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Thermal Behaviour of Anhydrous α-, β- and γ-Cyclodextrin at Low Temperature

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Abstract. Heat capacities of anhydrous α- and γ-cyclodextrin were measured by adiabatic calorimetry between 10 K and 300 K. The thermal behaviour of the two compounds exhibits significant differences. α-Cyclodextrin shows an anomalous excess heat capacity in the entire region between 70 K and 210 K. In the case of γ-cyclodextrin, an endothermic effect is observed at 240K. This effect is analysed through the study of the corresponding entropy change and discussed in terms of intramolecular organization. Using the known heat capacity values of anhydrous β-CD, a comparative analysis has been developed. For each cyclodextrin, the average behaviour of a bound α-D-glucopyranose has been calculated and compared. From a thermodynamic point of view, the degree of organization of the dehydrated macrocyclic compounds could be expressed as β-CD $>$ α-CD $>$ γ-CD.

Key words: anhydrous cyclodextrin, heat capacity, low temperature, thermodynamic, molecular organization.

1. Introduction

The α-, β- and γ-cyclodextrins are macrocyclic molecules respectively composed of six, seven, and eight α(1–4) linked α-D-glucopyranose units. They are known for their abilities to form non-covalent inclusion compounds in aqueous medium [1]. In the solid state, the cyclodextrins are described as non-defined hydrates [2, 3]. 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 [4, 5].

Despite some recent 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. A better understanding of these interactions needs more information on the anhydrous compounds behaviour. In a previous paper [8], we have described the behaviour of anhydrous β-CD at low temperature. From this work, we have assumed that intramolecular interglucopyranose H-bond interactions ($H\text{-}O_{(2)}$- - -$H\text{-}O_{(3)}$ flip-flop type) could exist even in the absence of water. This "ring" of hydrogen-bonds is probably

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the main factor governing the relatively good molecular organization of the β-CD macrocycle. It also explains its weak water solubility by restricting the possibilities of hydrogen-bond interactions with bulk water.

In the present work, our purpose is to develop a similar approach concerning α- and γ-cyclodextrin. The first objective is to deepen the knowledge 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 their relative molecular organization, according to the number of bound α-D-glucopyranose units.

2. Experimental

2.1. MATERIALS

α- and γ-Cyclodextrin powders of pharmaceutic grade were supplied by Wacker SA and used without further purification. The corresponding anhydrous compounds were prepared by dehydration under vacuum ($\approx 10^{-2}$ Pa) at 110°C during 24 h. The complete dehydration of the two samples was monitored by Differential Scanning Calorimetry (DSC) using a METTLER TA8000 apparatus. No mass loss or thermal effects relative to the dehydration were observed in the temperature range 20–200°C. These measurements confirm a hydration content lower than 0.3 H_2O per molecule of cyclodextrin. X-Ray diffraction studies on the resulting powders show that they are almost amorphous. The dehydrated samples were stored and handled in a glove box under dry argon gas. Another DSC control was done after the low temperature experiment. The molar masses were taken as 972.9 g mol^{-1} for anhydrous α-CD and as 1297.1 g mol^{-1} for anhydrous γ-CD.

2.2. ADIABATIC CALORIMETRY

The low temperature calorimetry experiments have been previously described in the case of β-CD [3, 8]. The same experimental method was used to measure the heat capacities of the anhydrous α- and γ-cyclodextrins between 10 K and 300 K. The sample masses were respectively 8.872 g and 7.986 g corresponding to 9.119 $\times 10^{-3}$ mole of α-CD and 6.157 $\times 10^{-3}$ mole of γ-CD. Four series of experiments were performed on each sample with the same cooling treatment at 2 K min^{-1}. A discontinuous heating mode was used to measure the heat capacities. In the first period, the sample – at the initial temperature T_i – receives a known quantity of energy Q. During the beginning of the second period, the temperature is increased to reach a constant final value T_i. The temperature jump $\Delta T = T_f - T_i$ is measured in the last part of the second period after a stabilization time of about 15 min. When the stabilization of the final temperature needs a longer time, it generally indicates that the sample relaxes from a non-equilibrium to an equilibrium state.

For each measurement, the energy Q is adjusted for having ΔT values around 4 K. The global heat capacity (cell + sample) is calculated for each temperature

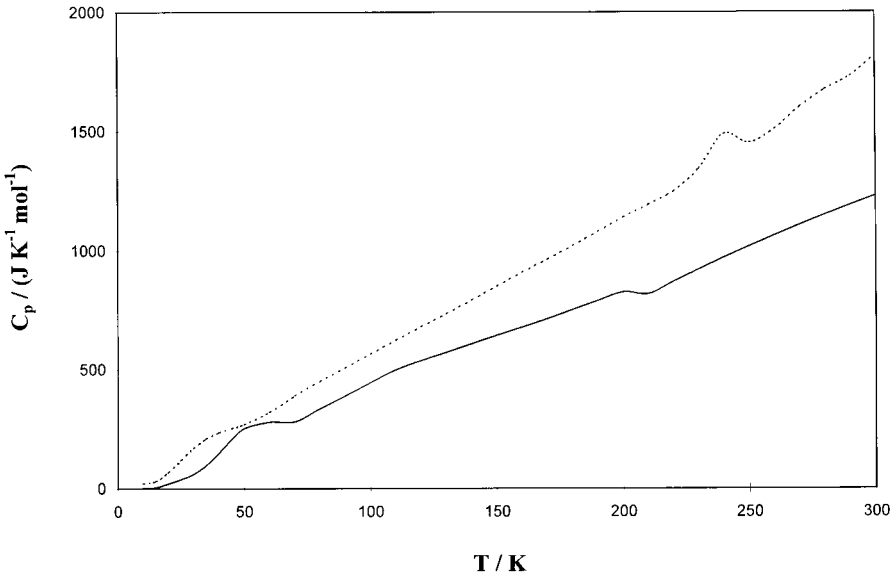

Figure 1. Molar heat capacities of: ——: anhydrous α-cyclodextrin , - - -: anhydrous γ-cyclodextrin.

$T = T_i + \Delta T/2$ as $C = Q/\Delta T$. Knowing the exact heat capacity of the gold-plated 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.

3. Results and Discussion

The smoothed heat capacity values for anhydrous α- and γ-cyclodextrin are plotted versus the temperature in Figure 1 in the range between 0 K and 300 K. The experimental results have been adjusted for curvature using the polynomial method of Tchebychev [9]. For temperatures lower than 15 K, C_p values have been obtained by extrapolation taking C_p (O K) = 0 J K^{-1} mol^{-1}.

3.1. ANHYDROUS α-CYCLODEXTRIN BEHVIOUR

The smoothed $C_p = f(T)$ curve reveals an anomalous thermal behaviour of anhydrous α-CD below 210 K which can be described by the following observations: a strong C_p increase followed by a plateau below 70 K and a more diffuse C_p "excess" over a large range of temperature between 70 K and 210 K.

The insert of Figure 2 shows the experimental data obtained around 200 K in series 1, 3 and 4. The obvious lack of reproductibility of the collected data requires a description of the cooling procedure of the three series even though the cooling rates were the same (2 K min^{-1}).

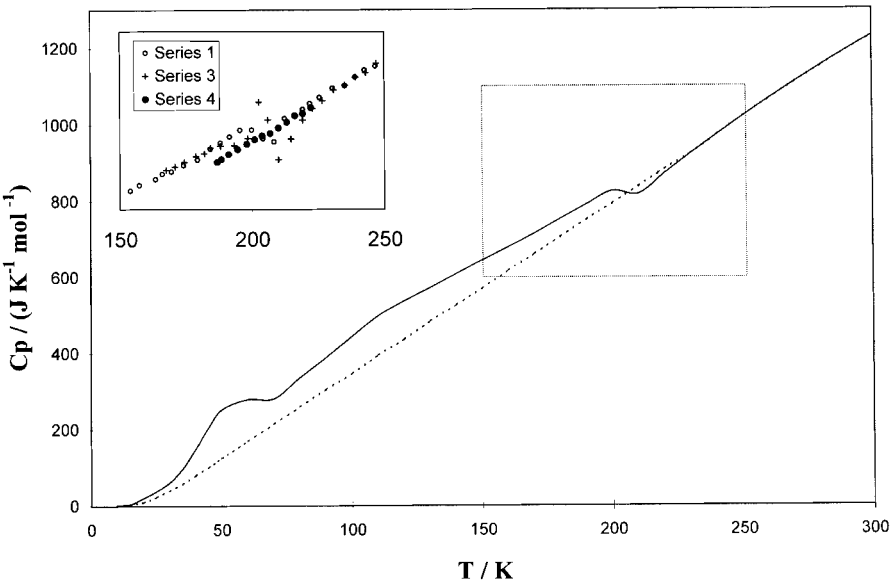

Figure 2. Molar heat capacities of anhydrous α-cyclodextrin: ——: smoothed Cp values, - - -: extrapolated baseline. The insert gives the experimental Cp values in series 1, 3, and 4 around 200 K.

Series 1 were registered after the sample had been cooled to 77 K for the first time. Before series 3, the sample was cooled to 4 K and slowly warmed to 65 K during series 2. Series 4 were carried out after a cooling procedure to 180 K.

Despite series 1 and 3 showing erratic experimental data around 200 K, series 4 does not present any sign of an excess heat capacity. These apparent differences of behaviour are probably due to experimental differences in the equilibration times during series 1, 3 and 4. This is usually the sign of a slow and incomplete phase transformation. In such a case, a too short equilibration time leads to an average heat capacity which may not correspond to the true heat capacity. The results of the series 4 experiment do not show the same behaviour. This observation is fortunate because the extrapolation of these experimental C_p values provides a baseline (illustrated by the dashed line in Figure 2) from which to consider the excess heat capacity in the lower temperature region. The lower curve could correspond to the behaviour of an ideally organized α-CD macrocycle. From our observations, this better degree of molecular organization could be associated with the thermal history of the sample. Saenger et al. [10–11] studied hydrated α-CD by X-Ray diffraction at room temperature. They concluded that one α-D-glucopyranose unit of the α-CD macrocycle is statistically in a rocking position with regard to the others. Since there is no further structural information concerning α-CD at low temperature, we hypothesise that the way the glucopyranose unit is frozen could condition the more or less random settling of intramolecular interglucopyranose ($H\text{-}O_{(2)}\text{- - -}H\text{-}O_{(3)}$) hydrogen bonds.

According to this hypothesis, the relaxational heat capacity anomaly observed up to 210 K could be explained by a random molecular arrangement in the frozen state depending on the cooling procedures in series 1, 2 and 3.

Above 230 K, series 1, 3 and 4 lead to similar data and the resulting curves show a steady increase till 300 K.

3.2. ANHYDROUS γ-CYCLODEXTRIN BEHAVIOUR

Anhydrous γ-CD exhibits an anomalous C_p "excess" between 10 K and 70 K and an endothermic effect, with an associated enthalpy change ΔH = 1.8 kJ mol^{-1}, that appears around 220 K and has its maximum at 240 K. In all the other temperature ranges, the C_p curve produces a steady evolution.

The thermal event occuring at 240 K may be interpretated as the consequence of a molecular change because γ-CD molecular organization appears to depend on temperature. The X-Ray and neutron crystal structure of γ-CD hydrate determined at room temperature [12], at 110 K [13] and 120 K [14], have shown that the γ-CD molecule adopts a round shape somewhat distorted from the ideal octagonal symmetry. At low temperature, the γ-CD molecule has one glucopyranose residue statistically disordered while at room temperature, no disorder has been observed. However, at 110–120 K, all the $O_{(2)}$ and $O_{(3)}$ hydroxyl groups are involved in a ring of intramolecular inter-residue H-bonds which stabilizes the conformation of the macrocycle whereas at room temperature only seven among the eight intramolecular H-bonds have been founded. The ring of intramolecular H-bonds is disrupted and consequently, its stabilizing effect disappears. Having no crystallographic information about anhydrous γ-CD (because the crystals almost completely disintegrate upon dehydration), we hypothesise that the intramolecular H-bond network remains in the anhydrous state because we observed this phenomenon in β-CD. We assume that, at room temperature, the hydrated conformation compensates for the loss of organization, following the replacement of the intramolecular H-bond ring, by an external H-bond network involving water molecules. In the anhydrous sample, stabilization by external interactions is more difficult and the molecular configuration is consequently more disordered at room temperature than around 120 K. In order to quantify the increase of disorder due to this molecular transition, the inherent entropy change ΔS has been calculated. Using our experimental heat capacity values, we have plotted the evolution of C_p/T versus T in Figure 3. The change in entropy $\Delta S = \int_{T_1}^{T_2} (C_p/T)\, \mathrm{d}T$ has been obtained from the area between the curve and a chosen straight base line in the temperature range between T_1 = 220 K and T_2 = 260 K. The calculated value of ΔS = 16.5 $\pm$ 0.9 J K^{-1} mol^{-1}, in agreement with an order-disorder transition. Any modification of a physico-chemical system can be correlated with its entropy through the Boltzmann relation $\Delta S = R$ ln (Ω_2/Ω_1) where Ω_1 and Ω_2 are respectively the number of statistical molecular arrangements before and after the transition. The experimental value of 16.5 $\pm$ 0.9 J K^{-1} mol^{-1} is in good agreement with the entropy change calculated in the

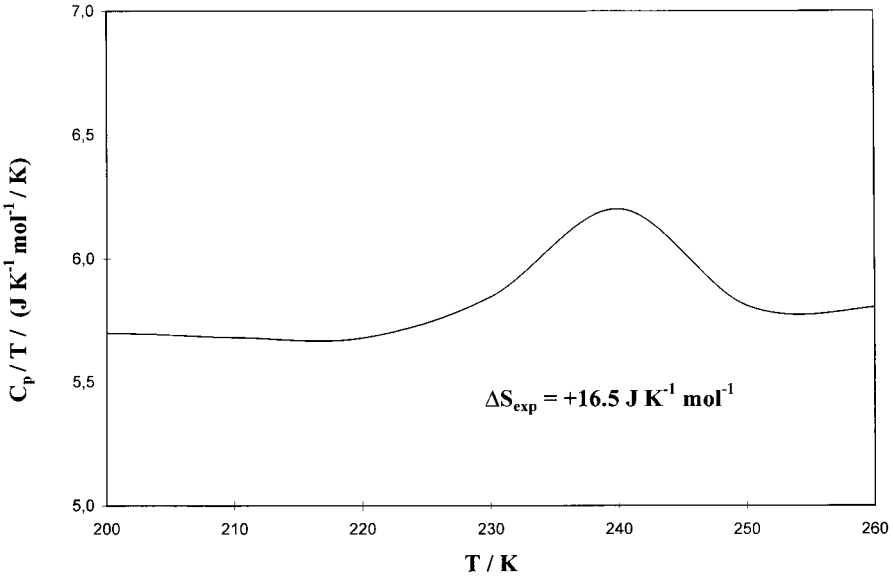

Figure 3. C_p/T vs T around the anomaly transition temperature for anhydrous γ-CD.

hypothesis of a transition from 1 to 8 molecular arrangements leading to $\Delta S_{\text{calc}} R \ln(8/1) = 17.35$ J K^{-1} mol^{-1}. This scheme corresponds to an initial configuration with one disordered glucopyranose unit to a final one with eight disordered units.

So, we assume from this result that the endothermic phenomenon observed at 240 K is directly related to the release of the motions of the eight glucopyranose units.

3.3. COMPARATIVE ANALYSIS OF ANYDROUS α-, β-, AND γ-CYCLODEXTRIN

In order to compare the molecular organization of the three anhydrous macrocycles, the entropies S_{CD} of α-, β-, and γ-cyclodextrin have been calculated from the smoothed heat capacity values using $S_T = \int_0^T (C_p/T)\, \mathrm{d}T$.

We have plotted, in Figure 4, the evolution of $S_n = (S_{\text{SD}}/n)$ against the temperature where n is the number of α-D-glucopyranose units in the macrocycle (respectively, n = 6, 7, and 8 for α-, β-, and γ-cyclodextrin). S_n represents the average entropy per α-D-glucopyranose unit for a given cyclodextrin. It can give some comparative information about the global organization of the macrocycles according to their size.

If the degree of molecular organization was the same in each cyclodextrin, we should obtain very similar values of S_n. Looking at the three distinct curves obviously indicates that this is not the case. The γ-CD curve exhibits the highest S_n values in all the studied range of temperature. At every temperature above 70 K, the lowest S_n values are for β-CD. From the relative positions of the curves, it can also be deduced that the degree of order of the cyclodextrins is not directly

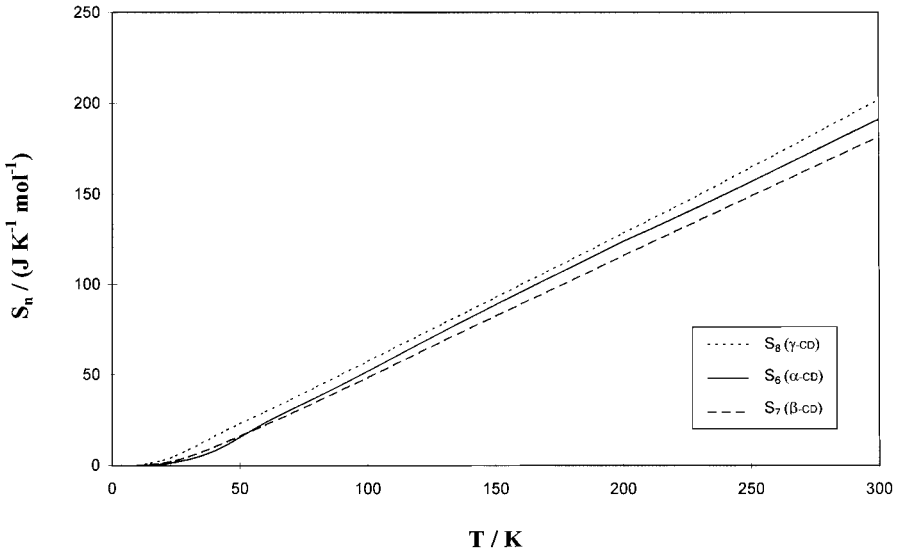

Figure 4. Average molar entropy per glucopyranose unit *Sn* vs *T*: ——: S_6 (α-CD), - - -: S_7 (β-CD), . . .: S_8 (γ-CD).

related to their size. The degree of molecular organization of the three anhydrous cyclodextrins could be ranked as: β-CD > α-CD > γ-CD.

Taking into account the few published structural studies and our work on the individual behaviour of anhydrous α-, β- and γ-cyclodextrin at low temperature, we assume that the observed differences in molecular organization are highly related to the intramolecular H-bond network. Of course, the existence of this network is itself dependent on the temperature and on the strain of the annular compounds.

Concerning both anhydrous and hydrated β-CD, no doubt exists about its high molecular symmetry associated with a regular ring of intramolecular intergluc-opyranose H-bonds.

Compared with β-CD, some conformational evidence [10–14] exist to explain the observed differences in the degrees of molecular organization of α-, and γ-CD. In these two compounds, respectively constituted by 6 and 8 glucopyranose units, the strain of the macrocycle implies at least one unit to be in a rocking position with regard to the others. Consequently, as detailed before, the global geometry of these two macromolecules and the intramolecular H-bond network are less regular in comparison with the 7 unit compound. Our thermodynamic approach to anhydrous cyclodextrins at low temperature confirms the important role of intramolecular hydrogen bonds to control their flexibilities and probably their reactivities in aqueous medium.

In conclusion, the present comparison between the thermal behaviours of anhydrous α-, β-, and γ-cyclodextrin at low temperature has revealed some major differences.

The relative solubilities of the three cyclodextrins in aqueous solution [15] can easily be correlated to the corresponding degrees of molecular organization of the dehydrated compounds. Our current thermodynamic study of hydrated solid α- and γ-cyclodextrin will provide other interesting information on the role of the water molecules in the stabilization of these peculiar polycyclic ring molecules.

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