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AQUEOUS SOLUBILITY BEHAVIOR OF THREE CYCLODEXTRINS

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ABSTRACT

The solubilities in water of α-, β-, and γ-cyclodextrins were measured over the temperature range 23.7–48°. Dissolution rate studies and microscopy showed that the stable solid phase was the hydrate. van't Hoff plots were linear, yielding these thermodynamic parameters of solution (in the order α, β, γ): ΔH^0 = 7.67, 8.31, 7.73 kcal/mol; ΔS^0 = 13.8, 11.7, 14.7 e.u. Methyl α-D-glucopyranoside, studied in the same way, gave ΔH^0 = 3.95 kcal/mol, ΔS^0 = 8.4 e.u. The cyclodextrins are less soluble than acyclic saccharides because of their relatively unfavorable enthalpies of solution, partially offset by more favorable entropies of solution. Among the cyclodextrins, cyclomaltoheptaose (β-cyclodextrin) is less soluble than the others because both its enthalpy and its entropy are less favorable. The following empirical equations satisfactorily correlate the thermodynamic parameters of the three cyclodextrins: $\Delta H^0/n = -0.155n + 2.232$, and $\Delta S^0/m = 1.95n - 8.10$, where n is the number of glucose residues per cyclodextrin molecule, and m is the number of cyclodextrin molecules per unit cell of the hydrate.

INTRODUCTION

The cyclodextrins are cyclic oligomers of D-glucose produced by the action of certain microbial enzymes on starch. The commercially available members of this series are cyclomaltohexaose (α-cyclodextrin, cyclohexaamylose), cyclomaltoheptaose (β-cyclodextrin, cycloheptaamylose), and cyclomaltooctaose (γ-cyclodextrin, cyclooctaamylose), having 6, 7, and 8 glucose units, respectively. Their possession of intramolecular cavities has led to much interest in these compounds as "hosts" in the formation of inclusion complexes with "guest" molecules of suitable size. Many reviews of the cyclodextrins are available[1–10].

The solubilities of the cyclodextrins in water are unusual; at room temperature these are[11], for α-cyclodextrin, 0.150M, β-cyclodextrin, 0.016M, γ-cyclodextrin, 0.179M. These values are all rather low for saccharides, but the peculiar feature is their alternating pattern, if it is permissible to infer a pattern from a series of three values. At any rate it is curious, and of considerable practical interest, that β-cyclodextrin is an order of magnitude less soluble than the other two. Nor is it obvious whether β-cyclodextrin is the "odd" member, or whether perhaps α-cyclodextrin

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behaves strangely relative to the other two. It was the purpose of the present study to enlarge our knowledge of the solubility properties of the cyclodextrins, with the hope that this might lead to an understanding of their unusual behavior. The study consisted of measurements of the equilibrium solubilities of the three cyclodextrins at several temperatures, from which the enthalpies and entropies of solution were obtained, and such other observations (microscopic, dissolution-rate, and thermal analysis studies) as were needed to confirm the identity of the solid phase in the equilibrium mixtures.

EXPERIMENTAL

Materials. — α-Cyclodextrin (cyclomaltohexaose) (Sigma Chemical Co., lot No. 92F-0165), β-cyclodextrin (cyclomaltoheptaose) (Aldrich Chemical Co., lot No. 061667; P. L. Biochemicals, lot No. 530401; Sigma Chemical Co., lot No. 102F-0821), γ-cyclodextrin (cyclomaltooctaose) (Sigma Chemical Co., lot No. 92F-0164; Chinoin, Budapest, lot No. 1026), and methyl α-D-glucopyranoside (Aldrich) were the saccharides used in this work. α-Cyclodextrin and β-cyclodextrin were recrystallized from water; γ-cyclodextrin was used directly. Methyl α-glucoside was recrystallized from water; m.p. 167–169°. Deionized distilled water (Barnstead PCS System) was used throughout.

Equilibrium solubility studies. — Appropriate amounts of solid were placed in 14-mL amber screw-top vials having Teflon®-lined caps; the threads of the vials were wrapped with Teflon® tape before sealing. The vials were agitated (either by shaking or rotation) in a constant-temperature water bath (temperature control ±0.1° at 25°) until equilibrium was assured (at least 48 h, but up to one week for some studies), and the solutions were analyzed for dissolved solid.

Analytical method. — Gravimetric analysis was employed to analyze the aqueous solutions. Excess solid phase was separated from the solution phase by means of a Gelman filtration system fitted with GA-8 Metricel® membrane filters (0.2 μm pore size) and 10-mL disposable plastic syringes. A measured portion (2 mL or 4 mL depending on solid content) of solution was transferred to a 5-mL volumetric flask and weighed. The samples were placed in a 90-degree oven until apparently dry (about 48 h); and then dried at 110° for 3 h prior to being weighed as the anhydrous solute. Recoveries were shown to be within 0.5% of 100% for all solutes, with standard deviations of 0.5–1%.

Dissolution rate studies. — The rates of dissolution of the anhydrous and hydrate forms of α-cyclodextrin and β-cyclodextrin were studied by preparing sample vials as described above, but withdrawing samples after appropriate times ranging from 15 s to 1 week. The solute concentrations were expressed on the molar basis so the anhydrous and hydrate systems could be compared.

Differential thermal analysis. — Samples of the anhydrous and hydrate forms of all three cyclodextrins were subjected to thermal analysis with a Mettler TA 2000 thermal analyzer equipped with a TA 10 DTA cell. The thermostat was

calibrated with indium. Samples of α-cyclodextrin ranging in composition from the fully crystalline hexahydrate to the amorphous anhydrous form were also examined.

RESULTS AND DISCUSSION

Solid phase properties. — The crystalline hydrates and amorphous anhydrous forms of the three cyclodextrins can be readily distinguished under the polarizing microscope[12]. Their melting ranges are all well above 200°, that is, far above the temperature range examined in the solubility studies.

DTA thermograms of the anhydrous α-cyclodextrin and γ-cyclodextrin showed exothermic peaks at 167° and 152°, respectively, whereas anhydrous β-cyclodextrin showed no peaks in this range; α- and β-cyclodextrins (anhydrous) gave small endothermic peaks at 230° and 225°, respectively. The hydrates, on the other hand, showed broad endothermic peaks representing loss of water. In these thermograms, β- and γ-cyclodextrins behaved similarly, giving undifferentiated peaks beginning at 30° and 50°, respectively. α-Cyclodextrin yielded three endothermic peaks, presumably reflecting adsorbed and bound water of different energies; these peaks were centered at 80°, 106°, and 129°. These thermograms demonstrated that under the drying conditions used in the analyses, the anhydrous forms of the cyclodextrins were obtained.

Dissolution rate studies. — The rates of dissolution of α- and β-cyclodextrins were studied at 23.7° and 40°. Fig. 1 shows a typical result, that for β-cyclodextrin at 40°. Solubility is plotted as a function of time for the anhydrous and hydrated forms. This profile, and the others we determined, shows that the more soluble anhydrous form dissolves faster than the hydrate, but conversion of the solid phase to the hydrate leaves the solution supersaturated with respect to the hydrate, and

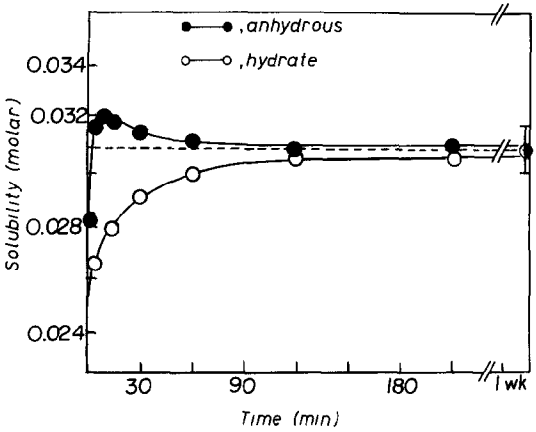

Fig. 1. Dissolution rate profile for β-cyclodextrin at 40°. The error bar at the 1-week point gives 95% confidence limits.

the concentration slowly decreases to the equilibrium value.

The thermodynamically stable solid phase at this temperature is the hydrate, for after one week both systems have the same solubility, and microscopic examination shows the solid to be the hydrate. Since the solubility of the anhydrous forms is greater than that of the hydrates, the transition temperature for the anhydrous–hydrate conversion lies outside the temperature range of the present studies. Examination of the solid phase at various times showed that anhydrous α-cyclodextrin formed the hydrate almost immediately upon the addition of water; β- and γ-cyclodextrins were converted to the hydrates within one hour.

Equilibrium solubilities. — Phase-solubility analysis of recrystallized α- and β-cyclodextrins showed that they contained no detectable impurities within the sensitivity limits of the technique[13].

Tables I–III give equilibrium aqueous solubilities of the three cyclodextrins at several temperatures, expressed as g solute/g solvent and in molar units; the densities of the saturated solutions at the experimental temperatures are also reported. From these data the solubilities in molar units, as mole fractions, and percent weight/volume can be calculated.

Thermodynamic parameters. — Fig. 2 is a van't Hoff plot of the data in Table I. The solubilities are expressed as mole fractions in order to eliminate the contributions of the free energy and entropy of mixing, so the resulting free energies and entropies of solution are unitary quantities in Gurney's terminology[14]. The data in

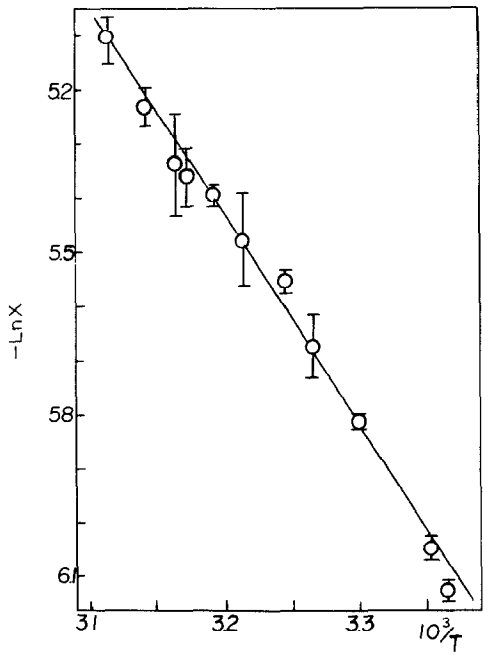

Fig. 2. Van't Hoff plot of α-cyclodextrin solubilities (Table I), where X is the mole fraction solubility, and the error bars represent 95% confidence limits. Correlation coefficient −0.996.

TABLE I

SOLUBILITY–TEMPERATURE DATA FOR CYCLOMALTOHEXAOSE (α-CYCLODEXTRIN)

Temp. (°C)	*n*[a]	*Density*[b] (g · mL^{-1})	*Solubility*[b]	
			(g/g H_2O)	(molar)
23.7	3	1.030 (.007)	0.1171 (.0014)	0.1118 (.0009)
25.0	10	1.037 (.003)	0.1282 (.0010)	0.1211 (.0008)
30.0	7	1.046 (.003)	0.1623 (.0008)	0.1501 (.0008)
33.0	11	1.052 (.003)	0.1860 (.0043)	0.1696 (.0033)
35.0	5	1.056 (.003)	0.2102 (.0016)	0.1885 (.0013)
38.0	4	1.064 (.004)	0.2268 (.0070)	0.2023 (.0051)
40.0	7	1.069 (.0002)	0.2463 (.0015)	0.2171 (.0011)
42.0	4	1.069 (.003)	0.2545 (.0043)	0.2229 (.0031)
43.0	6	1.073 (.005)	0.2604 (.0078)	0.2283 (.0055)
45.0	6	1.078 (.003)	0.2901 (.0042)	0.2492 (.0029)
48.0	7	1.086 (.004)	0.3305 (.0039)	0.2773 (.0026)

[a]Number of determinations. [b]Standard deviation in parentheses.

TABLE II

SOLUBILITY–TEMPERATURE DATA FOR CYCLOMALTOHEPTAOSE (β-CYCLODEXTRIN)

Temp. (°C)	*n*[a]	*Density*[b] (g · mL^{-1})	*Solubility*[b]	
			(g/g H_2O)	(molar)
23.7	3	0.999 (.005)	0.0178 (.00014)	0.0154 (.00022)
25.0	10	1.004 (.004)	0.0188 (.00009)	0.0163 (.00010)
35.0	7	1.003 (.003)	0.0283 (.00030)	0.0244 (.00031)
40.0	3	1.002 (.003)	0.0349 (.00040)	0.0298 (.00030)
45.0	11	1.008 (.002)	0.0453 (.00062)	0.0385 (.00051)
48.0	4	1.007 (.004)	0.0525 (.00022)	0.0443 (.00024)

[a]Number of determinations. [b]Standard deviation in parentheses.

TABLE III

SOLUBILITY–TEMPERATURE DATA FOR CYCLOMALTOOCTAOSE (γ-CYCLODEXTRIN)

Temp. (°C)	*n*[a]	*Density*[b] (g · mL^{-1})	*Solubility*[b]	
			(g/g H_2O)	(molar)
25.0	7	1.069 (.006)	0.256 (.0090)	0.168 (.0048)
30.0	2	1.084	0.322	0.204
35.0	2	1.111 (.005)	0.396 (.004)	0.243 (.0019)
40.0	1	1.117	0.452	0.268
42.0	1	1.142	0.547	0.311

[a]Number of determinations. [b]Standard deviation in parentheses.

TABLE IV

THERMODYNAMIC PARAMETERS FOR DISSOLUTION IN THE AQUEOUS SATURATED SOLUTION[a,b]

Compound	ΔG^0 *(kcal · mol^{-1})*	ΔH^0 *(kcal · mol^{-1})*	ΔS^0 *(e.u.)*
α-Cyclodextrin	3.58 (.004)	7.67 (.18)	13.8 (.6)
β-Cyclodextrin	4.81 (.003)	8.31 (.21)	11.7 (.7)
γ-Cyclodextrin	3.34 (.021)	7.73 (.27)	14.7 (.9)
Methyl α-glycoside	1.43 (.014)	3.95 (.26)	8.4 (.7)
α-D-Glucose[c]	1.47	4.65	10.7
Sucrose[d]	0.68	1.40	2.4

[a]Standard deviations in parentheses. [b]Based on mole fraction solubilities. [c]Calculated from data in ref. 15. [d]Ref. 16.

Tables II and III were treated similarly. In Table IV are given the resulting values of ΔH^0, ΔS^0, and ΔG^0 at 25°. Since methyl α-glucoside is often used as a "control" in cyclodextrin complexing studies, its thermodynamics of solution were also studied, and are reported in Table IV. For comparison, this table also gives results drawn from the literature for some other saccharides.

Several studies have been reported of the dependence of solubility on temperature for α- and β-cyclodextrins. The Corn Products Development Corporation described β-cyclodextrin solubilities over a wide range of temperatures, but no experimental details were given[17]. Wiedenhof and Lammers[18] published evidence that cyclomaltoheptaose dodecahydrate is the stable solid phase in contact with saturated aqueous solutions at 15–30°. They used refractometry as their analytical method, which necessitated extrapolation of a standard curve from lower concentrations to estimate the solubility at saturation. Wiedenhof and Lammers[19] also studied α-cyclodextrin, and proposed the existence of polymorphic hydrates over the temperature range of the study, based on shifts in refractive indices of some of the solutions. Their solubility data, however, give linear van't Hoff plots.

Because of this suggestion of Wiedenhof and Lammers, α-cyclodextrin was examined at many temperatures in the present study. The van't Hoff plot (Fig. 2) is best interpreted as a linear correlation over the temperature range studied. This does not rule out the possibility of polymorphism but there appears to be no strong evidence for more than one solid phase, which the dissolution rate study showed to be the hydrate.

There is evidence of non-ideality in cyclodextrin solutions in the results of Miyajima *et al.*[20], which provide estimates of 0.857 and 0.829 for the molal activity coefficients of α- and γ-cyclodextrin respectively, in saturated solutions at 25°. However, these departures from ideality are not manifested as non-linearities in the van't Hoff plots. Since the activity coefficients apply only at 25°, it is not possible to calculate corrected solubilities at other temperatures and make activity-based van't Hoff plots.

TABLE V

ENTHALPIES AND ENTROPIES OF SOLUTION FROM LITERATURE DATA[a]

Reference	*Cyclodextrin*	ΔH^0 *(kcal · mol^{-1})*	ΔS^0 *(e.u.)*
19	α	6.12 (0.17)	8.3 (0.6)
18	β	7.32 (0.05)	8.4 (0.2)
17	β	8.50 (0.24)	12.6 (0.3)

[a]Recalculated from literature solubilities expressed as mole fractions. Standard deviations in parentheses.

Table V lists thermodynamic parameters recalculated from literature data[17–19]. The discrepancies from the present results (Table IV) are probably in part a consequence of the smaller temperature range investigated by Wiedenhof and Lammers.

Referring now to Table IV, it is evident that the enthalpies of solution of α- and γ-cyclodextrin are not different at any level of significance. Since γ-cyclodextrin is definitely more soluble than α-cyclodextrin, the entropies of solution must be different, though this difference is masked by the uncertainties in the values. If the ΔS^0 values are different for α- and γ-cyclodextrin, it is clear that ΔS^0 for β-cyclodextrin is significantly smaller than either of these. Moreover ΔH^0 for β-cyclodextrin is significantly larger than ΔH^0 for α- and γ-cyclodextrins. Therefore, the much lower solubility of β-cyclodextrin (relative to α- and γ-cyclodextrins) is a consequence of a less favorable (more positive) ΔH^0 *and* a less favorable (more negative) ΔS^0.

D-Glucose, the monomeric unit of which the cyclodextrins are composed, has a higher solubility than the cyclodextrins because of its low enthalpy of solution; in fact it has a slightly less favorable entropy of solution. Suggert[21] has related the low ΔH^0 of solution to the conformational similarity of the glucose hydroxyls to structured water*. Methyl α-glucoside shows the same pattern; its ΔH^0 is favorable but ΔS^0 is less favorable, relative to the cyclodextrins.

These thermodynamic comparisons show that the low solubilities of cyclodextrins (compared with acyclic saccharides) are a consequence of relatively unfavorable enthalpies of solution, partially offset by more favorable entropies of solution. Among the cyclodextrins, α- and γ-cyclodextrins have very similar thermodynamic properties, while the solubility of β-cyclodextrin is depressed by its comparatively unfavorable enthalpy and entropy of solution.

Empirical correlations. — It is of interest to examine these thermodynamic results for regularities for two reasons: first, to learn if the three cyclodextrins constitute a "series" in the conventional sense; second, possibly to achieve a means

*Jasra and Ahluwalia[22] have reported calorimetric heats of solution for saccharides, but these are extrapolated to infinite dilution, and hence are not directly comparable with those in Table IV.

TABLE VI

CALCULATED THERMODYNAMIC PARAMETERS

Cyclodextrin	ΔH^0 *(kcal · mol^{-1})*	ΔS^0 *(e.u.)*	ΔG^0 *at 25° (kcal · mol^{-1})*	
			Calc.	*Exptl.*
α	7.81	14.4	3.52	3.58
β	8.03	11.3	4.72	4.81
γ	7.94	15.0	3.47	3.34
δ	7.53	18.9[a]	1.89	—

[a]Assuming $m = 2$.

of estimating solubilities efficiently. The availability of only the minimum number (three) of data points is a disadvantage, but in this case unavoidable. Numerous hypotheses were examined, and the most successful ones were then embodied in Eqs. *1* and *2*, where n represents the number of glucose units per cyclodextrin molecule and m is the number of cyclodextrin molecules in the unit cell of the crystalline hydrate.

$$\Delta H^0/n = -0.155n + 2.232 \quad (1)$$
(correlation coefficient −0.972)

$$\Delta S^0/m = 1.95n - 8.10 \quad (2)$$
(correlation coefficient 0.991)

α-Cyclodextrin has $m = 4$, whereas $m = 2$ for β- and γ-cyclodextrins[23–25]. Eq. *1* is significant only at about the 90% level, and Eq. *2* at about the 95% level[26], because of the small number of data points.

Table VI shows how well Eqs. *1* and *2* generate ΔH^0 and ΔS^0, and, from these, ΔG^0 values for the three cyclodextrins. The table also gives predicted values for δ-cyclodextrin ($n = 9$), for which the value $m = 2$ was assumed. The ΔG^0 calculated for δ-cyclodextrin corresponds to a solubility of mole fraction 0.040, which is equivalent to 3.4 g/g water, 2.3 molal, 0.53ρ molar, or 77ρ percent w/v, where ρ is the density of the saturated solution. This result is consistent with Pulley's observation[27] that δ-cyclodextrin is very soluble.

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