Calorimetric Behaviour of Anhydrous β-Cyclodextrin at Very Low Temperature

M. DIOT, C. de BRAVER and P. GERMAIN

Laboratoire de Thermodynamique Appliquée, INSA, Bât. 401, 69621 Villeurbanne, France.

Abstract. Heat capacities of anhydrous β-cyclodextrin were measured between 10 K to 300 K using an adiabatic low-temperature calorimeter. Two distinct types of thermal behaviour were observed in the temperature range 50–130 K, depending on the way the sample was cooled. In the absence of hydration water molecules, we assumed that the two types of thermal behaviour could be attributed to freezing in more or less ordered states of the hydroxyl groups engaged in the intramolecular interglucose flip-flop-type interactions.

Key words: Anhydrous β-cyclodextrin, heat capacity, hydrogen bonding, entropy.

1. Introduction

The cyclodextrins are a family of cyclic oligosaccharides composed of six to eight α (1–4) linked glucoses and called α-, β-, γ-cyclodextrins. Due to their torus-shaped structure, they easily form inclusion compounds with a large number of guest molecules of suitable size [1].

The cyclodextrins and their inclusion compounds are often studied as models of noncovalent interactions. β-Cyclodextrin crystallises from water with several hydration water molecules. A great number of O—H···O hydrogen bonds are responsible for intra- and intermolecular interactions. Many neutron and X-ray diffraction studies [2–9] have confirmed the existence of ordered and disordered hydrogen bonding networks in hydrated β-cyclodextrin crystals.

Adiabatic calorimetry at low temperature is an excellent way to investigate molecular interactions, as shown by Hanabata et al. [10] in a study of crystalline β-cyclodextrin·11 H_2O.

In this calorimetric study of dehydrated β-cyclodextrin, our aim is to understand the behaviour of the macrocyclic 'cage', free of hydration water molecules.

2. Experimental

2.1. MATERIALS

β-Cyclodextrin was supplied by Roquette Freres under the reference β-cyclo-dextrin-16. The anhydrous β-cyclodextrin was obtained by dehydration under vacuum at 90 °C during 24 h. The complete dehydration of the sample was con-

firmed by two distinct methods: thermogravimetric analysis using a Mettler TG 50 thermobalance and differential scanning calorimetry using a Mettler TA 2000 B apparatus. No mass loss or thermal effect that could be attributed to a dehydration was observed in the temperature range from 25 to 200 K. Taking into account the sensitivities of the techniques, these measurements confirm a hydration content lower than 0.1%. The dehydrated β-cyclodextrin was stored and conditioned in the calorimetric cell, in a glove box, under dry argon gas. In the following calculation, the molar mass was taken as 1135 g mol^{-1}.

2.2. CALORIMETRY

The anhydrous β-cyclodextrin sample was sealed in a gold-plated copper cell of 10 mL capacity. After evacuation, dry helium gas was added to facilitate thermal equilibrium. The sample mass was 8.201 g, corresponding to 7.226×10^{-3} mole. The calorimetric apparatus and measuring technique have been described elsewhere [12]. The heat capacity measurements were performed using a discontinuous heating mode consisting of two steps: heating and equilibration. In the first step, the sample cell receives a known quantity of energy. The beginning of the second step is necessary for the sample cell 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. When the nonlinear temperature drift persists longer than a few minutes, it indicates that the sample relaxes from a non-equilibrium to an equilibrium state.

Three series of experiments were performed on this sample. The only difference consists in the cooling treatment (final temperature and cooling rate). In series 1 the sample was cooled at 2 K/min to 77 K. Series 3 corresponds to the same treatment with a final temperature around 10 K. In series 2 the sample was cooled at the same rate and maintained at 80 K for several hours, then cooled at 0.5 K/min to 10 K, heated at 0.5 K/min to 50 K, and cooled again twice using the same conditions.

3. Results and Discussion

The heat capacity determinations (on the same sample) are listed in Table I in chronological order and graphically reported in Figure 1. These results have been adjusted for curvature. The heat capacities are considered to have a probable error of about 10% at 10 K, 1% at 20 K and less than 0.5% beyond 50 K.

Figure 1 shows differences in direct relation with the cooling treatment of the sample. On the upper curve, corresponding to series 1 and 3, a relaxational heat capacity anomaly was found to occur at 60–130 K. In the same range of temperature, the plotted values obtained in series 2 give the surprising lower curve. The lower and the upper curves meet at 130 K (Figures 2 and 3) and for higher temperatures, the three series of experiments do not display any differences. The heat capacity depends on the way the sample had been cooled from 130 to 10 K.

Table I. Molar heat capacities of anhydrous β-cyclodextrin.

| T K	C_p J K^{-1} mol^{-1}	T K	C_p J K^{-1} mol^{-1}	T K	C_p J K^{-1} mol^{-1}
78.53	363.70	256.70	1173.33	89.33	353.30
80.99	383.90	260.84	1193.00	93.64	364.48
84.67	400.74	264.69	1206.77	97.60	376.47
89.96	427.59	268.00	1224.17	101.31	410.73
93.77	442.12	271.54	1238.09	105.32	455.83
97.62	458.89	275.83	1255.48	109.53	497.19
98.15	457.03	280.35	1271.72	113.72	527.73
101.28	483.01	282.44	1282.89	117.75	552.02
105.17	510.02	285.52	1295.34	121.79	570.87
106.08	514.61	288.98	1311.43		
109.88	536.35	292.87	1326.93		series 3
113.81	580.79	295.38	1336.80	9.75	2.50
117.95	579.26			14.44	8.12
121.88	593.49		series 2	18.20	22.02
125.57	600.84	13.94	8.04	20.73	40.42
129.57	617.94	15.70	13.19	22.41	49.30
133.83	630.21	17.56	20.55	24.18	60.45
138.04	652.37	18.74	24.97	26.39	81.41
142.12	670.77	20.21	33.07	29.16	99.71
146.15	685.53	21.68	40.43	32.04	116.68
150.22	702.57	22.85	47.80	34.58	131.93
154.35	723.89	24.52	56.63	37.05	146.78
158.51	739.70	26.25	64.98	39.70	162.43
162.62	761.87	27.51	77.37	42.50	177.99
166.32	780.51	28.85	86.51	45.28	188.86
170.16	796.96	30.53	102.62	47.83	202.31
174.50	815.83	32.28	113.43	50.59	216.32
178.87	835.85	34.01	124.05	53.60	231.30
182.94	855.29	36.19	139.95	56.62	246.88
186.89	871.73	38.96	157.38	59.55	261.74
190.91	888.14	41.86	174.29	62.46	275.04
195.19	905.74	43.72	184.02	65.42	289.66
199.25	926.19	45.57	193.16	68.44	304.15
203.34	938.62	49.05	205.74	71.48	319.07
207.41	959.75	52.04	217.75	74.52	336.83
211.48	981.25	54.63	230.11	77.50	354.95
215.55	999.93	57.38	241.28	80.25	369.80
219.66	1013.54	60.39	256.72	82.80	388.27
223.81	1031.68	63.49	267.40	86.28	404.85
228.20	1052.70	66.50	281.76	90.90	426.68
232.02	1067.70	69.24	293.13	95.54	441.43
236.24	1083.79	72.11	302.11	99.59	461.91
240.43	1101.41	74.89	311.85	103.38	484.29
244.49	1117.87	77.93	322.18	107.52	515.2
248.52	1138.69	81.07	332.81	110.21	529.01
252.57	1151.42	84.40	343.50	114.02	548.45
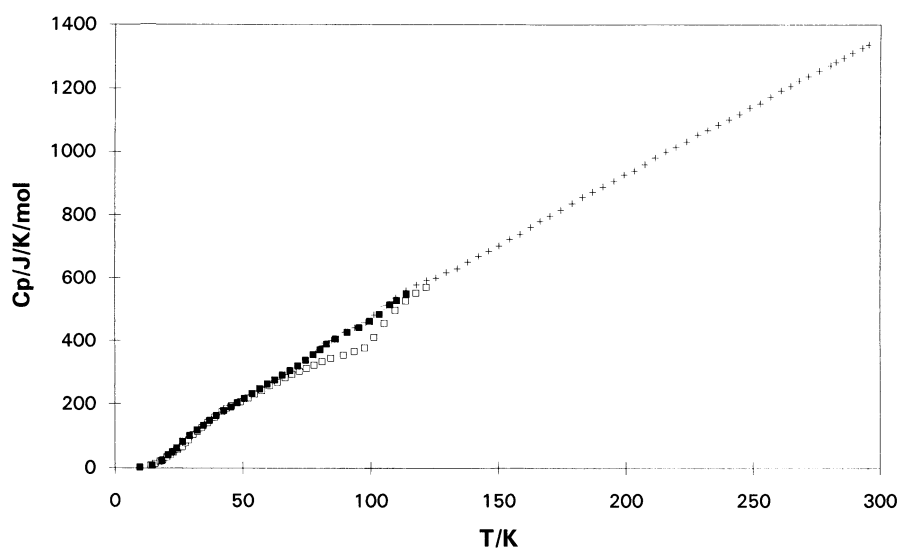
Figure 1. Molar heat capacity of anhydrous β-cyclodextrin: +, series 1; □, series 2; ■, series 3.

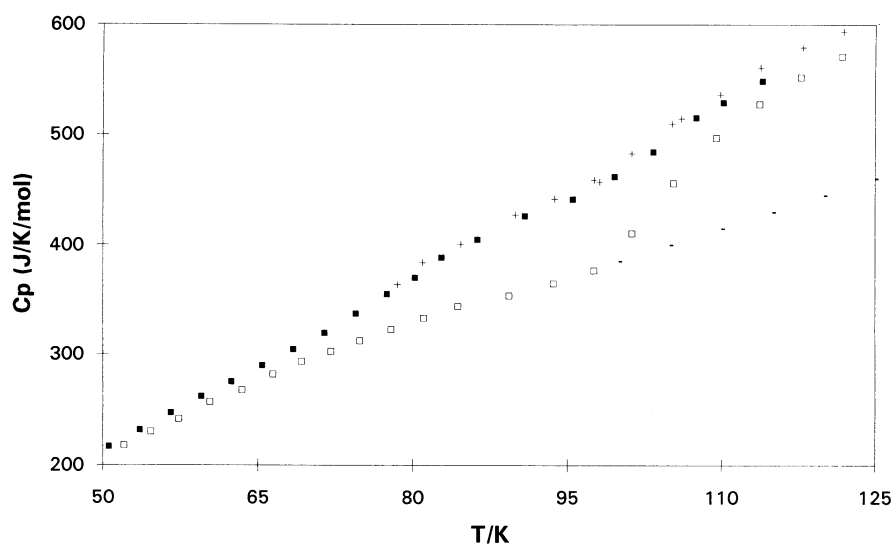*Figure 2.* Molar heat capacity of anhydrous β-cyclodextrin: +, series 1; □, series 2; ■, series 3.

In order to explain the observed heat capacity anomalies, we first compared our results with those of Hanabata *et al.* [10] concerning crystalline β-cyclodextrin undecahydrate. The thermodynamic behaviour of this compound exhibits a first order transition at 226 K and a glass transition phenomenon around 150 K. Cor-
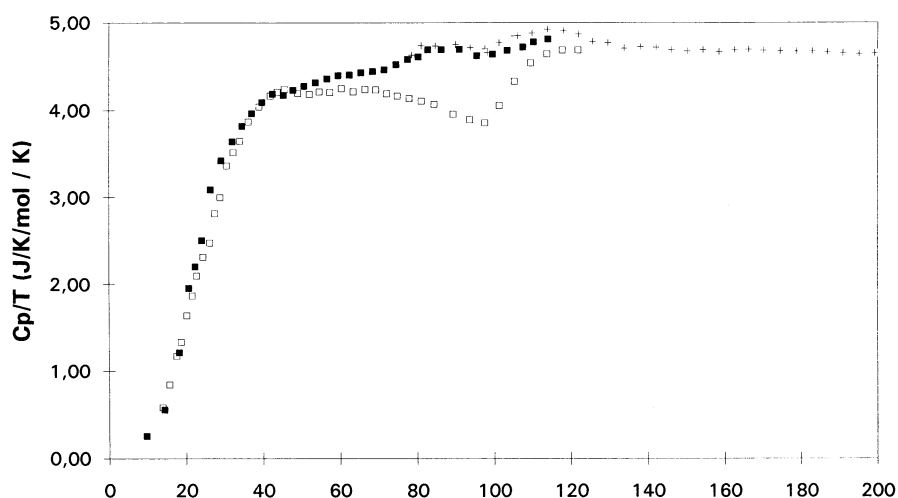
Figure 3. C_p/T vs T around the anomaly transition temperature. +, series 1; □, series 2; ■, series 3.

relating this finding with the diffraction data proposed by Lindner [2], Betzel [4], Zabel [5] and with DSC measurements done by Fujiwara *et al.* [11]. The two heat capacity anomalies are explained in terms of order–disorder phenomena involving both hydroxyl groups of the macromolecule and some of the hydration water molecules.

In the case of anhydrous β-cyclodextrin, the absence of a transition at 226 K confirms Hanabata's interpretation about the role of hydration water molecules in this first order transition. We assume that the relaxation phenomena observed on the upper curve between 70 K and 300 K are comparable to those noted by Hanabata around 150 K. Our interpretation of the lower heat capacity curve (Figure 2) is in direct correlation with some particular results of Lindner [2], Betzel [4] and Steiner [7, 9]. The X-ray [2] and neutron diffraction studies [4] on the dodeca-[2] and the undecahydrate [4] of β-CD led the authors to the conclusion that the conformation of the macrocycle is stabilised through a ring of hydrogen bonds between secondary hydroxyl groups O(2)—O(3) with all O(2)—O(3) distances lying in the normally accepted range of 2.7–3.0 Å. Betzel *et al.* [4] found that all these seven intramolecular interglucose O(2)—O(3) hydrogen bonds are of the flip-flop type. Steiner *et al.* [9] studied β-cyclodextrin hydrates with water contents in the range 12.3 H_2O to about 9.4 H_2O by X-ray diffraction. They concluded that the conformation of the β-CD molecule is not affected by dehydration. Steiner *et al.* [7] observed that a β-CD·10.5H_2O crystal became rugged due to dehydration in a silica gel dessicator and that X-ray diffraction on the resulting material indicated extensive but not complete disintegration of the crystalline structure. Since there is no further structural information about anhydrous β-CD, we hypothesise that even in the anhydrous sample, the intramolecular interglucose O(2)—O(3) flip-flop
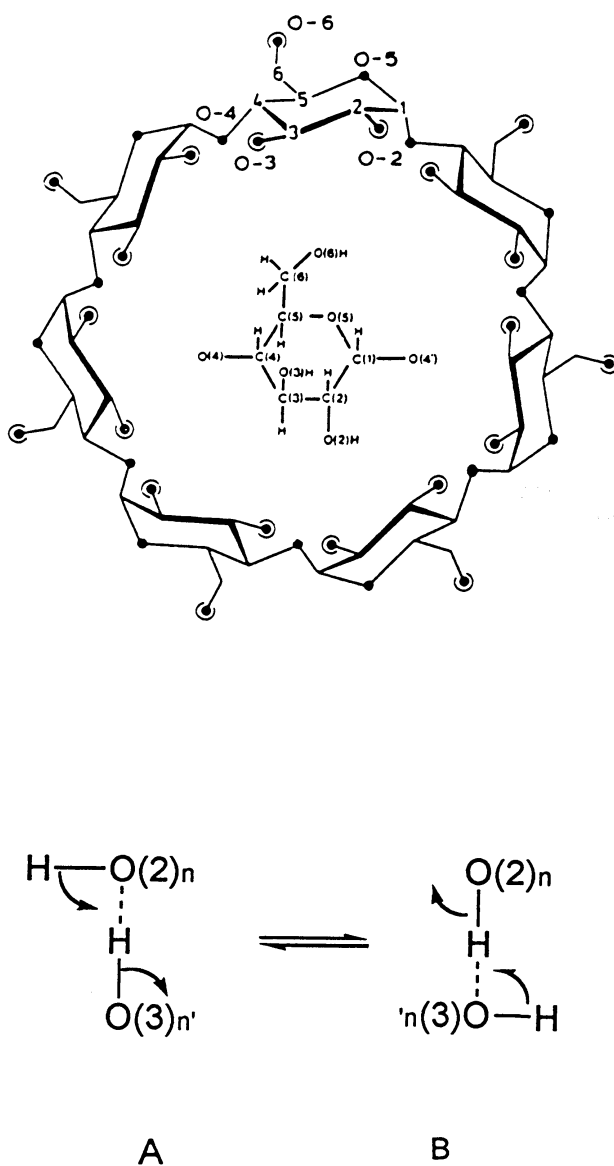
Figure 4. Chemical structure and numbering of the atoms in β-cyclodextrin (adapted from Ref. [2]). O(2)H$\cdots$O(3)H interglucose flip-flop hydrogen bonding: A or B equivalent positions (n and n' correspond to adjacent glucose units).

phenomenon still exists and that the 14 secondary hydroxyl groups concerned in the intramolecular flip-flop phenomenon are not engaged in any intermolecular bonds with a neighbouring β-CD molecule. In this flip-flop phenomenon, two alternative positions A and B exist (Figure 4), with an occupation frequency f_i

of about 0.5 at room temperature. We assume that a slow and elaborate cooling process (series 2) allows the O(2)$\cdots$O(3) hydroxyl groups to reorient and adopt fixed and independent positions A or B (Figure 4).

Under these conditions, the 14 secondary hydroxyl groups become perfectly ordered at very low temperature so that the flip-flop hydrogen interactions disappear. By heating to 130 K the disorder grows and flip-flop interactions can take place. Because the two states of flip-flop bonds are energetically equivalent, they should be favoured entropically with respect to individual O—H$\cdots$O hydrogen bonds. Disorder can be correlated with the entropy of the system through the Boltzmann relation ($R \sum f_i \ln f_i$). The two possible ways of arranging the protons in a completely random manner for the seven flip-flops lead to an entropy disorder $7R \ln 2 = 40.3$ J K^{-1} mol^{-1}. This should be considered as a limit value corresponding to the releasing of ideally well ordered hydroxyl groups without any other interaction, neither intra- nor intermolecular.

The experimental value (38 ± 3 J K^{-1} mol^{-1}) obtained from the upper and lower curves of C_p/T versus T (Figure 3) is in good agreement with the theoretical value. Comparing the two values, we are inclined to believe that the low temperature phase for series 2 was almost completely ordered whereas the excessively rapid cooling process of series 1 and 3 have probably frozen the room temperature disorder. It would be interesting to complement the calorimetric study of anhydrous β-cyclodextrin at very low temperature with X-ray diffraction and spectroscopic studies.

The model of intramolecular flip-flop type interactions developed for β-cyclodextrin hydrates seems to fit again in the case of anhydrous β-CD. The fact that the measured entropy is in agreement with the calculated entropy associated with the flip-flop phenomenon suggests that this motion is mainly due to the rocking of O(2)$\cdots$O(3) hydroxyl groups. These intramolecular interglucose interactions in solid β-cyclodextrin are suspected to limit the possibilities of hydrogen bond interactions with the bulk water and consequently the solubility of β-cyclodextrin in water.

In this sense, our current thermodynamic study of anhydrous γ-cyclodextrin will provide more information and enable a better understanding of the behaviour of cyclodextrins towards water.

References

1. M.L. Bender and M. Komiyama: *Cyclodextrin Chemistry*, Springer-Verlag, Heidelberg (1978).
2. K. Lindner and W. Saenger: *Carbohydr. Res.* **99**, 103 (1982).
3. W. Saenger, C. Betzel, B.E. Hingerty, and G.M. Brown: *Nature* (London) **296**, 581 (1982).
4. C. Betzel, W. Saenger, B.E. Hingerty, and G.M. Brown: *J. Am. Chem. Soc.* **106**, 7545 (1984).
5. V. Zabel, W. Saenger, and S.A. Mason: *J. Am. Chem. Soc.* **108**, 3664 (1986).
6. T. Steiner and W. Saenger: *J Am. Chem. Soc.* **114**, 10146 (1992).
7. T. Steiner, G. Koellner, S. Ali, D. Zakim, and W. Saenger: *Biochem. Biophys. Res. Commun.* **188**, 1060 (1992)
8. T. Steiner and W. Saenger: *Acta Crystallogr.* **B50**, 348 (1994).

9. T. Steiner and G. Koellner: *J. Am. Chem. Soc.* **116**, 5122 (1994).
10. H. Hanabata, T. Matsuo, and H. Suga: *J. Incl. Phenom.* **5**, 325 (1987).
11. T. Fujiwara, M. Yamazaki, Y. Tomizu, R. Tokuoka, K. Tomita, T. Matsuo, H. Suga, and W. Saenger: *Nippon Kagaku Kaishi* **2**, 181 (1983).
12. J. Bousquet, M. Diot, and M. Prost: *J. Chem. Phys.* **6**, 1004 (1972).