning calorimetry (DSC) results were obtained using a Mettler TA 8000 apparatus. Weighed samples (10–15 mg) were conditioned into 40 mL aluminium sealed crucibles. The experiments were performed with the same heating rate of 5 K/min between 170 and 300 K.

The low temperature calorimetry experiments have been previously described in the case of β-CD [2, 11]. The same experimental method was used to measure the heat capacities of the hydrated α- and γ-cyclodextrins between 10 and 300 K. The sample masses were 9.372 and 9.114 g respectively corresponding to 8.6706×10^{-3} mole of α-CD and 6.316×10^{-3} mole of γ-CD. Four series of experiments were

performed on each sample. A discontinuous heating mode was used to measure the heat capacities. It consists of two steps: heating and equilibration. In the first step, the sample cell receives a known quantity of energy Q. The beginning of the second step is necessary for the sample to reach a uniform temperature. The measurement of the temperature jump ΔT is performed in the last part of the step, when the calorimetric temperature drift observed becomes linear. The global heat capacity (cell + sample) is calculated for each temperature as $T = T_i + \Delta T/2$ as $C = Q/\Delta T$. Knowing the exact heat capacity of the goldplated cell, it is easy to obtain, by subtraction, the C_p values of the sample. The experimental accuracies on the heat capacities are considered to be about 10% at 10 K, 1% at 20 K and less than 0.5% beyond 50 K.

Results and discussion

The smoothed heat capacity values for α-, β-, and γ cyclodextrin (anhydrous and hydrated forms) are respectively plotted versus the temperature in Figures 1–3, in the range between 0 and 300 K. The experimental results have been adjusted for curvature using the polynomial method of Tchebychev [13]. For the temperature lower than 15 K, C_p values have been obtained by extrapolation taking C_p (O K) $= 0$ J K^{-1} mol^{-1}.

This presentation provides an easy comparison inside each couple of hydrated and anhydrous CD. It allows to point out the differences that are mainly connected to the presence of water molecules in the hydrated compound structure.

Comparative analysis of the heat capacity curves

From Figure 1, we can deduce that the thermal behaviours of hydrated and anhydrous α-CD are very similar at low temperature. The two $C_p = f(T)$ curves exhibit the same anomalous thermal event around 210 K. In a previous paper devoted to anhydrous α-CD [12], we hypothesise that this C_p anomaly seems to be associated with the more or less random settling of intramolecular interglucopyranose (H–O$_{(2)}$—H–O$_{(3)}$) hydrogen bonds during the frozen period in the calorimeter. This phenomena subsists in the case of α-CD, 6.0 H$_2$O and consequently cannot be attributed to water-CD interactions.

Concerning β-CD compounds, we can observe, in Figure 2, a very different behaviour above 230 K on the β-CD, 9.7 H$_2$O curve, with a maximum C_p "excess" around 273 K. This peculiar anomaly has been previously discussed [2]. Our conclusion was that, in β-CD hydrates with water content between 7 and 10, the interactions between water molecules and β-CD hydroxyl groups are less important than in more hydrated compounds. Around 273 K, these water molecules exhibit a thermal behaviour very close to that of ice melting in a porous solid material.

The $C_p = f(T)$ curves on Figure 3 point out a difference between the thermal behaviours of anhydrous and hydrated γ-CD. In the absence of hydration water, an endothermic event appears around 220 K with a maximum at 240 K. We have previously shown through entropic calculations
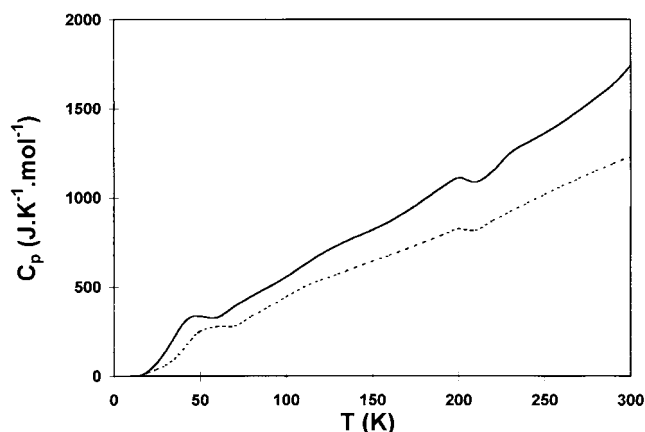

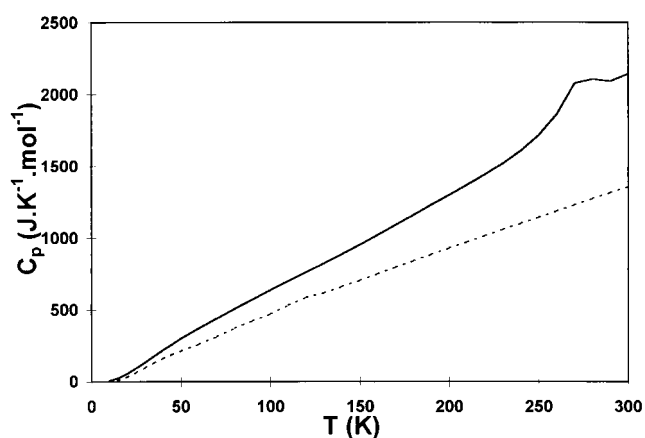*Figure 1.* Molar heat capacities of: —: α-Cyclodextrin, 6.0 H$_2$O, - - -: Anhydrous α-cyclodextrin.

Figure 2. Molar heat capacities of: —: β-Cyclodextrin, 9.7 H$_2$O, - - -: Anhydrous β-cyclodextrin.

that this endothermic phenomenon corresponds to an order-disorder transition related to the release of the motions of the eight glucopyranose units in the macrocyclic ring.

In order to understand the reason why this transition disappears for the γ-CD, 8.1 H$_2$O sample, we performed a series of DSC experiments on differently hydrated γ-CD microcristalline powders. The thermograms are effected in the temperature range 170–300 K . The hydration CD contents
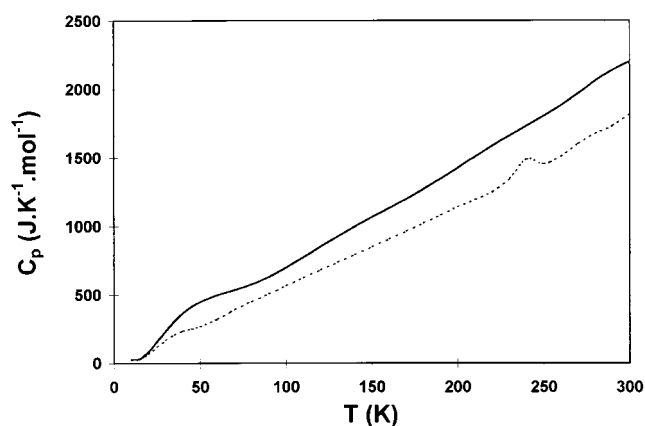

Figure 3. Molar heat capacities of: —: γ-Cyclodextrin, 8.1 H$_2$O, - - -: Anhydrous γ-cyclodextrin.
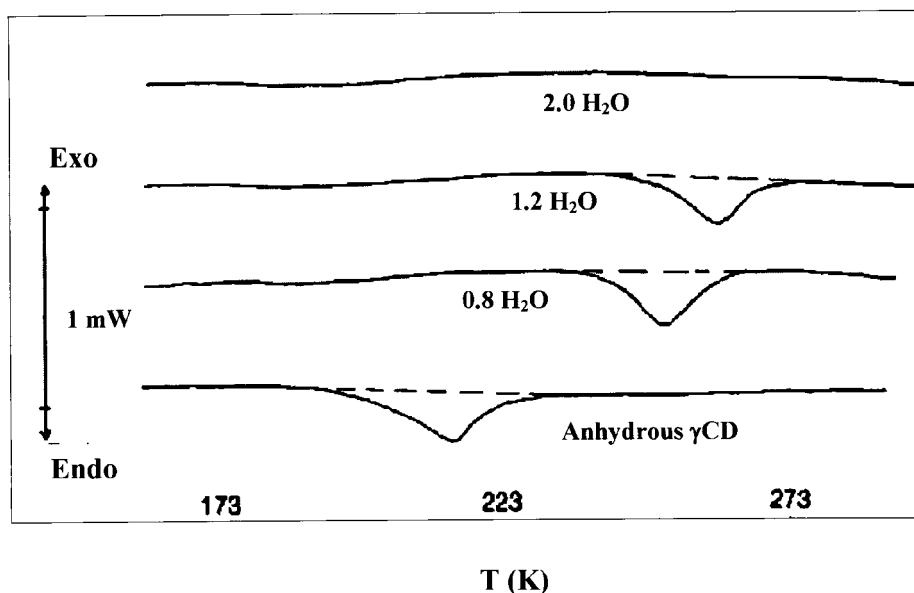
Figure 4. Thermal behaviour of γ-cyclodextrin according to water content: DSC curves.

were between 0 and 2 H_2O per CD. The results, presented on Figure 4, clearly show two important evolutions according to the hydration rate. The endothermic peak located around 220 K for the anhydrous CD, slightly moves to higher temperatures when the water content increases from 0 to 1.2 H_2O. However, this peak vanishes for γ-CD containing more than 2 H_2O. We have also noticed that the enthalpy change is around 1.8 kJ/mol for all the samples. The calculated entropy change tends towards zero when the hydration rate reaches 1.6. As such 1.6 H_2O are necessary to eliminate the order-disorder transition, we conclude that these water molecules are essential for the stabilisation of the γ-CD macrocycle.

Average contribution of the water molecules to the heat capacity of hydrated CD

The calculation method is based on the additivity of thermodynamic properties. The thermal behaviour of the hydrated compound can be envisaged as the sum of the contribution of the macrocycle skeleton, represented by the heat capacity of the anhydrous CD and of the energetic contribution of the hydration water molecules. This later contribution $\Delta C_p(w)$ includes both water–water and water–CD interactions. The average $\Delta C_p(w)$ value for one mole of water is so calculated from the experimental CDs heat capacities:

$$C_p \, (CD, nH_2O) = C_p \, (\text{anhydrous CD}) + n \, \Delta \, C_p(w).$$

$\Delta C_p(w) = f(T)$ curves for each cyclodextrin are plotted on the Figure 5 between 100 and 300 K.

For comparison and discussion, the heat capacity of hexagonal ice [14] is also represented in the same temperature range. Unfortunately, we didn't found equivalent numerical data at low temperatures for amorphous solid water.

From the shapes of the three curves, we assume that the energetic behaviour of the water molecules is very different above 200 K, according to the CD.
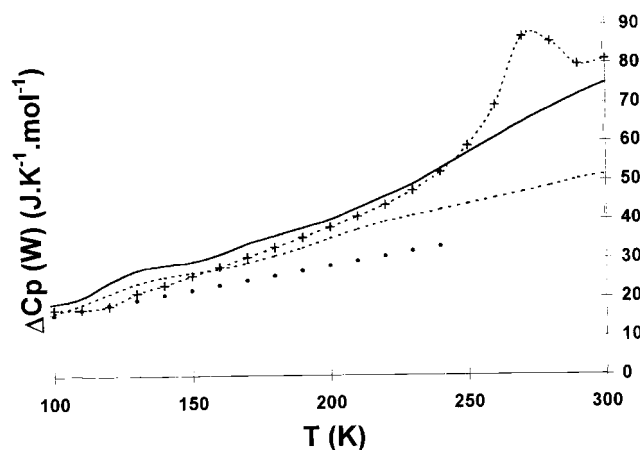

Figure 5. Average molar heat capacity of water ΔC_p vs T: —: (α-CD) , -+-: (β-CD), - - -: (γ-CD), $\cdots$: C_p of hexagonal ice [14].

We can find again, on the β-CD curve, the same anomaly we have previously attributed to solid water melting in a porous material. Furthermore, we can notice that the ΔC_p value at 298 K is comparable to the known C_p of liquid water (75.38 $JK^{-1}mol^{-1}$) [16].

The ΔC_p obtained for α-CD at ambient temperature is also very close to the heat capacity of liquid water. However, no melting peak is observed for this compound.

Around 273 K, the ΔC_p value calculated for γ-CD is closer to the known C_p value of solid water (37.2 $JK^{-1}mol^{-1}$) than to that of liquid water.

All these results suggest a more independent behaviour of water towards β-CD. The fact that no melting peak appears on the α-CD and γ-CD curves probably indicates tightest interactions between water and the macrocycles.

A convenient test of the validity of our calculations concerning the water contribution to the hydrated CDs heat capacities is provided by the extremely good agreement between our ΔC_p values and the known ΔC_p for both solid
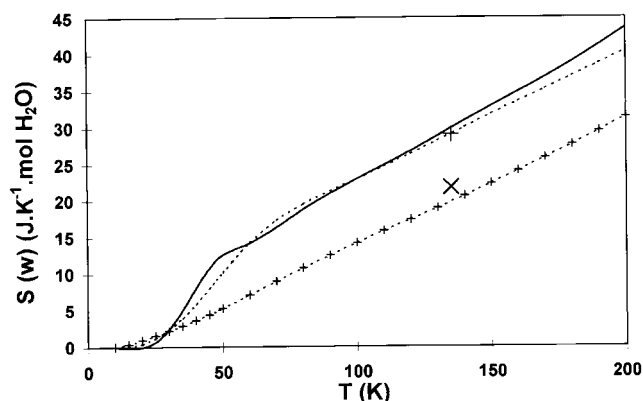
Figure 6. Average molar entropy of water S(W) vs T: —: $S_{(W)}$ ((α-CD), - + -: $S_{(W)}$ (β-CD), - - -: $S_{(W)}$ (γ-CD), +: S of amorphous solid H_2O at 135 K [15], ×: S of hexagonal ice at 135 K [15].

and liquid water around 273 K. In fact, the agreement is far better than should be expected from the uncertainties on ΔC_p which have been estimated to be around 10%.

Average entropic contribution of hydration water molecules to the entropy of the hydrated CD

The CD-water interactions have also been investigated from an entropic point of view. The calculations are also based on the additivity of thermodynamic functions using the following relation:

$$S(CD, nH_2O) = S \text{ (anhydrous CD)} + nS(w),$$

where S(w) represents the entropic "excess" of a hydrated CD relative to one mole of hydration water. S(w) = f(T) curves. are plotted on Figure 6 between 0 and 200 K. The choice of this temperature range is conditioned by the thermodynamic sense of the entropy itself. Entropic values of the CDs are calculated using the general formula

$$S(T) = \int_0^T \frac{C_p}{T} dT.$$

They are obviously cumulative data and we need to be careful in their interpretation. The entropic difference S(w) cannot be interpreted in terms of water influence above 150 K where the release of motions of glucopyranose units is not predominant. Below 150 K, we can consider that S(w) is more representative of the entropy "excess" associated to the water interactions and water motions released from 0 to 150 K. At first the comparison of the three S(w) = f(T) curves shows an obvious difference between β-CD hydrate and the two others CDs. S(w) values for α-CD and γ-CD hydrates are surprisingly very close in the temperature range 50–150 K. In order to go further in the physical interpretation of this result, the S(w) values are compared to the entropy of crystallised water (hexagonal ice) and of amorphous solid water given by F. Francks at 135 K [15].

The calculated entropy of one mole of water S(w) in α-CD and in γ-CD hydrates exactly corresponds to the molar entropy of amorphous solid water (29 $JK^{-1}mol^{-1}$) at the same temperature of 135 K.

In β-CD hydrate, the water molar entropy S(w) = 20 $JK^{-1}mol^{-1}$ is very close to the literature value of 21.8 $JK^{-1}mol^{-1}$ for the molar entropy of hexagonal ice.

At first, the above comparison allows to valid the calculation methods because the S(w) values are in good agreement with the known values for the entropy of solid water. Then, our interpretation of the observed differences are: at 135 K, water molecules in β-CD hydrate seem to be organised as in crystallised water. This result confirms our previous hypothesis that the H-bonds network between the hydration water molecules is quite independent from the β-CD macrocycles at low temperature. Concerning α-CD and γ-CD hydrates, we assume that stronger CD-water interactions exist and prevent an optimal organisation of the water–water bonds network as in β-CD. This hypothesis is also in agreement with the previous ΔC_p interpretation for these two CDs.

Conclusion

Our thermodynamic and comparative approach of hydrated an anhydrous native cyclodextrins at low and very low temperatures confirms the important role of water in their molecular stability and probably in their reactivities in aqueous and non-aqueous medium. Our results and hypothesis are in good agreement with the relative solubilities of the three cyclodextrins in water [17]. β-CD have a poor affinity with water molecules even in the solid state and its solubility is the lowest. On the contrary, for γ-CD, which solubility is the highest, strong water-CD interactions obviously exist in the solid state. α-CD shows an intermediate behaviour towards water, water-CD interactions exist, but their are not so strong as in γ-CD hydrates.

From our experimental conclusions, the inclusion properties of γ-CD, nH_2O would be very different in non-aqueous medium according to its hydration state (n < 2, or n > 2).

As previously discussed [2], the energetic properties of solid β-CD hydrates also depend on its hydration rate. But in this case, the number of hydration molecules seems to influence the water network through long distance interactions rather than the conformational feature of the macrocycle.

From a general point of view, the hydration state of a cyclodextrin must be taken into account because the thermodynamic parameters of an inclusion reaction are obviously influenced by the associated energetic state.

It would be interesting to complement the calorimetric studies of differently hydrated CD at very low temperatures (below 100 K) with X-ray or neutron diffraction and spectroscopy studies. These experiments would give another picture of the water interactions in the cyclodextrins molecular organisation.

References

1. M. Bilal, C. de Brauer, P. Claudy, P. Germain, and J.M. Letoffé: *Thermochim. Acta* **249**, 63 (1995).
2. P. Germain, C. de Brauer, M. Diot, and J.M. Letoffé: *J. Incl. Phenom. Mol. Recogn. Chem.* **31**, 205 (1998).

3. A.M. da Silva, T. Steiner, W. Saenger, J. Empis, and J. Teixeira-Dias: *J. Chem. Soc. Chem. Commun.* 1871 (1996).

4. T. Steiner and G. Koellner: *J. Am. Chem. Soc.* **116**, 5122 (1994).

5. T. Steiner, G. Koellner, S. Ali, D. Zakim, and W. Saenger: *Biochem. Biophys. Res. Commun.* **188**, 1060 (1992).

6. A.M. Da Silva, T. Steiner, W. Saenger, J. Empis, and J. Teixeira-Dias: *J. Incl. Phenom.* **25**, 21 (1996).

7. A.M. Da Silva, T. Steiner, W. Saenger, J. Empis, and J. Teixeira-Dias: *J. Chem. Soc. Chem. Commun.* 465 (1997).

8. A. Szafranek and J. Szafranek: *J. Incl. Phenom. Mol. Recogn. Chem.* **30**, 163 (1998).

9. P. Claudy, P. Germain, and J.M. Letoffé: *Thermochim. Acta* **161**, 75 (1990).

10. A. Marini, V. Berbenni, V. Massarotti, P. Mustarelli, and R. Riccardi: *Solid State Ionics* **63**, 358 (1993).

11. M. Diot, C. de Brauer, and P. Germain: *J. Incl. Phenom. Mol. Recogn. Chem.* **30**, 143 (1998).

12. C. de Brauer, M.P. Merlin, P. Germain, and T. Guerandel: *J. Incl. Phenom. Mol. Macro. Chem.* **37**, 75 (2000).

13. Y.N. Linnik: *Méthode des moindres carrés*, Dunod ed., Paris (1963), pp. 290–306.

14. W.F. Giauque and J.W. Stout: *J. Am. Chem. Soc.* **58**, 1144 (1936).

15. F. Francks: *Amorphous Solid Water and its Relationship to Liquid Water* (Water and Aqueous Solutions at Subzero Temperatures, ed.), Plenum Press, New York (1982), pp. 83–214.

16. M.W. Chase: NIST–JANAF Thermochemical Tables, 4th edn., *J. Phys. Chem. Ref. Data*, monograph 9, 1–1951 (1998).

17. M.J. Jozwiakowsky and K.A. Connors: *Carbohydr. Res*. **143**, 51 (1985).