

006259

CARBOHYDRATE RESEARCH

Polymerized cyclomaltoheptaose (β -cyclodextrin,
 β -CD_n) inclusion complex formation with
chlorogenic acid: solvent effects on
thermochemistry and enthalpy–entropy
compensation

Polymerized cyclomaltoheptaose (β -cyclodextrin,
 β -CD_n) inclusion complex formation with
chlorogenic acid: solvent effects on
thermochemistry and enthalpy–entropy
compensation

Abstract

The inclusion of chlorogenic acid (CA) by epichlorohydrin-polymerized cyclomaltoheptaose (β -cyclodextrin, β -CD_n) was studied with regard to temperature, solvent, and water activity ($a_{\text{H}_2\text{O}} \sim$ mole fraction $= X_{\text{H}_2\text{O}} = 0.8-1$ using MeOH as the diluent; 0.1 M sodium phosphate buffer). We discovered that the extreme convex curvature in K (the apparent stability constant) as a function of temperature was nearly eliminated at the lowest $X_{\text{H}_2\text{O}}$. The latter finding argues that this unusual CD behavior in aqueous media was due to perturbations in β -CD's spatial organization in the polymeric matrix with temperature. Related to this we found, from the dependence of K on $X_{\text{H}_2\text{O}}$ ($K = K' X_{\text{H}_2\text{O}}^z$), that the β -CD_n · CA complex's stoichiometric coefficient, z , for water, varied between 5 and 8, depending on the temperature of the solution ($K' = 400-800 \text{ M}^{-1}$; $T \sim 295-315 \text{ K}$). Our determinations of z were similar to those reported previously for β -CD · (+)-limonene ($z \sim 7$), soluble β -CD · CA ($z \sim 6$) or obtained by molecular dynamics calculations for β -CD · CA reported herein ($z \sim 5$). However, β -CD_n · CA's z values did show a significant positive correlation with temperature not evident in equivalent solution experiments. Calculations of ΔH and ΔS at various $X_{\text{H}_2\text{O}}$ values show linear enthalpy–entropy compensation (ΔH plotted against ΔS) but with a slope ($T_c = \partial\Delta H/\partial\Delta S \sim 228 \text{ K}$) significantly less than T_c values determined from either standard aqueous thermodynamic experiments ($T_c \sim 305$

K on either β -CD or β -CD_{*n*} or variable $X_{\text{H}_2\text{O}}$ ($T_c \sim 272$ K) experiments. To the best of our knowledge, this is the smallest T_c value detected in a multitude of CD-guest studies. This evident solvent effect on T_c strongly argues that the chemical part process of inclusion complex formation involves changes in the solvation of the β -CD_{*n*}'s binding site.

Keywords: Cyclomaltoheptaose; β -Cyclodextrin; Chlorogenic acid; Thermodynamics; Binding; Water activity; Molecular modelling; Enthalpy-entropy compensation; Compensation temperature

1. Introduction

In most plant-derived food products, such as juices, enzymatic browning results from the oxidation of substrates (e.g., chlorogenic acid, CA) and their subsequent condensation to form undesirable pigments. Polyphenol oxidase (PPO) is the major component in an enzyme-induced cascade brought on by the exposure of plant cellular contents to O₂. The control of enzymatic browning in fresh plant products presents a dilemma for the food processing industry since the utilization of sulfites, the most effective inhibitors of browning, has been severely restricted by the Food and Drug Administration (FDA) [1]. Thus, the amelioration of enzymatic browning in minimally processed fruit and vegetable products offers a significant economic use for sulfite alternatives such as CDs [2]. Some of the most effective, and commercially feasible, types of CDs for this application are insoluble forms, such as epichlorohydrin-polymerized β -CD (β -CD_{*n*}) [3]. β -CD_{*n*} has a great commercial advantage over other CD forms since it can be removed from the treated juice, regenerated and reused numerous times. Also, because β -CD_{*n*} is removed from the final product, it is not a true 'food additive', and hence approval by the FDA may be simplified. Since it is desirable to regenerate the β -CD_{*n*} matrix, one of the limiting factors for its utilization is a lack of knowledge concerning the dissociation of PPO substrates as influenced by such factors as differing solvents and water activity. In previous work the behavior of various cyclomalto-oligosaccharide (cyclodextrins or CDs)-chlorogenic acid (CA) inclusion complexes were characterized with respect to binding geometry, stoichiometry (1:1), and thermodynamics (variable pH, buffer, and ionic strength) [4]. The CD inhibition of enzymatic browning in apple juice was also shown to be directly correlated with the β -CD-CA's stability, or equilibrium, constant (K). Our present work mainly focuses on the thermodynamic characterization of the β -CD_{*n*}-CA inclusion complex as influenced by temperature and solvent in a model buffer system (0.1 M sodium phosphate). Thermodynamics is of practical value because it deals with the free energy ($-RT \log_e K$) of binding, a measure of the extent to which CDs can effectively remove some substrate from the solution. If one knows the free energies in the system, one can predict whether a reaction will proceed spontaneously. Pragmatically, knowing K under various circumstances, one can predict the extent of the reaction. Thus, chemical thermodynamics provides an exact theoretical environment for predicting the probability that certain chemical reactions, such as CD binding, will occur.

Manifold mechanisms have been proposed as the driving force for CD inclusion complex formation [5-7] with various guest molecules. Most of these are related to the effect of the solvent, usually H₂O, on the complex [5,8]. In our previous work [8], the

complexation reaction of soluble β -CD with CA was studied by characterizing the dependence of K on water activity, $a_{\text{H}_2\text{O}}$ (assuming $a_{\text{H}_2\text{O}} \sim$ the mole fraction of water, $X_{\text{H}_2\text{O}}$), using simple diluents such as methanol (MeOH) or formamide. These experiments were performed to help determine the localization of that hypothetical [9] species of H₂O which is believed to be uniquely associated with, and which may contribute to, the stability [10,11] of various CD inclusion complexes. Data [8] were presented which argued that water plays a major structural role in stabilizing the soluble β -CD-CA inclusion complex. From the dependence of K (assuming $K = K' X_{\text{H}_2\text{O}}^z$) on $X_{\text{H}_2\text{O}}$, it was estimated that the β -CD-CA complex's stoichiometric coefficient, z , for water was ca. 7 [8], which agrees with what has been found for a similar parameter in the (+)-limonene- β -CD [12,13] system. It was further argued that this bound water was associated with the CD's hydroxyl groups since a 50% hydroxyethyl substitution resulted in an equivalent loss in z . This hypothesis [8] was supported by ¹H NMR spin-lattice relaxation data. Clearly, from these [4,8] and other [5-7,10-14] studies, water plays a significant role in CD inclusion complex stability. Since $a_{\text{H}_2\text{O}}$ modulates K in the β -CD system, we were interested in determining the relationship between solvent and β -CD_{*n*}-CA complexation for the reasons outlined above. Therefore, in this work we have extended previous studies [8] on solvent effects on β -CD-CA inclusion complex formation to incorporate the potentially important polymerized form.

2. Experimental

General methods.— β -CD_{*n*} (40-60 mesh) was donated by the American Maize Products Company (1100 Indianapolis Blvd., Hammond, IN 46320-1094, USA). CA [3-*O*-(3,4-dihydroxycinnamoyl)-1,3,4,5-tetrahydroxycyclohexanecarboxylic acid] was purchased from Sigma Chemical Company. The MeOH utilized in these studies was HPLC grade. All H₂O used in these experiments was processed from deionized-distilled H₂O to have a low conductivity (ca. 10⁻⁷ Ω⁻¹).

Several experiments were performed on buffer-washed and -equilibrated β -CD_{*n*}, using MeOH ($X_{\text{H}_2\text{O}} = 0.8$ and 1) as the diluent [8], in order to determine the proportionality between hydrated and dry weight. For each binding experiment, described below, ca. 100 mL of β -CD_{*n*} matrix was washed with 1 L of H₂O. Excess fluid was removed by filtering the slurry through an acid-washed, coarse, sintered glass-filter apparatus. The matrix was then washed with ca. 300 mL of the buffer solution ($X_{\text{H}_2\text{O}} = 0.8, 0.85, 0.9, 0.95, 0.99$, or 1) and refiltered for gravimetric determination of each β -CD_{*n*} level. These samples were then loaded into 5 mL gas-tight Reactivials. Once the buffer solutions containing CA were added, the vials were tightly capped, shaken, and submerged in a refrigerated water bath to maintain the temperature of the various slurries ± 1 °C; samples were shaken several times throughout the equilibration period (ca. 1 h).

UV studies were performed on a Perkin-Elmer 552 dual-beam spectrophotometer. For the binding studies, CA's initial concentration, [CA]_{*n*}, was fixed at 8 × 10⁻⁵ M; [β -CD_{*n*}]_{*n*} was either 0 (control), 0.33, 0.67, 1, 2, 4, 8, 12, 16, 20, or 24 mM in a 0.1 M sodium phosphate buffer (pH ~ 6.7); [β -CD_{*n*}]_{*n*} was calculated on a β -CD monomer

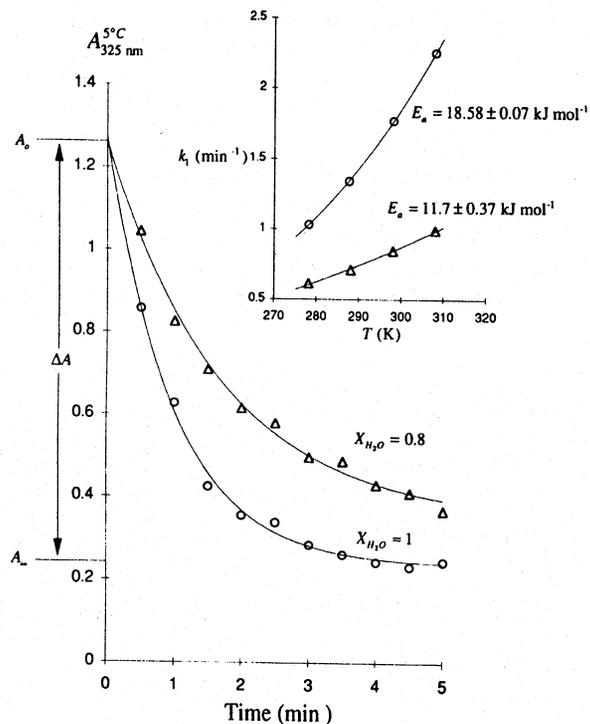


Fig. 1. Change in CA's absorbance or optical density at 325 nm ($A_{325\text{ nm}}$) as a function of time (min) and $X_{\text{H}_2\text{O}}$ (0.8 and 1; open triangles and circles, respectively) at 5 °C in the presence of 12.5 mM $\beta\text{-CD}_n$. Inset: temperature (T , K) dependence of the pseudo first-order rate constant (k_1) in media of differing $X_{\text{H}_2\text{O}}$ (0.8 and 1).

basis. ¹³C CPMAS NMR [4] spectroscopy (800 μs contact time) of $\beta\text{-CD}_n$ indicated that virtually all of the dry weight of the polymer was due to the CD moiety. All our calculations are based upon the assumption that 97% of the dry weight of the polymer was due to the monomer. The $\beta\text{-CD}_n$ -treated solutions were pipetted into the reference cuvette. The sample cell contained an identical solution but without exposure to $\beta\text{-CD}_n$ (i.e., the control). In these experiments UV spectral traces were identical to CA's normal UV spectrum but with A_λ varying proportionally to the sequestered species. Thus, our observed data (Fig. 1).

$$\Delta A = A_{325\text{ nm}}^{\text{control}} - A_{325\text{ nm}}^{\beta\text{-CD}_n}$$

reflect the reduction in absorbance due to the removal of CA from the bulk solution by $\beta\text{-CD}_n$.

Fig. 1 (12.5 mM $\beta\text{-CD}_n$, 8×10^{-5} M CA, $X_{\text{H}_2\text{O}} = 0.8$ or 1) also illustrates that the minimum time (5 °C) necessary to reach an apparent chemical equilibrium was ca. 5 min ($[\text{CA}]_{\text{free}}$ half-life in $X_{\text{H}_2\text{O}} \sim 1$ was ca. 40 ± 3 s). This rate of loss of CA from the

medium containing $\beta\text{-CD}_n$ was much slower than its soluble counterpart under similar circumstances [15]. Ordinarily, $\beta\text{-CD} \cdot \text{guest}$ equilibria are reached almost instantaneously, and, therefore, such a slow approach to an apparent equilibrium argues that the $\beta\text{-CD}_n \cdot \text{CA}$ binding process is mainly modulated by physical aspects such as porosity of the $\beta\text{-CD}_n$ beads and by the fact that the reaction is heterogeneous. Kinetic data were obtained by fitting $A_{325\text{ nm}}$ to the three-parameter equation

$$A_{\tau \rightarrow \infty} + \frac{A_0 - A_{\tau \rightarrow \infty}}{e^{\tau k_1}}$$

MeOH had a substantial effect on the disappearance of CA from the test solutions, whereupon both k_1 (the pseudo first-order rate constant) and activation energy (E_a) were affected. When $X_{\text{H}_2\text{O}} \sim 0.8$, the rate of CA disappearance from the bulk solution diminished ca. 41% (half-life ~ 68 s). From its temperature dependence this process had an E_a of ca. 12 kJ mol⁻¹ ($X_{\text{H}_2\text{O}} \sim 1$, $E_a = 19$ kJ mol⁻¹). Thus, the rate of binding of CA by $\beta\text{-CD}_n$, when $X_{\text{H}_2\text{O}} \sim 1$, was significantly more temperature-sensitive than when $X_{\text{H}_2\text{O}}$ was low and argues that this process was not modulated by CA diffusion since neither k_1 nor E_a would vary this much due to $X_{\text{H}_2\text{O}}$ alone.

Determination of K , ΔH and ΔS .—If CA binds to $\beta\text{-CD}_n$ with a 1:1 stoichiometry [4,8], then

$$K = \frac{[\beta\text{-CD}_n \cdot \text{CA}]}{[\beta\text{-CD}_n][\text{CA}]}$$

Expressing $[\text{CD}]$ and $[\text{CA}]$ in terms of initial concentrations, $[\text{CD}]_0$ and $[\text{CA}]_0$,

$$K = \frac{[\beta\text{-CD}_n \cdot \text{CA}]}{\{[\beta\text{-CD}_n]_0 - [\beta\text{-CD}_n \cdot \text{CA}]\}\{[\text{CA}]_0 - [\beta\text{-CD}_n \cdot \text{CA}]\}}$$

Since $[\text{CD} \cdot \text{CA}]$ is proportional to the ratio $\Delta A : \Delta A_{\text{max}}$,

$$K = \frac{[\text{CA}]_0 \Delta A / \Delta A_{\text{max}}}{\{[\beta\text{-CD}_n]_0 - [\text{CA}]_0 \Delta A / \Delta A_{\text{max}}\}\{[\text{CA}]_0 - [\text{CA}]_0 \Delta A / \Delta A_{\text{max}}\}}$$

Rearranging and solving for ΔA gives

$$\Delta A_{\text{max}} \left\{ 1 + [\beta\text{-CD}_n]_0 K + [\text{CA}]_0 K - (1 + 2[\beta\text{-CD}_n]_0 K + 2[\text{CA}]_0 K + [\beta\text{-CD}_n]_0^2 K^2 + [\text{CA}]_0^2 K^2 - 2[\beta\text{-CD}_n]_0 [\text{CA}]_0 K^2)^{1/2} \right\} / 2[\text{CA}]_0 K$$

The parameters ΔA_{max} and K were evaluated by fitting the experimental values of ΔA to the above equation utilizing a modified Gauss-Newton algorithm [16-18]. ΔA_{max} is the $[\beta\text{-CD}_n]_{\tau \rightarrow \infty}$ asymptote and is approximately equivalent to $A_{325\text{ nm}}$ for CA alone (i.e., for $X_{\text{H}_2\text{O}} \sim 1$: $A_{325\text{ nm}} = 1.48 \pm 0.02$ and $\Delta A_{\text{max}} = 1.4 \pm 0.06$). In fact, if the binding stoichiometry for $\beta\text{-CD}_n$ were substantially different than 1:1, ΔA_{max} would diverge from CA's optical density at this λ . All binding experiments were replicated thrice at each temperature, and the associated parameters (K , ΔA_{max} and z) are reported \pm the standard deviation of the mean (s_τ).

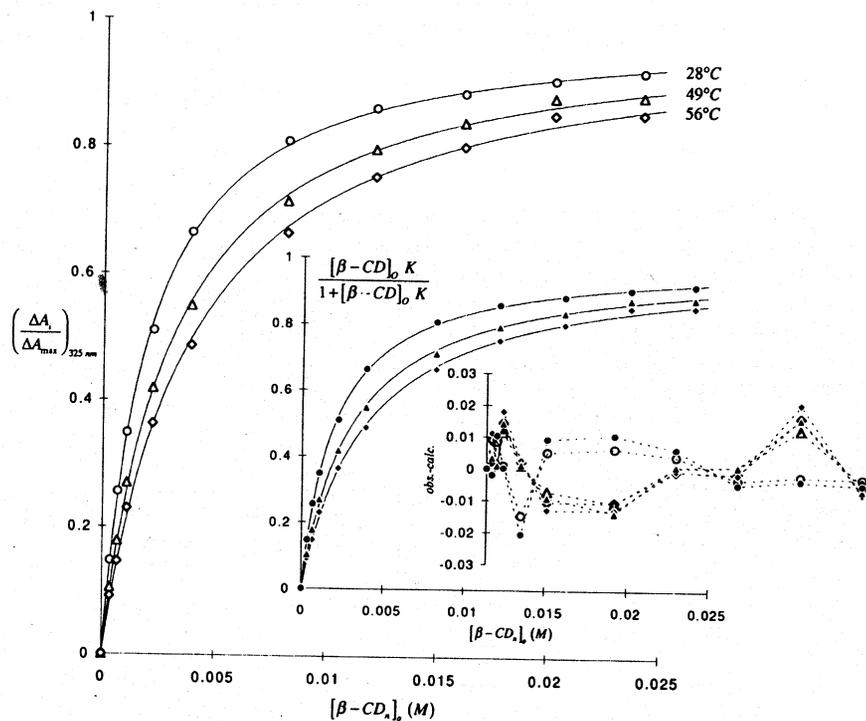


Fig. 2. Dependency of the normalized change in CA absorbance ($\Delta A/\Delta A_{\max}$) on β -CD_n concentration ($[\beta\text{-CD}_n]_0$, M) at three temperatures ($t = 28, 49$ and 56 °C). Normalization was performed to more clearly demonstrate the effect of t on the apparent stability constant ($K \sim 504, 326, 256 \text{ M}^{-1}$ at $28, 49$ and 56 °C, respectively) based on the curvature of the function alone. The parameter $\Delta A/\Delta A_{\max}$ is equivalent to the fraction of β -CD_n sites filled with CA. Inset: plot of $\Delta A/\Delta A_{\max}$ fit to the Langmuir adsorption isotherm ($K \sim 491, 320, 252 \text{ M}^{-1}$ at $28, 49$ and 56 °C, respectively). A third inset figure is provided (observed—calculated at each $[\beta\text{-CD}_n]_0$) demonstrating the deviation of the observed relative to the nonlinear regression fits for both binding equations (see Experimental section).

Typical fits to the above expression for ΔA data obtained at three temperatures are provided in Fig. 2 (data normalized to ΔA_{\max}). β -CD_n is a polymeric solid, and, therefore, our data should also fit well to an expression frequently used under such circumstances (Fig. 2, inset), the Langmuir adsorption isotherm, which results in virtually identical K values. The Langmuir adsorption isotherm,

$$\frac{\Delta A}{\Delta A_{\max}} = \frac{K[\beta\text{-CD}_n]_0}{1 + K[\beta\text{-CD}_n]_0},$$

is based on the assumption that every binding site is equivalent in the polymeric matrix and that the occupation of one site is unaffected by the occupation at another. This expression is ordinarily utilized for measuring the fractional coverage of an adsorbed substance, usually a gas, onto a surface. The deviation of the observed from the

calculated for both binding expressions is also presented in Fig. 2. Clearly, the fits for these two expressions are nearly identical and argues that our method [4,8] of obtaining K is reasonable. Based upon all these observations, we conclude that the binding of CA to β -CD_n is 1:1 (from ΔA_{\max} data) and that the binding sites are mutually independent. However, our observed K values only represent an ensemble average. For instance, if a matrix had two independent binding sites in equal proportion with K values of 200 and 700 M^{-1} , the fitting of such data to one of the above expressions would provide an average K of ca. 450 M^{-1} , assuming that the maximum $[\beta\text{-CD}_n]_0$ was saturating. There is little doubt that β -CD_n is heterogeneous with respect to the monomeric β -CD linkages. We also know that the substitution of hydroxyl groups does have an effect on K [8]. Because of this there are probably many different, but independent, site stability constants (K_i), and our data represent but an average such that

$$K = \frac{\sum_{i=1}^N K_i}{N}.$$

Standard enthalpy and entropy changes due to complexation (ΔH and ΔS , respectively) were calculated from the dependence of K on temperature (T , K) using nonlinear regression analysis [18]. Using this technique, we obtained ΔH and ΔS by fitting the observed values of K to the function

$$K = e^{-\Delta H/RT} e^{\Delta S/R}.$$

The advantages of utilizing such a direct method over linear regression of mathematically transformed data have been discussed in detail previously [8,18]. All ΔH and ΔS values are reported \pm the asymptotic standard error (ϵ).

Molecular modeling.—A molecular modeling study of the $\beta\text{-CD} \cdot \text{CA} \cdot z\text{H}_2\text{O}$ complex was conducted on a Silicon Graphics (Mountain View, CA) 4D/35 workstation using the SYBYL (version 6.03, Tripos Associates, St. Louis, MO) software package. The system was prepared by docking a CA molecule (undissociated) within the cavity of β -CD with the aromatic ring in contact with the hydrophobic inner surface of β -CD. Fourteen water molecules were placed near the hydroxyl groups on the outer surface of β -CD. Seven waters were positioned so as to form hydrogen-bond bridges of the type $\text{O-3} \cdots \text{HOH} \cdots \text{O-2}$, where O-2 and O-3 are the oxygen atoms of the hydroxyl groups attached to carbon atoms C-2 and C-3, respectively, of the pyranose ring; here O-2 and O-3 are not in the same ring but in adjacent glucopyranose rings. The remaining seven water molecules were placed so as to form hydrogen-bond bridges of the type $\text{O-5} \cdots \text{HOH} \cdots \text{O-6}$, where O-5 is the hemiacetal oxygen atom and O-6 is associated with the C-6 hydroxyl group; in this case O-5 and O-6 were in the same glucopyranose ring.

The inclusion complex + $14\text{H}_2\text{O}$ system was then energy-minimized. The force-field used in this and subsequent calculations was as follows: the parameters of King and Warshel [19] were used for H_2O ; the atomic charges of Homans [20] were used for β -CD; the atomic charges of CA were assigned with the Gasteiger and Huckel [21] method. All other parameters were those of the Tripos force-field [22] except that the van der Waals radii of sp^3 oxygen atoms were increased from 1.52 to 1.65 \AA . The minimized inclusion complex + $14\text{H}_2\text{O}$ system was next surrounded by two layers of

additional water molecules. At this point the system contained a total of 182 water molecules. A molecular dynamics (MD) simulation was carried out on this system. The total MD trajectory was of 30 ps in length. In the first 10 ps the temperature of the system was gradually increased from 0 to 300 K. For the final 20 ps the temperature was held at 300 K. No restraints were placed on the positions of the water molecules relative to the inclusion complex. As a result, about half of the water molecules drifted away from the inclusion complex over the course of the MD simulation.

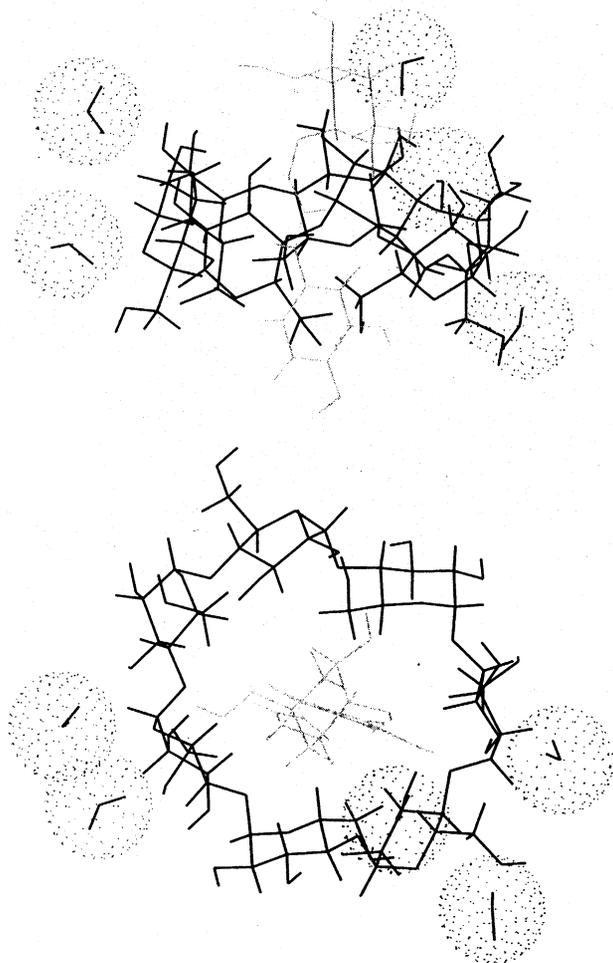
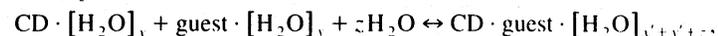


Fig. 3. View of the side and top of the β -CD·CA· z H₂O complex after 30 ps of molecular dynamics (MD) simulation. The displayed five water molecules (z) stayed in their initial positions for the entire MD time period. In a previous study [8] we determined that z was about 6 for this same system with a plurality of the water molecules associated with the hydroxyl group associated with C-3.

3. Results and discussion

Inclusion complex formation as a function of X_{H_2O} .—Water activity (a_{H_2O}) is known to have substantial effects on CD binding of azo-dyes [6], *p*-nitrophenol [23] and (+)-limonene [12,13]. A small isotopic effect on K in D₂O has also been reported [4]. Assuming the major effect of increasing the activity of any nonaqueous solvent is to decrease a_{H_2O} , these results support the equilibrium relationship [8]



where K is defined as

$$K = K' a_{H_2O}^z;$$

in this relationship K is the apparent stability, or equilibrium, constant and is equivalent to the stability constant K' (K as $X_{H_2O} \rightarrow 1$) multiplied by a_{H_2O} to the power of H₂O's stoichiometric coefficient, z . We have defined the total amount of complex-bound H₂O as $[\text{H}_2\text{O}]_{x'+y'+z}$ rather than $[\text{H}_2\text{O}]_{x+y+z}$ [6,14] since both the host and guest molecules probably [14] lose water (i.e., $x > x'$; $y > y'$) during inclusion complex formation. The small isotopic effect [4] on K is evidence for this model since D₂O H-bonds are more effective than H₂O. The dependence of K on ionic strength (IS) is also consistent with this model since an increase in IS should disorder the CD·guest·[H₂O]_{x'+y'+z} H-bond network [4]. For our purposes we have chosen MeOH as a simple diluent to alter a_{H_2O}

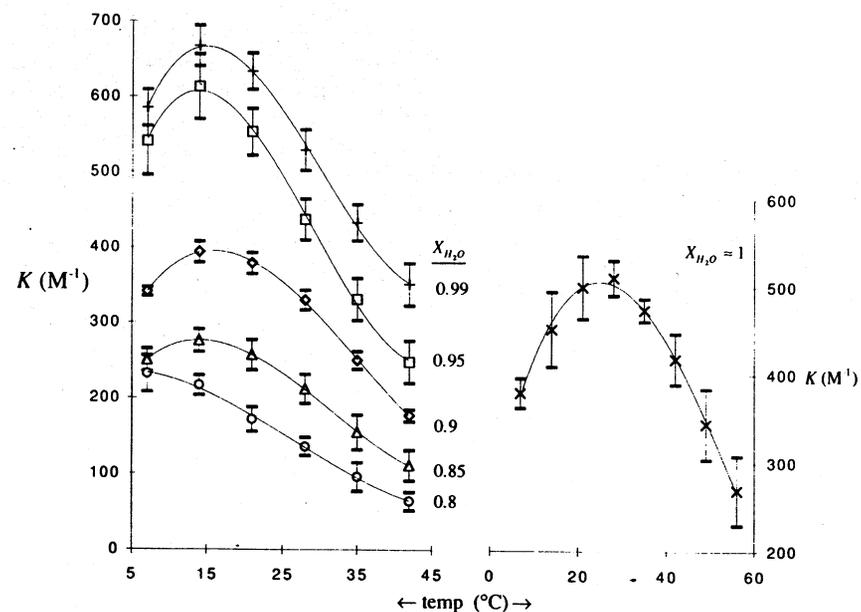


Fig. 4. Effect of differing X_{H_2O} on K as a function of t . Inset: $K-t$ plot for the standard buffer solution ($X_{H_2O} \sim 1$).

(assuming, as argued previously [8], that $a_{H_2O} \sim X_{H_2O}$ for the narrow X_{H_2O} range of 0.8–1). This method of determining z by measuring K values at various X_{H_2O} values appears [6,8] to be valid since, utilizing entirely different techniques, Yoshii et al. [13] found that an equivalent number of water molecules were needed to stabilize a similar β -CD complex. Other evidence [8] that this method is a reasonable approach to the study of the role of water, at least in a relative comparison, in CD-guest stability was that a 50% substitution of β -CD's hydroxyl groups produced an equivalent loss in z . Lastly, MD calculations on the β -CD \cdot CA \cdot z H₂O system (Fig. 3) indicate that z was about 5 and is close to what was determined [8] experimentally ($z \sim 6$) using MeOH as a

diluent. Of the 14 water molecules that were strategically placed near β -CD hydroxyl groups when the model system was prepared, five remained in close proximity to their original position throughout the MD calculations (2 near O-3 at 1.8 and 2.0 Å, 2 near O-6 at ca. 1.8 Å, and 1 near O-5 at 1.8 Å). Thus, MD calculations also predict that a small number of water molecules H-bond to β -CD with a relatively long lifetime of association.

Table 1 and Fig. 4 provide data on the dependence of β -CD_{*n*} \cdot CA complex K values on X_{H_2O} at various temperatures (t , °C). The β -CD_{*n*} \cdot CA complex displayed, as first shown in a previous study [4], an unusual K - t dependence ($X_{H_2O} \sim 1$; Table 1) whereupon the K values increased substantially from 7 to 28 °C and decreased between 28 and 56 °C. Such convex curvature in K - t plots is indicative that ΔH and ΔS vary substantially as a function of t . Because of this; the thermodynamic values reported in Table 1 were calculated only from the four highest temperatures and had reasonably small ϵ values. This curvature in K with respect to t is more apparent when plotted (Fig. 4, inset). As X_{H_2O} was decreased this curvature was nearly reversed to provide the customary (negative slope and ΔH) K - t dependence shown elsewhere [4,8,14]. Similar t dependences are common in protein systems whereupon different folding patterns take place as a function of binding and temperature in aqueous solution thereby resulting in different ΔH values as measured directly by titration microcalorimetry (personal communication from Dr. John Brandts, Microcal, Inc.). If the protein analogy holds true for the β -CD_{*n*} \cdot CA system, our data indicate that substantial perturbations in β -CD_{*n*}'s spatial organization occur with increasing t and are less significant as a_{H_2O} decreases. From a pragmatic perspective, such changes in microscopic polymeric structure with t could have commercial consequences for the utilization of β -CD_{*n*} since food systems

Table 1
Apparent stability constants (K), $[\beta\text{-CD}_n]_{t \rightarrow \infty}$, asymptotes for the relative change in CA optical density due to complexation (ΔA_{\max}), and entropy/enthalpy (ΔH and ΔS) change associated with CD-CA complexation as a function of temperature (t) and mole fraction of water (X_{H_2O})

| X_{H_2O} | t (°C) | ΔA_{\max} | K (M ⁻¹) | Thermodynamic Parameters |
|------------|----------|-------------------|------------------------|---|
| 0.8 | 7 | 1.44 ± 0.17 | 232.00 ± 77.93 | ΔH (kJ mol ⁻¹) |
| | 14 | 1.49 ± 0.07 | 216.50 ± 43.42 | -33.57 ± 3.61 |
| | 21 | 1.46 ± 0.06 | 171.56 ± 30.77 | ΔS (J mol ⁻¹ K ⁻¹) |
| | 28 | 1.38 ± 0.05 | 135.98 ± 27.14 | -71.12 ± 12.05 |
| | 35 | 1.33 ± 0.08 | 96.35 ± 28.21 | |
| | 42 | 1.34 ± 0.18 | 63.84 ± 28.75 | |
| 0.85 | 7 | 1.38 ± 0.09 | 250.08 ± 6.22 | |
| | 14 | 1.44 ± 0.08 | 276.06 ± 14.01 | -29.02 ± 3.24 |
| | 21 | 1.40 ± 0.06 | 257.28 ± 14.42 | |
| | 28 | 1.35 ± 0.07 | 212.33 ± 12.52 | -52.34 ± 10.8 |
| | 35 | 1.31 ± 0.08 | 155.23 ± 11.66 | |
| | 42 | 1.25 ± 0.08 | 111.12 ± 7.76 | |
| 0.9 | 7 | 1.40 ± 0.04 | 341.45 ± 15.20 | |
| | 14 | 1.41 ± 0.02 | 393.65 ± 14.60 | -25.61 ± 4.15 |
| | 21 | 1.38 ± 0.02 | 378.53 ± 20.35 | |
| | 28 | 1.32 ± 0.03 | 329.75 ± 19.02 | -37.43 ± 13.81 |
| | 35 | 1.28 ± 0.02 | 249.98 ± 23.14 | |
| | 42 | 1.23 ± 0.02 | 176.82 ± 20.09 | |
| 0.95 | 7 | 1.37 ± 0.01 | 540.50 ± 23.86 | |
| | 14 | 1.39 ± 0.01 | 613.39 ± 12.51 | -28.58 ± 1.46 |
| | 21 | 1.37 ± 0.02 | 553.01 ± 16.34 | |
| | 28 | 1.33 ± 0.01 | 337.24 ± 200.38 | -44.6 ± 4.86 |
| | 35 | 1.31 ± 0.02 | 331.07 ± 19.01 | |
| | 42 | 1.27 ± 0.01 | 247.90 ± 11.63 | |
| 1 | 7 | 1.48 ± 0.05 | 359.54 ± 19.25 | |
| | 14 | 1.47 ± 0.04 | 444.16 ± 47.08 | -17.16 ± 2.56 |
| | 21 | 1.45 ± 0.05 | 495.76 ± 37.84 | |
| | 28 | 1.42 ± 0.06 | 494.93 ± 39.07 | -4.79 ± 8.25 |
| | 35 | 1.39 ± 0.06 | 456.46 ± 41.38 | |
| | 42 | 1.35 ± 0.07 | 397.82 ± 56.44 | |
| | 49 | 1.33 ± 0.05 | 316.60 ± 73.49 | |
| | 56 | 1.32 ± 0.03 | 249.44 ± 57.88 | |

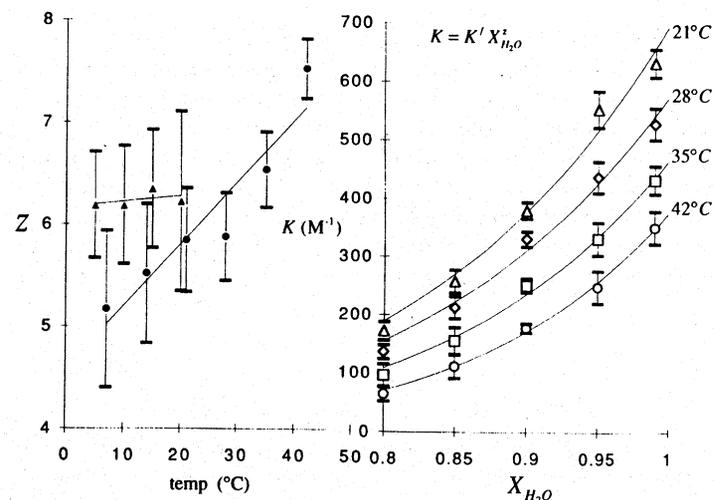


Fig. 5. Dependence of β -CD_{*n*}'s (closed circles) and β -CD's (closed triangles) stoichiometric coefficient ($z \pm \epsilon$) on t . Error bars represent $z \pm \epsilon$ or $z \pm s_z$ for β -CD or β -CD_{*n*}, respectively. Inset: plot of average K ($\pm s_T$) versus X_{H_2O} for various temperatures and their resultant curve fits ($K = K' X_{H_2O}^n$).

are extremely heterogeneous from the standpoint of a_{H_2O} and solute concentration and, thus, could alter the binding potential of the matrix.

It is interesting to note that ΔA_{\max} (Table 1), the $[\beta\text{-CD}_n]_{T \rightarrow \infty}$ asymptote (see the Experimental section), also had a slight t dependence whereupon ΔA_{\max} values decreased ca. 7–13% as a function of increasing t while corresponding $A_{325\text{ nm}}$ values for CA controls remained relatively constant (1.49 ± 0.05 , averaged across 96 observations). These data might indicate that the number of binding sites decreased slightly as t increased albeit that temperature-related deviations in ΔA_{\max} were within the range $\Delta A_{\max} \pm \varepsilon$ (from curve-fitting K versus $[\beta\text{-CD}_n]_o$) and $\Delta A_{\max} \pm s_{\bar{x}}$ (deviations of the means of 3 ΔA_{\max} calculations). The $[\beta\text{-CD}_n]_o$ (ca. 200 mM for 99% binding; $K \sim 500\text{ M}^{-1}$; data shown in Fig. 2, open circles) where ΔA approaches ΔA_{\max} provides the binding capacity (which changes as a function of K) of the $\beta\text{-CD}_n$ matrix. For a K of ca. 500 M^{-1} , more than 92% of CA is bound at 25 mM $[\beta\text{-CD}_n]_o$ therefore $[\beta\text{-CD}_n]_o/[CA]_o \sim 300$.

Using a nonlinear regression technique [18], we found that the average value of z for $\beta\text{-CD}_n \cdot \text{CA}$ ($z = 6.1 \pm 0.8$) was similar to z estimations reported previously [8] for the soluble $\beta\text{-CD} \cdot \text{CA}$ system (6.2 ± 0.04) and is similar ($z \sim 7$) to what has been reported to be the minimum number of waters requisite for (+)-limonene $\cdot \beta\text{-CD}$ stability [14].

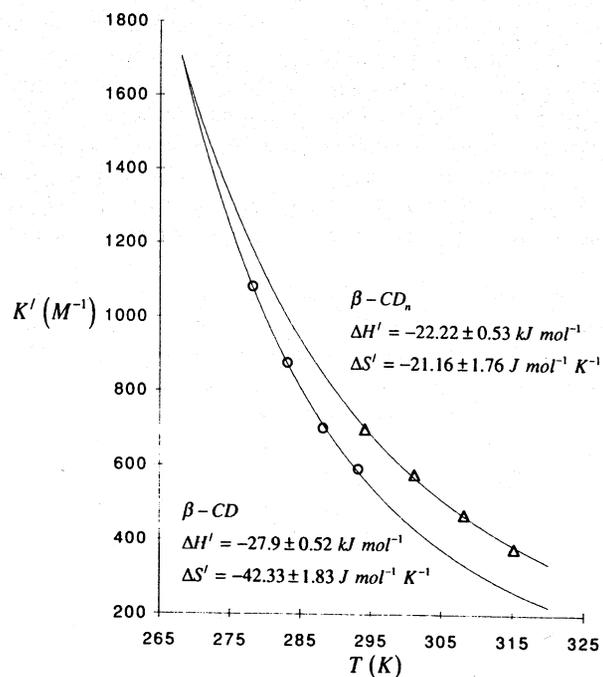


Fig. 6. Temperature (T , K) dependence of $\beta\text{-CD}_n \cdot \text{CA}$ (triangles) and $\beta\text{-CD} \cdot \text{CA}$ (circles) K' values. Data were fitted to the double exponential, $K = e^{-\Delta H'/RT} e^{\Delta S'/R}$, using nonlinear regression analysis which produced the displayed curves. $\Delta H'$ and $\Delta S'$ are provided $\pm \varepsilon$.

However, the $\beta\text{-CD}_n \cdot \text{CA}$ complex (Fig. 5, closed circles) displayed a significant effect of t on z ($\Delta z \sim 2$ as t was increased from 7 to 39 °C) not seen in solution studies (Fig. 5, closed triangles) [8] and further argues that the $\beta\text{-CD}_n$ spatial architecture changes with temperature. From the $K-X_{H_2O}$ fits shown in Fig. 5, we obtained the K' values plotted in Fig. 6 along with similar data [8] for the equivalent solution $\beta\text{-CD}$ system. From nonlinear regression analysis of K as a function of T we estimated that $\beta\text{-CD}_n$'s $\Delta H'$ (-22 kJ mol^{-1}) was only somewhat different from its soluble counterpart's $\Delta H'$ (-28 kJ mol^{-1}). However, $\Delta S'$ values were substantially different ($-21\text{ J mol}^{-1}\text{ K}^{-1}$ for $\beta\text{-CD}_n$ and $-41\text{ J mol}^{-1}\text{ K}^{-1}$ for $\beta\text{-CD}$) and resulted in the two curves intersecting at approximately the freezing point of the buffer (ca. 268 K).

$\beta\text{-CD}_n$'s entropy–enthalpy compensation behavior in the MeOH–H₂O binary solvent system.—Enthalpy–entropy compensation [9] describes the behavior of ΔH and ΔS for a series of similar reactions, such as CD \cdot guest inclusion, driven by changes in solvation. Often the enthalpy change for such processes is directly correlated with changes in entropy. According to Lumry and Rajender [9], this linear relationship is not

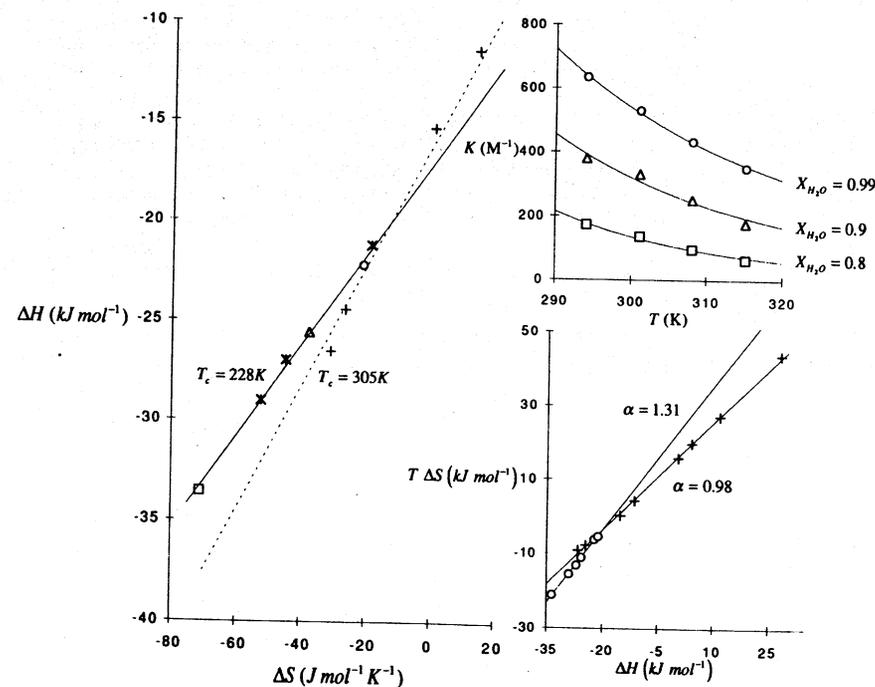


Fig. 7. Enthalpy–entropy compensation plot for both $\beta\text{-CD}_n \cdot \text{CA}$ (square, stars, triangle, and circle; $T_c = 228\text{ K}$) and $\beta\text{-CD} \cdot \text{CA}$ (+; $T_c = 305\text{ K}$) thermodynamic parameters as a function of X_{H_2O} (0.8–1 for $\beta\text{-CD}_n$ and ~ 1 for $\beta\text{-CD}$). Top inset: T -dependence of $\beta\text{-CD}_n \cdot \text{CA}$ K values for 3 X_{H_2O} treatments. Bottom inset: $T\Delta S - \Delta H$ plot for $\beta\text{-CD}_n \cdot \text{CA}$ (circles; $\alpha = 1.31$) and $\beta\text{-CD} \cdot \text{CA}$ (+; $\alpha = 0.98$) thermodynamic parameters as a function of X_{H_2O} (0.8–1 for $\beta\text{-CD}_n$ and ~ 1 for $\beta\text{-CD}$).

an obvious consequence of thermodynamic laws since, for any i th species involved in a simple reaction, ΔH_i and ΔS_i are related by the expressions

$$\Delta H_i = \Delta H_i^0 + \int_{T^0}^T \Delta C_{p,i} dT$$

and

$$\Delta S_i = \Delta S_i^0 + \int_{T^0}^T \frac{\Delta C_{p,i}}{T} dT.$$

Clearly these quantities are linear with respect to each other only in the case where ΔC_p is zero for all i . Thus, the compensation behavior described herein and elsewhere [4.8–11.14] is indicated only if a linear relationship exists between ΔH and ΔS and the ΔC_p values are nonzero. In most simple reactions, a series of ΔH – ΔS pairs can be created by altering the solvent with monohydroxy alcohols [9], changing the pH [4.9] with different buffers, or by working with different derivatives of the interacting species [9–11]. Regardless of the method used to create these ΔH – ΔS data sets, all should produce only one line [9] for each class of reaction. Thus, for most CD-driven binding reactions, the slopes (T_c) of these ‘compensation’ plots are all approximately 300 K [14]. Different reactions, such as simple hydrolysis, would produce a different T_c but in the range of 270–320 K [9].

Fig. 7 shows calculated values of ΔH , plotted against ΔS for various types of β -CD_n· and β -CD·CA complexes [4] (+: data pairs created using different pH values, CD types, and buffers) where $X_{H_2O} \sim 1$. Data from the variable X_{H_2O} studies (β -CD_n), reported herein, are plotted in the same figure. The uppermost regression line represents the least-squares fit for the thermodynamic parameters associated with β -CD_n utilizing MeOH to alter X_{H_2O} ($T_c = 228$ K). The lowermost line in Fig. 7 represents the line of best fit for data on various CD·CA equilibria but where $X_{H_2O} \sim 1$ ($T_c = 305$ K). A similar thermodynamic parameter, α ($\alpha = T\partial\Delta S/\partial\Delta H = 1.31$ for $X_{H_2O} \leq 1$; $T = 298$ K; Fig. 7 inset), calculated from changes in $T\Delta S$ – ΔH pairs measured at various X_{H_2O} values, was greater than α derived from standard aqueous thermodynamic measurements ($\alpha = 0.98$) in a similar system. Our α estimates were also much larger than those [10,11] reported for chemically modified and normal CD·naphthalenesulfonate complex equilibria ($\alpha = 1.11$ and 0.9, respectively). Thus, calculations of ΔH and ΔS for β -CD_n at various X_{H_2O} s show linear enthalpy–entropy compensation but with a slope significantly less than T_c values determined from either standard aqueous thermodynamic experiments ($\Delta T_c \sim 77$ K) or variable X_{H_2O} experiments reported previously for solution β -CD studies ($\Delta T_c \sim 44$ K) [8]. To the best of our knowledge, this is the smallest T_c value observed in a host [4.8,10,11,14] of CD·guest studies. As reported previously [8], when one follows the progression from most to least negative ΔH – ΔS pairs (Fig. 6: $X_{H_2O} \sim 0.8$ to 1; left to right), the data points generally represent the progression in X_{H_2O} treatments. This behavior is typical for enthalpy–entropy compensation [9].

4. Conclusions

Thermodynamically, many processes, no matter how simple, can be considered to be a sum of ‘part processes’ [9]. For any CD·guest system these parts should consist of

‘chemical’ part process and a ‘solvation’ part process. According to Lumry and Rajender [9], the mutual nonlinearity (e.g., $X_{H_2O} < 1$ data having a different T_c than $X_{H_2O} \sim 1$ data) in our compensation data could result from more than one part process depending upon changes in solvation. Thus, for both β -CD [8] and its polymerized form (Fig. 7), the chemical part process appears to be thermodynamically linked to the partial desolvation of β -CD’s binding site. The observation that β -CD_n undergoes a greater thermodynamic perturbation than its soluble counterpart is probably related to its insolubility and differences in unoccupied binding site solvation. Differences between the soluble and polymeric β -CD forms were also observed during the ‘chemical’ part process since β -CD_n z values were shown to vary substantially with temperature which might reflect changes in the conformation of the monomeric unit as a function of temperature and a_{H_2O} .

Acknowledgements

We would like to thank Drs. D. Downing, A. Hedges and R. Friedman of American Maize Products for the donation of various CD products.

References

- [1] G. Sapers, *J. Food Technol.*, 54 (1991) 75–84.
- [2] K. Hicks, G. Sapers, and P. Seib, U.S. Patent 4 975 293 (1990).
- [3] P. Shaw and B. Buslig, *J. Agric. Food Chem.*, 34 (1986) 837–840.
- [4] P. Irwin, P. Pfeiffer, L. Doner, G. Sapers, J. Brewster, G. Nagahashi, and K. Hicks, *Carbohydr. Res.*, 256 (1994) 13–27.
- [5] K. Connors and D. Pendergast, *J. Am. Chem. Soc.*, 106 (1984) 7607–7614.
- [6] W. Gerasimowicz and J. Wojeik, *Bioorg. Chem.*, 11 (1982) 420–427.
- [7] J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht, 1988, pp 79–185.
- [8] P. Irwin, J. Brouillette, and K. Hicks, *Carbohydr. Res.*, 269 (1995) 201–216.
- [9] R. Lumry and S. Rajender, *Biopolymers*, 9 (1970) 1125–1227.
- [10] Y. Inoue, Y. Yiu, L.-H. Tong, B.-J. Shen, and D.-S. Jin, *J. Am. Chem. Soc.*, 115 (1993) 10637–10644.
- [11] Y. Inoue, T. Hakushi, Y. Liu, L.-H. Tong, B.-J. Shen, and D.-S. Jin, *J. Am. Chem. Soc.*, 115 (1993) 475–481.
- [12] T. Furuta, H. Yoshii, A. Miyamoto, A. Yasunishi, and H. Hirano, *Supramol. Chem.*, 1 (1993) 321–325.
- [13] H. Yoshii, T. Furuta, A. Yasunishi, and H. Hirano, *J. Biochem.*, 115 (1994) 1035–1037.
- [14] R. Clarke, J. Coates, and S. Lincoln, *Adv. Carbohydr. Chem. Biochem.*, 46 (1988) 205–249.
- [15] F. Cramer, W. Saenger, and H.-C. Spatz, *J. Am. Chem. Soc.*, 89 (1967) 14–20.
- [16] H. Hartley, *Technometrics*, 3 (1961) 269–280.
- [17] N. Draper and H. Smith, *Applied Regression Analysis*, 2nd ed., Wiley, New York, 1981, pp 462–465.
- [18] P. Irwin, W. Damert, and L. Doner, *Concepts Magn. Reson.*, 6 (1994) 57–67.
- [19] G. King and A. Warshel, *J. Chem. Phys.*, 91 (1989) 3647–3661.
- [20] S.W. Homans, *Biochemistry*, 29 (1990) 9110–9118.
- [21] J. Gasteiger and M. Marsili, *Tetrahedron*, 36 (1980) 3219.
- [22] M. Clark, R.D. Cramer, III, and N. Van Opdenbosch, *J. Comput. Chem.*, 10 (1989) 982.
- [23] M. Eftink and J. Harrison, *Bioorg. Chem.*, 10 (1981) 388–398.