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THERMOCHEMICAL INVESTIGATION OF β-CYCLODEXTRIN COMPLEXES WITH BENZOIC ACID AND SODIUM BENZOATE

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ABSTRACT

The formation of complexes of β-cyclodextrin with benzoic acid and sodium benzoate in water has been studied at 30° and 50°C using the flow-mix cell of a heat conduction microcalorimeter (LKB 2277). Values for the dissociation constant, change of enthalpy and entropy have been determined. Values for the dissociation constant vary widely, proving that the carboxyl group and carboxylate ion of benzoic acid and sodium benzoate enter the cavity of β-cyclodextrin first. It has been shown that direct calorimetric measurements can be used successfully to determine both the thermodynamic values for formation of the complex and values for the dissociation constant.

INTRODUCTION

Different methods are used for investigation of cyclodextrin complexes and determination of the values of dissociation constants and thermodynamical parameters. In the opinion of Szejtli [1] the direct measurement of thermodynamic data should be regarded as reliable and the collection of a large set of calorimetric data is desirable. Lewis and Hansen [2] have determined ΔH and ΔS values for many inclusion complexes of α- and β-cyclodextrins and have found the correlation between these values. Interesting results have been also obtained by Takeo and Kuge [3].

The complexes of β-cyclodextrin (β-CD) with benzoic acid and sodium benzoate have been chosen for study in this work. Using data from the literature it is possible to assume that carboxyl group and carboxylate ion enter the cyclodextrin cavity first followed by the benzene ring. Bender and Komiyama [4] have expressed their surprise at such a structure for the complex (since the hydrophilic carboxylate ion usually prefers to be in aqueous media) saying that "if the proposed structures of the complexes are correct, it will require a complete reconsideration of the binding forces of cyclodextrins". For the benzoic acid–CD complex the position of the

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carboxyl group inside the cavity seems to be proved. Tucker and Christian [5] have found from vapour pressure studies that the value of the formation constant for the 1 : 1 complex of benzene with β-CD is considerably greater than that for α- or γ-CD and they consider that benzene penetrates deeply into the cavity of β-CD. It has been shown by Connors and Pendergast [6] that the COOH group of terephthalic acid in the α-CD complex is also deeply inserted in the cavity. The great increase in the pK value (1.09 units) of benzoic acid [7] indicates a decrease in acidity as a consequence of complex formation and that the anion complex is probably less stable than the acid. Inoue et al. [8] have determined the orientation and position of benzoic acid in the α-CD inclusion complex by ^{13}C NMR chemical shifts and have shown that the carboxyl group is completely within the cavity of CD.

In this work we show that the flow-mix cells of heat conductive microcalorimeters can be used for determination of thermodynamic parameters and values of dissociation constants of these complexes.

THEORY

The scheme of a flow-mix cell is given in Fig. 1.

The solutions of β-CD with initial concentration $[C]'_0$ and substrate (benzoic acid or Na-benzoate) with initial concentration $[S]'_0$ are pumped into the measuring cell using a peristaltic pump with flow rates q_1 and q_2 (ml s^{-1}) respectively, where they are perfectly mixed. The reaction mixture leaves the cell with flow rate $q = q_1 + q_2$ (ml s^{-1}). Initial concentration of CD after mixing is

$$[C]_0 = [C]'_0 q_1/q \text{ (mmol ml}^{-1}) \qquad (1)$$

and of substrate

$$[S]_0 = [S]'_0 q_2/q \text{ (mmol ml}^{-1}) \qquad (2)$$

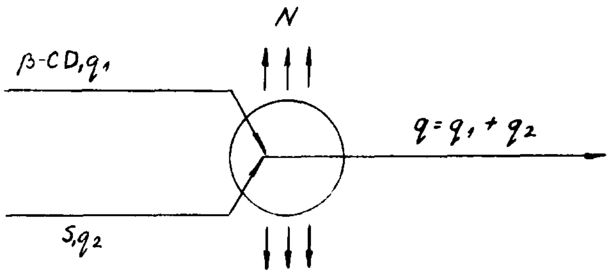

Fig. 1. Scheme for the flow-mix cell of the microcalorimeter.

The contact time of solutions in the measuring cell (e.g. about 1.4 min at $q = 0.005$ (ml s^{-1}) in this work) is sufficient for complex formation, which is a rapid process and is probably limited by diffusion rate. The steady concentrations of complex and free β-CD and substrate are [CS], [C], and [S] respectively.

For the reaction

$$\mathrm{C} + \mathrm{S} \rightleftarrows \mathrm{CS} \tag{3}$$

the coefficient of dissociation K_d can be expressed as follows

$$K_d = \frac{[\mathrm{C}][\mathrm{S}]}{[\mathrm{CS}]} \tag{4}$$

To find the K_d value two equations can be used:

$$[\mathrm{C}]_0 = [\mathrm{C}] + [\mathrm{CS}] \tag{5}$$

$$[\mathrm{S}]_0 = [\mathrm{S}] + [\mathrm{CS}] \tag{6}$$

and the third equation is necessary to calculate the [CS] value from experimental data.

The summary heat flow consists of the three components: heat flow caused by the complex formation N and two effects caused by dilution of β-CD and substrate which have to be measured separately. The value of N can be found by subtracting the dilution effects from the summary effect.

We can assume that the heat flow value N depends linearly on the quantity m of complex which is formed in 1 s in the measuring cell of the calorimeter

$$N = mQ_c(\mu\mathrm{W}) \tag{7}$$

where Q_c $(= -\Delta H)$ is the thermal effect of the complex formation (mJ mol^{-1} or μJ mmol^{-1}), and consequently

$$m = N/Q_c \ (\mathrm{mmol\ s^{-1}}) \tag{8}$$

It is evident that the steady concentration of the complex [CS] is equal to

$$[\mathrm{CS}] = m/q \ (\mathrm{mmol\ ml^{-1}\ or\ M}) \tag{9}$$

Using eqns. (4–6) and (8), the value of dissociation constant K_d can be expressed as follows

$$K_d = \frac{[([\mathrm{C}]'_0 q_1/q) - (m/q)][([\mathrm{S}]'_0 q_2/q) - (m/q)]}{m/q} \tag{10}$$

This expression can be transformed to

$$K_d = \frac{1}{q}([\mathrm{S}]'_0 q_1/m - 1)([\mathrm{C}]'_0 q_2 - m) \ (\mathrm{mmol\ ml^{-1}\ or\ M}) \tag{11}$$

The heat flow value N is measured directly by experiment. For the value m the thermal effect of the complex formation Q_c must be known. To measure this value directly a great excess of substrate is needed to guarantee the complexation of practically all β-CD molecules. This is often impossible because the solubility of the substrate may be insufficient or the K_d value is great. In this case eqn. (11) will have two unknown values, K_d and m (Q_c), and a system of eqn. (11), formed of the single equations for each experiment with different values for $[S]'_0$ and $[C]'_0$, can be solved by a non-linear regression method.

From the values for complex formation constants K ($K = K_d^{-1}$) ΔG values can be calculated

$$\Delta G = -RT \ln K \tag{12}$$

and from ΔG and ΔH values the change of entropy ΔS can be found. On the other hand if the values of K are determined at two temperatures (T_1 and T_2), it is possible to calculate ΔH values using the equation

$$\ln\left(K_{T_2}/K_{T_1}\right) = -(\Delta T/RT_1T_2)\ \Delta H \tag{13}$$

and compare this value with that found directly from calorimetric measurements.

EXPERIMENTAL

Apparatus

A LKB 2277 microcalorimeter (“Bioactivity Monitor”) was used in flow-mix mode at 30 and 50 °C. The solutions of β-CD and substrate were pumped into the measuring cell using a LKB 2132 microperspex pump with equal flow rates $q_1 = q_2 = 2.94 \times 10^{-3}$ ml s^{-1} (10.6 ml h^{-1}), summary flow rate 5.89×10^{-3} ml s^{-1} (21.2 ml h^{-1}). The heat flow was monitored with an LKB 2210 recorder potentiometer.

Fresh solutions of β-CD (Chinoin, Hungary), benzoic acid and sodium benzoate of chemically pure grade in distilled water were used.

RESULTS AND DISCUSSION

The results of the measurements carried out at 30 °C are presented in Tables 1 and 2 and illustrated in Figs. 2 and 3. From these data the values of the enthalpy change ΔH and dissociation constant of the complex K_d have been calculated according to eqn. (11) using a non-linear regression method. The results of calculation are summarized in Table 3 which also shows the results of investigation of the β-CD–benzoic acid complex at 50 °C (experiments at 50 °C were carried out under similar conditions).

TABLE 1

Thermochemical study of β-CD–benzoic acid complex in water at 30 ° C

Concentration of benzoic acid (M)		Molar ratio benzoic acid: β-CD	Heat flow corresponding to the complex formation, $N(\mu W)$	Percentage β-CD in complex
Before dilution $[S]_0'$	After dilution $[S]_0$			
0.00363	0.00182	1.70	32.3	34.9
0.00605	0.00303	2.83	45.0	48.2
0.00909	0.00455	4.25	55.1	59.2
0.0129	0.00645	6.03	62.0	67.9
0.0182	0.0091	8.50	70.0	75.4

Concentration of β-CD after dilution in all experiments 0.00107 M (1.21 g l^{-1}).

Using the K_d value the steady concentrations, [C], [S], [CS], and the amount of β-CD fixed by complexing can be found. The last values are presented in Tables 1 and 2 for experiments carried out at 30 ° C.

Benzoic acid and β-CD form a strong complex in water with significant change of enthalpy and negligible change of entropy. The value for the dissociation constant at 30 ° C is 0.0027 M. This indicates that the use of the flow-mix mode is also possible for analytical purposes, for the determination of β-CD content in solutions in the range of concentrations 1–3 g l^{-1} (8.8×10^{-4}–2.6×10^{-3} M). The solubility of benzoic acid in water at room temperature is only about 3 g l^{-1} (maximum possible concentration ~ 0.025 M). However, a molar ratio of benzoic acid: β-CD of 10–30 is sufficient to

TABLE 2

Thermochemical study of β-CD–sodium benzoate complex in water at 30 ° C

Concentration of sodium benzoate (M)		Molar ratio sodium benzoate: β-CD	Heat flow corresponding to the complex formation	Percentage β-CD in complex
Before dilution	After dilution			
0.0138	0.0069	6.5	6.2	6.4
0.0231	0.0116	10.8	9.6	10.3
0.0347	0.0174	16.3	13.4	14.7
0.0520	0.0260	24.3	18.1	20.5
0.0694	0.0347	32.4	22.0	25.6
0.1055	0.0528	49.3	29.7	34.4
0.1416	0.0708	66.2	34.0	41.3
0.210	0.105	98.1	44.5	51.1
0.279	0.140	131	51.3	58.2

Concentration of β-CD after dilution in all experiments 0.00107 M (1.21 g l^{-1}).

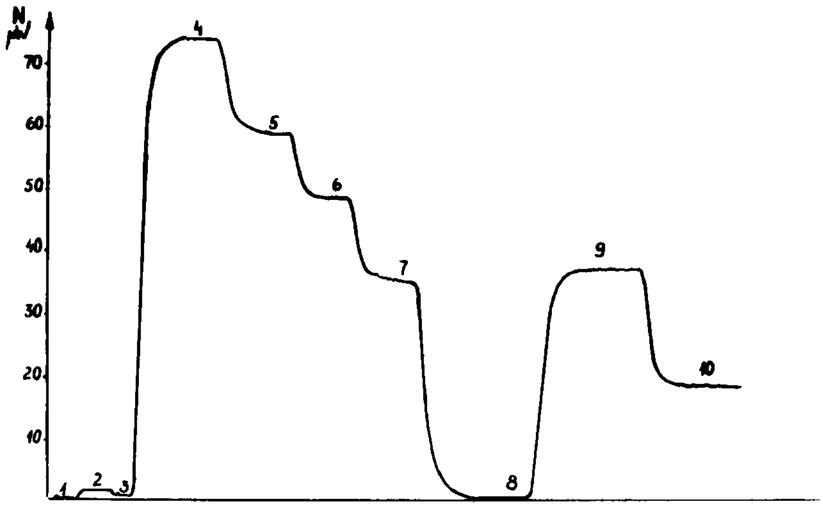

Fig. 2. Determination of heat flow by means of the flow-mix system. Sensitivity 100 μW, paper feed 2 mm min^{-1}. Concentration of β-CD in all experiments before dilution 0.00214 M. W, water; BA, benzoic acid; SB, sodium benzoate. 1, W + W; 2, W + BA 0.018 M; 3, β-CD + W; 4, β-CD + BA 0.018 M; 5, β-CD + BA 0.0090 M; 6, β-CD + BA 0.00605 M; 7, β-CD + BA 0.0036 M; 8, W + W; 9, β-CD + SB 0.052 M; 10, W + SB 0.052 M.

bind 75–77% of β-CD in the complex and nearly linear dependence of heat flow N on concentration of β-CD can be obtained at these conditions.

In Fig. 4 the experimental data are presented. An initial concentration of benzoic acid (before dilution in the calorimetry cell) of 2.2 g l^{-1} (0.018 M)

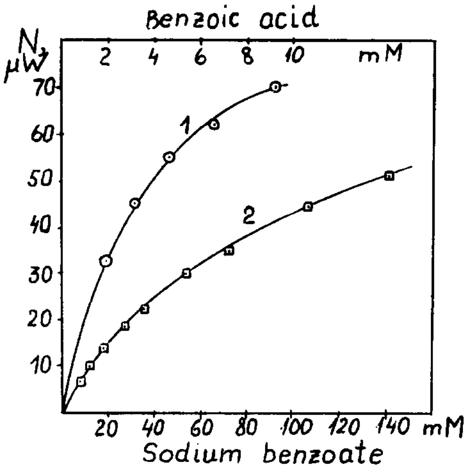

Fig. 3. Dependence of heat flow, corresponding to complex formation, on concentration of substrate after dilution. 1, benzoic acid; 2, sodium benzoate.

TABLE 3

The values of thermodynamic parameters and dissociation constants for β-CD complexes with benzoic acid and sodium benzoate

Temperature (°C)	Dissociation constant (M)	ΔG Calculated by eqn. (12)		ΔH Found from experiment		ΔS Calculated (e.u.)
		(kJ mol^{-1})	(kcal mol^{-1})	(kJ mol^{-1})	(kcal mol^{-1})	
Benzoic acid						
30	0.0027 ± 0.0002	−14.9 ± 0.2	−3.6 ± 0.1	−15.0 ± 1.0	−3.6 ± 0.2	0
50	0.0040 ± 0.0003	−14.8 ± 0.2	−3.5 ± 0.1	−19.0 ± 1.4	−4.5 ± 0.3	−3 ± 1
Sodium benzoate						
30	0.10 ± 0.01	−5.8 ± 0.3	−1.4 ± 0.1	−15.0 ± 1.5	−3.6 ± 0.4	−7 ± 1

was used in all experiments. The concentration range of β-CD (also before dilution) was 0.49–3.1 g l^{-1} (4.32×10^{-4}–2.73×10^{-3} M), flow rates were $q_1 = q_2 = 2.86 \times 10^{-3}$ ml s^{-1} (10.3 ml h^{-1}), temperature was 30 °C.

By linear regression the next equation has been found with correlation coefficient value 0.9995

$$N = 1.7 + 28.8[\mathrm{CD}]'_0 \qquad (14)$$

where β-CD concentration is expressed in g l^{-1} and heat flow in microwatts. Consequently, the concentration of β-CD can be calculated as follows

$$[\mathrm{CD}]'_0 = 0.034N - 0.06(\mathrm{g\ l^{-1}}) \qquad (15)$$

On average 20 min (including time for dilution effects measurements) are

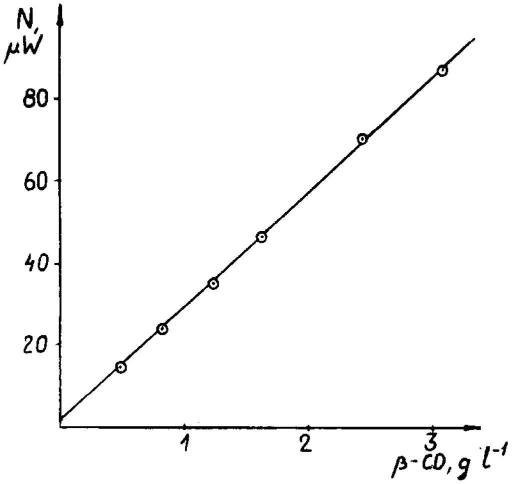

Fig. 4. Calorimetric determination of β-CD content in water solutions at 30 °C. Concentration of benzoic acid 2.2 g l^{-1} (0.018 M).

needed for one determination of β-CD in water solution and 5 ml of β-CD solution (containing maximum 15 mg of β-CD) is sufficient for the experiment.

As seen in Table 3, the value for the dissociation constant of the benzoic acid–β-CD inclusion compound increases 1.48 times as the temperature rises by 20 °C. Using eqn. (13), the ΔH value can be calculated (-16.0 kJ mol^{-1}) which is in good agreement with the value measured directly.

There is considerable difference between the complex formation of benzoic acid and sodium benzoate. The dissociation constant of the sodium benzoate–β-CD complex at 30 °C is 0.1 mol l^{-1} which is 37 times greater than that for the benzoic acid–β-CD compound. The significant entropy change has been observed. This significant difference in complex formation can be explained by the fact that the carboxyl group of benzoic acid and the carboxylate ion of benzoate are located in the cavity of β-CD which they enter first in the process of complex formation. However, the reason why β-CD with its hydrophobic cavity demonstrates such (though reserved) "hospitality" to the hydrophilic carboxylate group remains undetermined.

We recommend the systematic use of microcalorimetry for the direct determination of thermodynamic values and dissociation constants of molecular complexes. It is helpful that if flow-mix systems are used the dependence of heat flow on substrate concentration contains enough information to calculate three thermodynamic parameters (ΔG, ΔH, ΔS) as well as values for the dissociation or complex formation constants.

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