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Solubility of cyclomaltooligosaccharides (cyclodextrins) in H_2O and D_2O: a comparative study

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Abstract—Cyclomaltooligosaccharides (cyclodextrins, CDs) are cyclic oligomers having six, seven, or eight units of α-D-glucose, named as cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD) and cyclomaltooctaose (γ-CD), respectively. The molecule of CD has a cavity in which the interior is hydrophobic relative to its outer surface. The solubility of cyclodextrins in water is unusual, as an irregular trend is observed in the series of the cyclic oligomers of glucose. β-CD is at least nine times less soluble than the others CDs. This intriguing behavior has been investigated, and some interesting explanations in terms of the effect caused by CD on the water lattice structure have been proposed. In this work a comparative study on the solubility of α, β, and γ-cyclodextrins was carried out in H_2O and D_2O and reveals a much lower solubility of the three CDs in D_2O. The solid-phase structure of the CDs in equilibrium with the solution is quite similar with both solvents. The results are discussed in terms of the CD molecular structure and the differences in the hydrogen bonds formed between H_2O and D_2O.

Keywords: Solubility of cyclodextrins in H_2O and D_2O; Hydrogen bonding; Hydrophobic effect

1. Introduction

Cyclomaltooligosaccharides (cyclodextrins, CDs) are cyclic oligosaccharides consisting of six (cyclomaltohexaose, α-CD), seven (cyclomaltoheptaose, β-CD), or eight (cyclomaltooctaose, γ-CD) glucose units linked by 1,4-α-glucosidic bonds. The structural consequence of this bonding mode is the formation of a shallow truncated 'cone shape' with *n* (number of glucose residues) primary hydroxy groups lined in one rim of the cone, and 2*n* secondary hydroxy groups lined at the other rim. A cavity arises from this structure containing rows of CH groups (C-3 and C-5) and a row of glucosidic oxygens. Therefore, the cavities are nonpolar relative to their outer surface.[1,2] The cavities can act as hosts for a great variety of monomer guests,[3] and also to polymeric chains. In the latter case supramolecular adducts are produced by the threading of the polymer chain into the cavity of the CD, forming interesting structures such as *necklaces*.[4–8] In spite of the great scientific interest in such supramolecular chemistry, the understanding of the driving forces that promote the guest–host complex formation in water is unclear. The role of the interaction between the CDs and water molecules, and even their anomalous solubility is still inconclusive.

The solubility of cyclodextrins in water is unusual, as an irregular trend is observed in the series of the cyclic oligomers of glucose. β-CD is nine and 11 times less soluble in water in comparison to α and γ-CD, respectively.[3,9] This intriguing behavior has been discussed in terms of the effect caused by CDs on the water lattice structure. The local water density around the molecules of the three CDs was determined using molecular dynamics, and the result reveals that β-CD induces stronger ordering on the surrounding water in comparison with the others.[10] The enthalpy of solutions of the three CDs is very similar, but the entropy of solution of β-CD is lower relative to the others, as shown in Table 1.[9] Therefore, the lower solubility of β-CD

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Table 1. Thermodynamic parameters for dissolution of cyclodextrins in aqueous saturated solution (adapted from Ref. 9)

Cyclodextrin	$\Delta G°$/kcal mol^{-1}	$\Delta H°$/kcal mol^{-1}	$\Delta S°$/cal mol^{-1} K^{-1}
α-CD	3.58	7.67	13.8
β-CD	4.81	8.31	11.7
γ-CD	3.34	7.73	14.7

in water is driven by the less favorable entropy.[11] The effect can be even reinforced if D_2O is considered. D_2O forms stronger hydrogen bonds than H_2O, as a consequence of the smaller quantum mechanical zero-point energy. The effects that arise from the difference in the hydrogen bonding between D_2O and H_2O influence a wide range of molecular phenomena in aqueous solution, ranging from gas solubility, formation of membranes and folding of proteins.[12]

The rate of threading of the poly(ethylene glycol) chain into the α-CD cavities, forming the polypseudorotaxanes, is higher in D_2O, in comparison to the rate of threading in H_2O. This result has been attributed to the stronger hydrogen bonding formed with D_2O compared to H_2O.[13,14] Harada pointed out that the formation of the complex is entropically unfavorable as the polymer chain must be fit into several host units.[5] The entropic gain related to the release of free water molecules to the bulk phase is not enough to drive the process. On the other hand, the difference in the kinetics of the complexation in light and heavy water suggests an important role of the hydrophobic effect. In order, to investigate the behavior of the CDs in both solvents, we developed a systematic study of the solubility and the kinetics of crystallization of CDs in H_2O and D_2O. In this work we present results in which remarkable differences in these properties are observed. The results are discussed in terms of the numbers of the glucose units that form the cyclic oligomers, and in the difference in the hydrogen bonds formed between H_2O and D_2O molecules.

2. Experimental

2.1. Purification of CDs

α-CD (lot 60T005), β-CD (lot 70P229), γ-CD (lot 80P200) were supplied by Wacker Chemical Co. α-CD and γ-CD were purified using five steps of recrystallization from concentrated aqueous solutions by adding small amounts of EtOH (E. Merck). Supersaturated solutions (25% w/w) of α-CD and γ-CD were prepared in water at 70 °C, and kept at this temperature for 24 h. The solutions were centrifuged for 10 min at 3,000 rpm at room temperature, and then the supernatants were separated from the solid phase. EtOH (E. Merck) was then added to the solutions, in order to precipitate the CDs. The solid phases were filtered, washed with cold water and dried at 80 °C for 24 h. Due to the low solubility of β-CD in water and the low recovery by addition of EtOH, it was purified by recrystallization from a saturated solution (7% w/w). Part of the purified samples were used to prepare supersaturated solutions in D_2O (Goss), and recrystallized in this solvent in order to prepare D_2O hydrates of the samples.

2.2. Equilibrium solubility studies

An adaptation of the method proposed by Jozwiakowski and Connors[9] was used in the determination of the solubility of the three CDs. The proportions of 15%, 4%, and 28% (w/w), respectively, of purified α, β, and γ-CD were placed in vials with H_2O and with D_2O. The samples were agitated by rotation in a constant-temperature bath at 25.00 ± 0.01 °C for 10 days. The supernatants were then separated from the solid phase at 25 °C and filtered with Milli-Q membrane filters (0.45 μm pore size) upon injection from 3-mL disposable plastic syringes. Supernatant (1 g) of each sample was placed in at least eight different Eppendorf vials. The samples were dried at 70 °C for 5 days and weighed to within ±0.0001 g.

2.3. Kinetics of crystallization

Supersaturated solutions of α-CD in H_2O and in D_2O were prepared by heating the solutions at 70 °C and then centrifuging for 1 h at 10,000 rpm at 25 °C. The supernatants of both solutions were then distributed among several Eppendorf vials and kept in a constant-temperature bath at 25 °C without agitation. The supernatant present in each Eppendorf vial was then sampled at different times. These samples were dried in an oven at 80 °C for 5 days, and the mass of solid was then measured. The concentration of α-CD (weight of α-CD/weight of solution) present in the supernatant was determined from the average of several duplicate runs.

2.4. Sorption experiments

Dried CD samples were kept in contact with the vapors of H_2O and D_2O for 10 days in a constant-temperature bath at 25 °C without agitation. Thermal gravimetric analysis (TGA) of the dehydrated and hydrated CD were obtained by using a TA instruments model 2950 for temperatures ranging from 25 to 300 °C using a heating rate of 10 °C/min in order to determine the number of water molecules (H_2O and D_2O) of the stable hydrates. X-ray diffraction measurements of the hydrates were performed with a powder diffractometer (Shimadzu XRD 7000, Cu $K_\alpha = 1.5406$ Å, 40.0 kV, 30.0 mA).

3. Results and discussion

In the preparation of supramolecular complexes between α-CD and poly(ethylene glycol), it is quite common to make use of saturated aqueous CD solutions. During the preparation of such solutions we have observed a clear difference in the rate of crystal formation and growth rates in D_2O relative to H_2O. In order to investigate such effects, a comparative kinetic study was carried out. Using the same experimental conditions, supersaturated solutions of α-CD were prepared in both solvents, and the results are shown in Figure 1. The rate of crystallization is approximately 10 times higher in D_2O than in H_2O, and almost 15 and 5 days, respectively, are necessary for the solutions in H_2O and in D_2O to reach equilibrium. In addition, not only the kinetics of crystallization are different, but also the equilibrium solubility of α-CD. Thus, a set of experiments was performed to determine accurately the values of the solubility in both solvents at 25 °C. The experiments of the solubility in H_2O and D_2O were also extended to β and to γ-CD (Table 2) in order to investigate the effect of the number of glucose units and the hydrophobic cavity size on the solubility in H_2O and D_2O. The values in light water are in good agreement with those described in the literature.[9] The solubility of the CDs in H_2O is clearly higher than in D_2O, and the anomalous trend in the solubilities is also found in D_2O. However, the relative difference in solubility in both solvents is 41% for α and β-CD, but the difference is only 20% in the case of γ-CD. The higher solubility of γ-CD in D_2O could be, in principle, attributed to the lower ability of γ-CD to structure the water molecules inside the cavity and around itself, relative to the other two CDs. This result is in agreement with that observed by Naidoo et al.,[10] in which the anomalous solubility of the CDs in H_2O is associated with the amplitude and the relaxation time of macrocyclic ring motion. γ-CD is more flexible and, therefore, is less likely to trap water molecules within its hydrophobic cavity and in the first hydration shell compared with the others. Therefore, despite the stronger hydrogen bonds formed among D_2O, in comparison to the H_2O molecules, the higher flexibility of γ-CD molecules allows a favorable local accommodation of D_2O molecules around themselves. Therefore, the enthalpic–entropic balance in this case, associated with the structuring of the water molecules around the γ-CD molecule is more favorable, resulting in a higher solubility in comparison with the solubility of the two others CDs in D_2O.

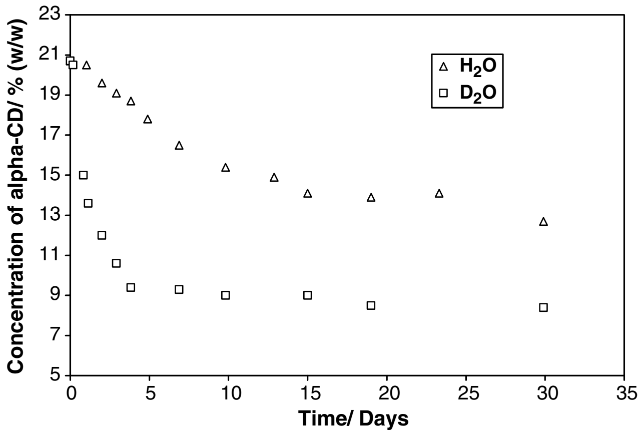

Figure 1. Kinetics of crystallization of α-CD in H_2O and D_2O from saturated solutions at 25 °C. The concentration α-CD is expressed as weight of α-CD/weight of solution.

Table 2. Values of the solubility (g/g solvent) of the three CDs in H_2O and D_2O at 25 °C

Solute	H_2O	D_2O	Relative difference/%
α-CD	0.1295 ± 0.0007	0.0758 ± 0.0005	41
β-CD	0.0184 ± 0.0002	0.0108 ± 0.0001	41
γ-CD	0.2492 ± 0.0002	0.1988 ± 0.0006	20

In order, to verify if the different behaviors of the CDs are only associated with the liquid phase, the solid phases in equilibrium with the solution in both solvents were also investigated. The structures of the stable CD hydrates, as well the number of H_2O and D_2O molecules present in the crystals, were investigated. The number of water molecules that form the stable CD hydrates was determined by measuring the loss of water in thermal gravimetric experiments involving hydrates formed after maximum sorption of vapors of D_2O and H_2O. The thermal gravimetric curves of α-CD–H_2O and α-CD–D_2O are shown in Figure 2A. Stepwise dehydration is observed to both hydrates, indicating that distinctly different energy levels are present. However, the released temperatures to H_2O and D_2O are quite close, and similar results were observed to the other CDs (Fig. 2B and C). These results indicate that the energy of the interactions between CDs in the solid state and water molecules are not different to H_2O and D_2O. The number of water molecules present in the hydrates of the three CDs is shown in Table 3. For the hydrate α-CD–H_2O the stoichiometry is very similar to that indicated in the literature,[1,15] but small differences were observed for β and γ-CD. In relation to the isotopic effect, we observed that, for each CD, the total number of water molecules present in the lattice of the crystal is the same for H_2O and D_2O. Therefore, it is reasonable to suppose that for each CD, the distribution of water molecules inside and outside the cavity is independent of the isotopic composition of the solvent. From the H_2O molecules that form the hydrates, 2, 6, and 8.8 water molecules are present in the α, β, and in the γ-CD cavities, respectively, the others being distributed around the exterior of the CD molecules.[1]

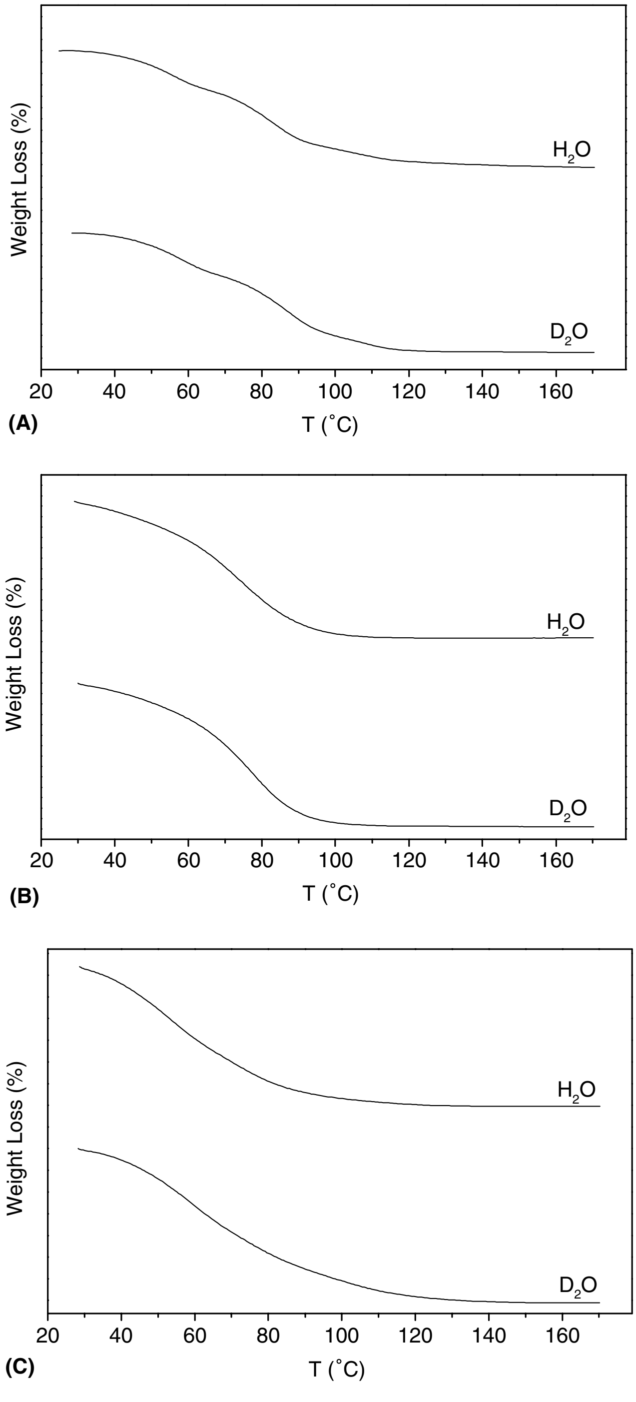

Figure 2. Thermal gravimetric curves of stable hydrates formed by sorption of H_2O and D_2O to α-CD (A), β-CD (B), and γ-CD (C).

Table 3. Number of water molecules (H_2O and D_2O) present in the stable hydrates of the CD lattices[a,b]

Cyclodextrin	H_2O	D_2O	Values from the literature (to H_2O)
α-CD	6.4	5.8	6[a,b]–6.6[a]
β-CD	9.6	9.2	10–11[a], 12[b]
γ-CD	14.2	14.4	17[a,b]

The number of H_2O molecules in the lattice of the stable hydrates obtained from the literature are also indicated.

[a] Ref. 1.

[b] Ref. 15.

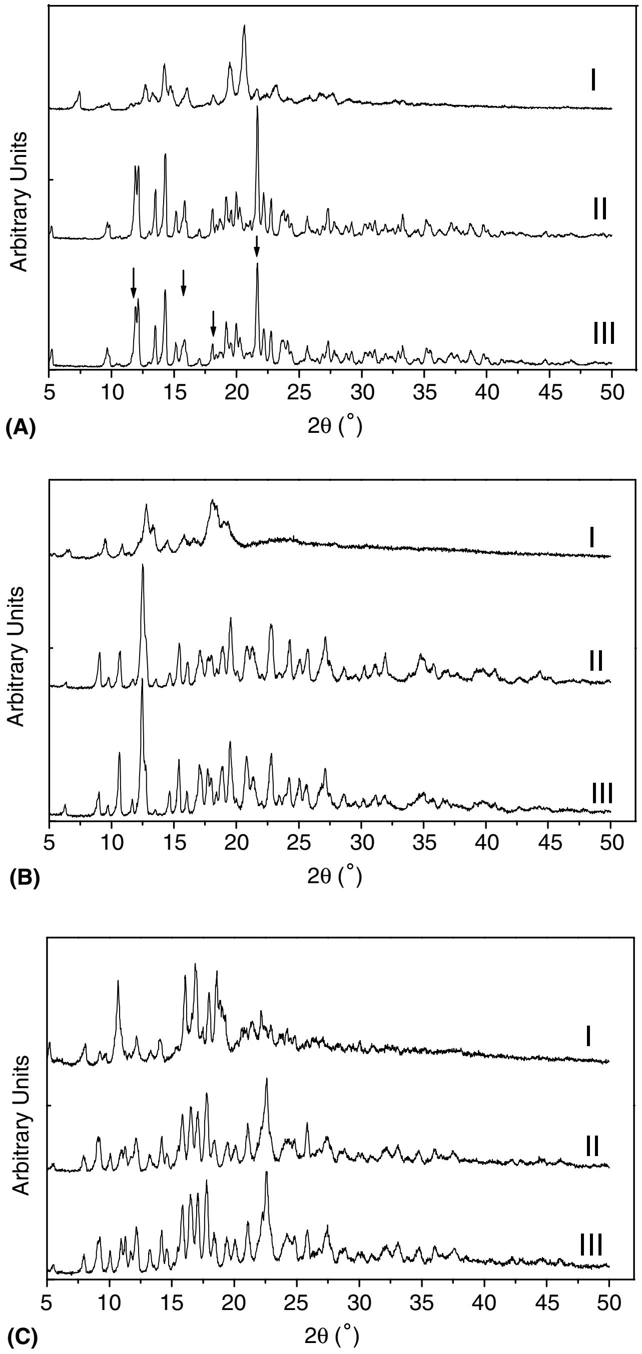

Figure 3. X-ray diffractograms of the dehydrated CD (I), CD–H_2O (II), and CD–D_2O (III) to α-CD (A), β-CD (B), and γ-CD (C). The characteristic peaks of the α-CD hydrated form are indicated by arrows.

Eleven D_2O molecules were found in the lattice of β-CD unit crystallized from D_2O.[16] This is the same number of H_2O molecules present in the hydrates of β-CD produced by vapor sorption (Table 3, Refs. 1 and 15). This means that the stoichiometry of the hydrate is also maintained when the solid phase is in contact with the saturated solution. This supposition is corroborated by the calorimetric results obtained by Bastos et al.,[17] in which the enthalpy involved in the formation of the α-CD–$6H_2O$ is exothermic ($-91\ \mathrm{kJ\ mol^{-1}}$), whereas the dissolution enthalpy α-CD–$6H_2O$ in H_2O at 298 K is an endothermic process ($+31\ \mathrm{kJ\ mol^{-1}}$). This means that the formation of hydrates with higher number of water molecules than six (in the case of α-CD) is enthalpically unfavorable.

The X-ray diffractogram of the dehydrated and hydrated (H_2O and D_2O) crystals of α-CD are shown in Figure 3A. The pattern of the diagrams is quite similar to the hydrates. The peaks of the dehydrated form ($2\theta = 13.8°$, 14.6°, and 20.5°) indicated that the CDs adopt a cage structure.[7] The characteristic peaks of the hydrated forms ($2\theta = 11.9°$, 15.8°, 18.1°, and 21.6°) are present in the diffractograms of the α-CD crystals formed by H_2O and D_2O, which means that the crystal structure of the solid phases in equilibrium with both solvents is very similar. The diffractograms of the hydrates of β and γ-CD formed with H_2O and D_2O are also quite similar as shown, respectively, in Figure 3B and C.

We envisage that crystallization of the CDs is driven by the increase of the entropy caused by the release of the water molecules from the CD hydration shell to the bulk of the solution. As D_2O forms stronger hydrogen bonds in comparison to H_2O, the effect is more intense in D_2O. This view is in agreement with the interpretation of Frank et al. in which the crystallization temperature of a saturated solution of β-CD is 9 °C higher in D_2O than in H_2O.[18]

4. Conclusions

The solubility of CDs is significantly higher in H_2O, in comparison to their solubility in D_2O. The differences are explained in terms of the hydrophobic effect. D_2O forms stronger hydrogen bonds in comparison to H_2O. CDs change the local water structure, and hence, the entropic–enthalpic balance involved in dissolution of the CDs is more unfavorable in D_2O. The anomalous solubility trend of the CDs in H_2O is also observed in D_2O, but the difference between both solvents is lower for γ-CD relative to α- and β-CD. This is probably associated with the higher flexibility of γ-CD, in comparison to the two others, allowing a favorable local accommodation of D_2O molecules. The kinetics of crystallization of CDs is also different in both solvents. In the case of α-CD the rate of crystallization is almost 10 times higher in D_2O in comparison to H_2O. The number of water molecules present in the stable hydrates of the three CDs is similar in H_2O and D_2O. The structures of the crystal hydrates are not affected if either light or heavy water is present in the lattice. As D_2O forms stronger hydrogen bonds than H_2O, in the crystallization process, the release of the water molecules from the shell of hydration to the bulk promotes an increase in the entropy of the system.

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