Analytical estimation of water contents, specific heat capacity and thermal profiles associated with enzymatic model compound β-cyclodextrin

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Studies of water contents of solid β-cyclodextrin (β-CD) in crystallized and vacuum dried conditions are reported. Water contents were estimated using Karl–Fischer (KF) titration and thermo-gravimetric analysis (TGA) techniques. It was found that the water contents for crystallized and vacuum-dried samples were 6.93 and 0.86 moles of water per β-CD molecule respectively. TGA studies gave values of 6.83 and 0.52 moles of water per β-CD molecule respectively. The results agree with those reported from X-ray diffraction studies. Also, the molecular weight of the vacuum dried sample was determined using vapour pressure osmometry, which agrees well with the actual molecular weight of β-CD. The thermal profiles of α-cyclodextrin (α-CD) and β-CD are presented. The differential scanning calorimetry data was used to calculate specific heat at constant pressure (C_p) at different temperatures (338.15–468.15 K). These results are compared with similar data for α-CD and discussed in terms of motional contribution to C_p values and related conformational effects.

Keywords: Cyclodextrins, differential scanning calorimetry, specific heat capacity, thermo-gravimetric analysis.

SUPRAMOLECULAR chemistry, broadly the chemistry of multi-component molecular assemblies in which the component structural units are typically held together by a variety of water (non-covalent) interactions, has developed rapidly and is finding application in many fields[1]. It is being studied and applied to understand complementarily, recognition, self-assembly, pre-organization and self-replication phenomena frequently occurring in the field of molecular biology. In our previous studies, we discussed the measurements of thermodynamic properties (such as osmotic coefficient, activity coefficient, free energy changes and volume change due to complexation) of aqueous solutions of supramolecules like crown ethers, cyclodextrins and 2,2,2-cryptand in the presences of salts or neutral molecules[2–10]. Such studies enabled us to understand the nature of forces operating through hydro-

phobic interaction and complexation in the case of host–guest binding equilibria[2–10].

Cycloamylases have hydrophobic cavities that can form inclusion complexes with various kinds of guest molecules, i.e. cyclodextrins can recognize guest molecules. In such host–guest complex formation of molecular recognition, the process is controlled by the structure of the guest molecule (e.g. hydrophobicity, functional group, charge effect, etc.) and the cavity diameter of the host as well[6,8]. As amylase, the glucose units in cyclodextrins are linked by α-(1–4) bonds and adopt the $^4C_1$ chair conformation, they are considered as fairly rigid building blocks[4–8].

The peculiarity of cyclodextrins is that they have specific distribution of hydrophilic and hydrophobic groups. As hydrophilic hydroxyl groups occupy both rims of the cone, they render the cyclodextrins soluble in water. The core of the cavity, however, is hydrophobic in character as it is covered by C_1–H, C_5–H and C_6–H hydrogen, and by the ether like oxygen O_4 (refs 11–14). Consequently, the cavities provide a hydrophobic matrix in aqueous solution. There is a difference in the amount of strain in the rings of α-, β- and γ-cyclodextrins due to orientation and degree of hydrogen bonding between the hydroxyl groups on the C_1 and C_3 atoms of adjacent glucose molecules that differ in each type of cyclodextrin. We have depicted the structure of β-cyclodextrin (β-CD) prepared by Linder and Saenger[12] in their study based on X-ray diffraction data in Figure 1. The hydroxyl groups on the C_2 and C_3 atoms of adjacent glucose units of β-CD (Figure 1) are oriented such that they interact very strongly with each other. As a result, they do not interact with bulk solvent molecules in order to solvate the β-CD molecule and the solubility decreases considerably when
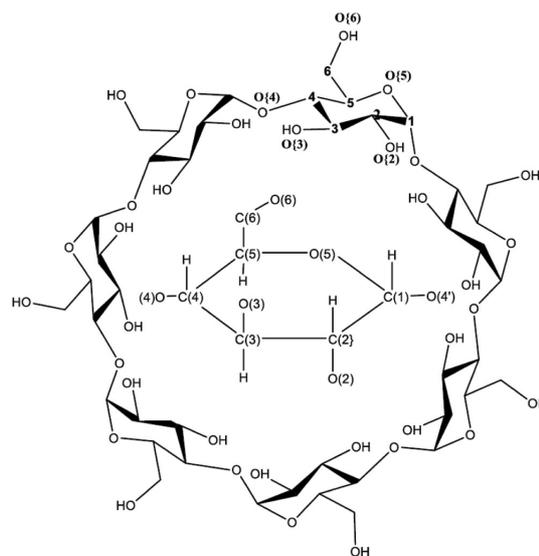
Figure 1. Chemical structure and numbering of the atoms for β-CD. The details about specific configurations of the atoms constituting the glucose ring and the glucosidic linkage is shown at the centre of the figure for clarity[12].

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compared to the other two forms[15]. However, it is reported that solid β-CD contains appreciable amount of water. Therefore, in order to study the structural aspects and interactions of β-CD, one is concerned with the exact composition or molecular weight of commercially available samples. We report here, the estimation of water content for commercial and dried samples using Karl–Fischer (KF) titration and thermo-gravimetric analysis (TGA) techniques. The results are supplemented by molecular weight determination using vapour pressure osmometry.

Heat capacity at constant pressure (C_p) is one of the basic thermodynamic properties specifying the state of important molecules like diamond, fullerene, metals and proteins. By definition, C_p is equal to the amount of heat required to raise the temperature of a unit mass of a substance by one degree Celsius. C_p is an intensive characteristic of the material constituting a body because it does not depend on the size. As in the case of protein research, protein denaturation is accompanied by changes in the heat capacity – the heat capacity of the unfolded state is always higher than that of the native state[16].

Measurement of heat capacities of liquids is complicated by chemical equilibria, vapour–liquid equilibria, and thermal expansion of the liquid. The type of calorimeter used determines the significance of each of these complications. There are several different factors that can contribute to the measured heat capacity; it is known that expansivity, compressibility and specific heat properties are made up of two parts, one is the geometrical part and the other is the structural part. We can write for total C_p as

$$C_{p\text{ total}} = C_{p\text{ geometrical}} + C_{p\text{ structural (relaxitional)}}.$$

The utility of C_p in terms of geometrical and relaxational components was discussed by Jolicoeur et al.[17] for 18-crown-6-K^+ complexes in aqueous solutions. Here we report our results on the calculations of C_p values for β-CD at different temperatures using heat flow measurements. The C_p values are compared with α-CD values reported by Briggner and Wadsö[18] as well as with our own data for α-cyclodextrin (α-CD) reported in this communication.

To characterize the composition of β-CD hydrate, the β-CD samples were tested without drying so as to quantitatively get information on the number of water molecules per β-CD molecules, which was named as 'crystallized sample'. β-CD (98% pure) procured from Merck–Schushardt was used without further purification. The sample was dried at 100°C under vacuum for about 24 h and labelled as 'vacuum dried sample'. KF titration and TGA techniques were used for determination of water contents in both samples.

To determine water content in β-CD, methanol (99.9%, purity) procured from Merck–Schushardt was used for extraction of water. Pyridine-free KF reagent (AR grade, S.D. Fine-Chem Ltd.) was used for determining the water content. The KF reagent was standardized with solutions of water in methanol for which doubly quartz distilled deionized water was used. The water content in the sample of β-CD was determined using microprocessor controlled automatic KF Titrator (mode: TKF-55, chemito from Toshnival Company). The detection limit of the instrument was ± 2 μg ml^{-1}. Also the water content in solid β-CD samples (crystallized and vacuum dried) was calculated using TGA data obtained with Universal V25H TA instrument.

The molecular weight of the vacuum dried sample of β-CD was determined using a colligative property, i.e. osmotic pressure for which the KNAUER K-7000 vapour pressure osmometer (K-7000 VPO) was used. The instrument was calibrated with aqueous NaCl solutions of known osmomolalities. The details of calibration and working of the instrument are described elsewhere[4,7].

The TGA-differential scanning calorimetry (DSC) analysis of α-CD and β-CD was performed on TA instrument (model: SDT Q 600 V 20.9), which was kept in an air conditioned lab having a humidity of 5–10% with sample temperature accuracy ± 0.5 K under N_2 atmosphere.

Sample weights in the range 4–10 mg were used for measurements. The sample volumes of the alumina pans were 0.01 ml with a cell volume of 3.4 ml and were subjected to nitrogen purging with a flow rate of 50 ml min^{-1}. The heating rate of the sample was always maintained at 5 K min^{-1}. The instrument was calibrated for differential temperature using the empty pan, sapphire and fusion point of Zn, while heat flow in the range 313.15–1073.15 K was measured. The parameters, uncalibrated temperature difference and actual temperature, were measured with reference to the standard sample of sapphire (59.719 mg).

The α-CD and β-CD samples as well as the KCl standard were subjected to DSC analysis over a temperature range of 298.15–773.15 K. A systematic and efficient computing was adopted to calculate C_p for solids using the DSC-SDT-Q-600 instrument. Other details of calculations of sample deflections and the appropriate corrections are as described below: (a) Performance of thermistors was checked for a selected temperature range and the signal obtained in μV. (b) The calibrated weight analysis data performed for a selected temperature range and data obtained in $\mu V/mg$. (c) A measure of empty pan signal was studied and its contribution in μV at different temperatures obtained. (d) Sapphire was studied as a calibrant standard and its DSC profile obtained in a temperature range between 55°C and 400°C (328.15–673.15 K). The corresponding signal was obtained in $\mu V/mg$. (e) The correction for pan signal data was applied by appropriate corrections due to matching of thermistors. (f) The signal of weights was subtracted from the signal due to sapphire. The resulting signal was multiplied by weight of sapphire, finally converting the emf into volts (V). Then the pan signal in volts was subtracted

Table 1. Water content in β-CD by using Karl–Fischer titration technique

Crystallized sample		Vacuum dried sample	
Amount of β-CD (g)	Water content (g)	Amount of β-CD (g)	Water content (g)
0.0123	1.356×10^{-3}	0.0184	0.273×10^{-3}
0.0125	1.375×10^{-3}	0.0218	0.273×10^{-3}

Table 2. Water content in terms of number of moles of water per mole of β-CD by using KF titration and TGA techniques

Sample of β-CD	Karl–Fischer method	Thermal analysis
Crystallized sample	6.93	7.30
Vacuum dried sample	0.86	0.52
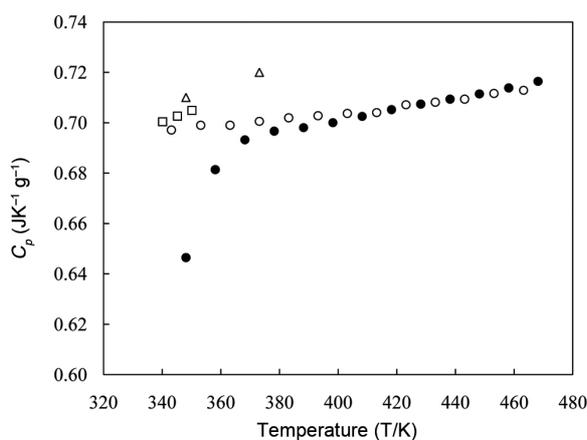
Figure 2. Comparison of specific heat at constant pressure (C_p) data for KCl (●, KCl Expt.) with literature (△, Burns and Verall[20]; □, Dortmund Data Bank (Skuratov et al.)[21]; ○, Dortmund Data Bank (Egorov et al.)[21]).

from sapphire signal in volts. (g) Zinc metal was used to assess the temperature difference, i.e. dT (°C) between the two pans. A DSC scan of zinc metal was obtained in a temperature range of 55–500°C (328.15–773.15 K). The zinc melts at 425°C (698.15 K). The area under the endothermic curve was calculated and further used to obtain heat of fusion of zinc metal as 107.1 J g^{-1}. The data is in excellent agreement with the literature data[19]. (h) From the C_p values for sapphire, and measured dT values at different temperatures, the enthalpy change for sapphire as a function of temperature was calculated in J s^{-1}. The data yielded the values of current (I) generated via the use of equation $\Delta H = I^2 Rt$. Using the average of current values, signal values for sapphire were converted into power (watt) values at the studied temperatures. From the measured current, the DSC signals for the pan and sapphire were appropriately converted into the power unit of watt. The appropriate amplitudes for the sapphire and pan were obtained and a pan correction for amplitude data of sapphire was applied. (i) The amplitude ratios for salt/sapphire (corrected) as well as the weight ratio (sapphire/salt) were calculated and further used to obtain C_p

as a function of temperature for the studied salts, using the equation

$$\frac{\text{Amplitude ratio} \times \text{Weight ratio}}{\text{Scanning rate}} = K \times C_p,$$

where K is the sensitivity in J^{-1} g s taken as unity (i.e. 1). The above methodology was applied to the data of KCl. The calculated values of specific heat capacity for KCl show excellent agreement above 65°C (338.15 K) up to 345°C (618.15 K). We observed that sapphire as a standard for specific heat capacity measurements yielded reliable results above 65°C (338.15 K), as there are structural transitions reported at about 40–50°C (313.15–323.15 K) for sapphire.

Based on this methodology, the specific heat values at different temperatures were calculated using eq. (2). The accuracy of the instrument was verified by determining the specific heat capacity of KCl. The specific heat capacity values as a function of temperature for KCl are plotted in Figure 2. The estimated accuracy of the measured values is ±2% over this temperature range, when the data were compared with the literature data[20,21].

The molecular weight of β-CD estimated using the vapour pressure osmometry was found to be 1122 g mol^{-1}, which is in good agreement with the actual molecular weight of 1135 g mol^{-1}. The estimated amounts of water in β-CD using KF titration technique are presented in Table 1, and the thermograms for α-CD and β-CD are shown in Figure 3 a and b. The results show that the water content in β-CD is 10.86% and 0.827% for crystallized and vacuum dried samples respectively. The water content in solid β-CD samples estimated using KF titration and TGA techniques in terms of number of moles of water per mole of β-CD are compiled in Table 2. The water contents estimated using both the techniques agree with each other. The water contents for α-CD using TGA data was found to be 10.93%, indicating that one molecule contains 5.9 water molecules.

The results obtained show that there are 7 ± 0.2 water molecules per molecule of β-CD. These results are consistent with X-ray analysis by Linder and Saenger[12] and Saenger[13]. It has been suggested that oxygen atoms of the glucose ring (the hydroxyl groups) may be exerting weak interactions with the dipole of water molecules. However, this is not completely true as the cavity is hydrophobic and the hydroxyl groups of the glucose ring can interact amongst themselves by forming intramolecular hydrogen
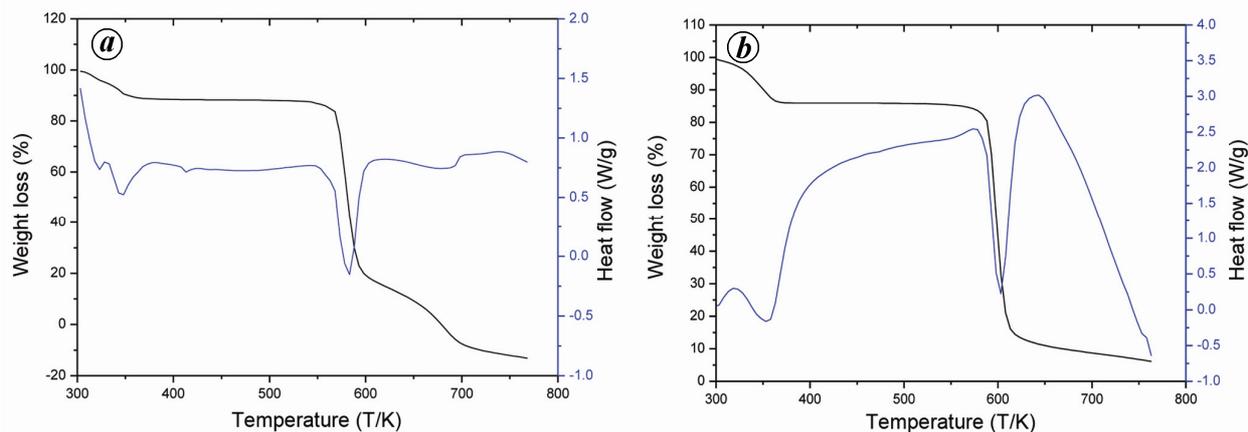
Figure 3. Thermograms of α-CD (***a***) and β-CD (***b***).
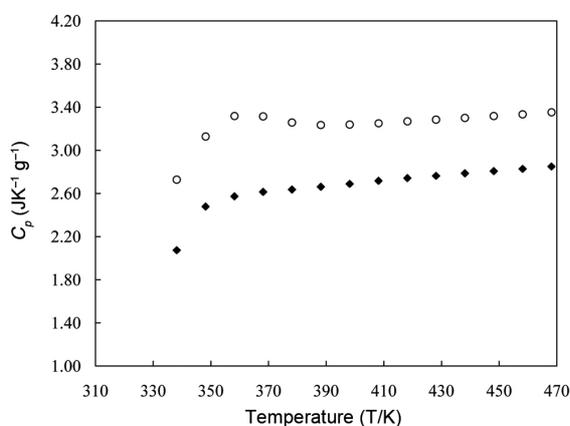
Figure 4. Specific heat at constant pressure (C_p) of α-CD and β-CD as a function of temperature.

Table 3. Specific heat at constant pressure (C_p) at different temperature (338.15–418.15 K) of α-CD and β-CD

Temperature		α-CD	β-CD
°C	T/K	JK^{-1} g^{-1}	JK^{-1} g^{-1}
65	338.15	2.07	2.73
75	348.15	2.48	3.13
85	358.15	2.57	3.32
95	368.15	2.61	3.31
105	378.15	2.64	3.26
115	388.15	2.66	3.24
125	398.15	2.69	3.24
135	408.15	2.72	3.25
145	418.15	2.74	3.27
155	428.15	2.76	3.28
165	438.15	2.79	3.30
175	448.15	2.81	3.32
185	458.15	2.83	3.33
195	468.15	2.85	3.35

bonds as mentioned earlier. We feel that as there are seven glucose units in β-CD, each glucose unit is bonded with other glucose units by glycosidic linkages and these can exert a sort of affinity for water molecules through dipole–dipole interactions. Therefore, a stoichiometric composition of β-CD·H$_2$O complex of 1 : 7 is expected which is in excellent agreement with the present experimental results. One can suggest an alternative explanation that a cluster of seven water molecules (H-bonded among themselves) resides in the cavities of β-CD, stabilized by van-der Waals forces.

Examining the TGA profile (Figure 3), we note that there is a 13.66% loss, meaning that 7.3 water molecules are lost at 365.55 K, after which the weight remains constant up to 549.15 K and later decomposition was observed. In heat flow measurements, we observe an endothermic peak at 354.42 K, and this can be attributed to the energy requirements for removal of water molecules. Beyond 550.14 K, another peak due to decomposition products occurs and may be attributed to fragmented residues at about 602.60 K and subsequent changes noted in heat flow might be due to interactions amongst the fragments.

The C_p data for α-CD and β-CD (Table 3) as a function of temperature are depicted in Figure 4. An examination of Figure 4 reveals that C_p values of β-CD hydrate increase slightly up to 373.15 K and thereafter remain more or less constant while for α-CD hydrate a slight increase in C_p values with rise in temperature was observed up to 373.15 K. The extrapolated C_p values at 298.15 K for both the hydrates are 1.18 and 1.28 JK^{-1} g^{-1} for α-CD and β-CD respectively, which are in good agreement with those reported in the literature (1.15 and 1.22 JK^{-1} g^{-1} obtained using automated drop heat capacity microcalorimeter)[18]. The relatively higher value of β-CD as compared to α-CD is expected as it contains one additional glucose unit. Also, one can visualize that the removal of water molecules from cavities of β-CD and β-CD molecules, causes rotational motion of the glucose moieties (rotation due to methylene proton) and hydroxyl group which is best seen as a conformation change imparting higher values for the specific heat capacity parameter. A similar interpretation for methylene group

rotation was advanced for neutron scattering results obtained for aqueous 18-crown-6 solutions[22]. Accordingly, the ring structure exists for 18-crown-6 only in water. It has also been demonstrated that in CCl$_4$ in case of 18-crown-6 the methylene groups are inside the cavity (nature abhors vacuum)[2,23]. Similar arguments can be advanced to explain the thermal profile of β-CD. On increasing temperature, when water molecules are removed, a conformational change in the form of rotation of one glucose unit inside the cavity of β-CD occurs. Such a conformation change makes the vacuum-dried sample very active due to the strain involved. It is suggested that for precise measurements of thermodynamic properties, vacuum-dried samples should not be used for solutions involving β-CD, and its complexes. The commercial crystallized samples can be used after estimation of water in the sample using KF-titrimetry or TGA analysis. The vacuum-dried sample yields erratic results for many physico-chemical properties in aqueous solutions meaning a strong activation due to conformational change when one glucose molecule is rotated outward to make the cavity (6.5 Å) in which water molecules can be accommodated.

The water contents in the crystallized (commercial) and vacuum dried samples of β-CD were determined by KF-titrimetry and TGA measurements. It was observed that β-CD incorporates 7 ± 0.3 water molecules. For the first time C_p measurements were made for β-CD at different temperatures and compared with α-CD which contains 6-glucose moieties. It is observed that C_p values increase initially up to 100–120°C (373.15–393.15 K). due to removal of H$_2$O molecules and thereafter remain more or less constant. These results are important as β-CD and its complexes are used as energy storage material. It is further suggested that vacuum-dried samples should not be used for measurements of thermodynamic properties involving β-CD complex equilibria as the β-CD cavity is occupied by glucose methylene groups as a result of the rotational motion around the glucosidic bonds.

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