

## CALORIMETRIC STUDIES OF BENZOIC ACID–CYCLODEXTRIN INCLUSION COMPLEXES

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### ABSTRACT

Direct calorimetric measurements have been used to study the formation of complexes of  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrin with benzoic acid at 30°C in water solutions by means of the flow-mix cell of the microcalorimeter LKB 2277 (Bioactivity Monitor). The values of the dissociation constants and the changes of thermodynamic parameters (Gibbs energy, enthalpy and entropy) have been calculated and compared. It has been shown that  $\alpha$ - and  $\beta$ -cyclodextrin form stable 1:1 complexes with benzoic acid, and that  $\gamma$ -cyclodextrin forms more unstable 1:1 and 1:2 inclusion compounds.

### INTRODUCTION

The structure of cyclodextrin (CD) inclusion complexes depends directly on the sizes of CD cavities and of the guest molecules. Precise vapour pressure–solubility measurements by Tucker and Christian [1] show that 1:1, 2:1 and 1:2 complexes form in water solutions between cyclodextrins and benzene. 1:1 inclusion compounds of  $\alpha$ -CD are known with benzoic acid, *p*-hydroxybenzoic acid, *p*-nitrophenol [2], some azo compounds [3] and different substituted benzene derivatives [4].

The calorimetric determination of the thermodynamic parameters of complex formation seems to be reliable. Takagi and Maeda [5] investigated the transfer process of alcohols to  $\alpha$ - and  $\beta$ -CD using a twin-microcalorimeter. The same type of calorimeter has been used by Takeo and Kuge [6]. They measured the heats of complex formation of  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD with tri-iodide ion and *p*-nitrophenolate. We have shown that flow-mix systems of Calvet-type microcalorimeters can be successfully used for direct calorimetric measurements; for example, the results for  $\beta$ -CD–benzoic acid 1:1 complex have been published [7].

In the present study the same method was used for determining the values of the thermodynamic parameters and dissociation constants for the benzoic acid inclusion compounds with  $\alpha$ - and  $\gamma$ -CD.

## THEORY

Two models are considered for the complex formation between cyclodextrin C and substrate S after mixing of solutions in the flow-mix cell of the microcalorimeter. Initial concentrations of these compounds after mixing are  $[C]_0$  and  $[S]_0$ , respectively.

*Model I: the complex 1 : 1 is formed*

For the reaction



with dissociation constant of complex

$$K_d = \frac{[C][S]}{[CS]} \quad (\text{M or mmol ml}^{-1}) \quad (2)$$

the material balance is

$$[C]_0 = [C] + [CS] \quad (3)$$

$$[S]_0 = [S] + [CS] \quad (4)$$

From eqns. (2–4) the next root equation can be obtained

$$[CS]^2 + ([C]_0 + [S]_0 + K_d) [CS] - [C]_0[S]_0 = 0 \quad (5)$$

In calorimetric experiments carried out in flow-mix mode at constant total flow rate  $q$  ( $\text{ml s}^{-1}$ ), the quantity of complex CS formed in 1 s is equal to  $q[CS]$  ( $\text{mmol s}^{-1}$ ) and if the heat effect of complex formation is  $Q$  ( $= -\Delta H$ ) ( $\text{mJ mol}^{-1}$  or  $\mu\text{J mmol}^{-1}$ ), the observed heat flow  $N$  caused by complex formation can be expressed

$$N = q[CS]Q \quad (\mu\text{W}) \quad (6)$$

By use of eqns. (5) and (6), from experimental data  $N = f([S]_0, [C]_0)$  the values of  $K_d$  and  $Q$  can be calculated by non-linear regression methods, minimizing the sum  $(N_{\text{calc}} - N_{\text{exptl}})^2$ , where  $N_{\text{calc}}$  and  $N_{\text{exptl}}$  are calculated and experimental values of heat flow.

*Model II: the complexes 1 : 1 and 1 : 2 are formed simultaneously*

For the reaction



the material balance is

$$[C]_0 = [C] + [CS] + [CS_2] \quad (8)$$

$$[S]_0 = [S] + [CS] + (1/2)[CS_2] \quad (9)$$

and the dissociation constants  $K_1$  and  $K_2$  of complexes CS and  $CS_2$  have the values

$$K_1 = \frac{[C][S]}{[CS]} = \frac{([C]_0 - [CS] - [CS_2])([S]_0 - [CS] - (1/2)[CS_2])}{[CS]} \quad (10)$$

$$K_2 = \frac{[CS][S]}{[CS_2]} = \frac{[CS]([S]_0 - [CS] - (1/2)[CS_2])}{[CS_2]} \quad (11)$$

For [CS] the next cubic equation can be derived using eqns. (10) and (11)

$$\begin{aligned} [CS]^3(-1/2K_2 - 1/4K_1) + [CS]^2K_2(K_2 + 3/2[S]_0 - 1/2[C]_0 - K_1) \\ + [CS]K_2(-[S]_0K_2 - [C]_0K_2 - K_1K_2 + 1/2[C]_0[S]_0 - [S]_0^2) \\ + [C]_0[S]_0K_2^2 = 0 \end{aligned} \quad (12)$$

At flow rate  $q$ , the quantities of complexes CS and  $CS_2$  formed in 1 s are equal to  $q$  [CS] and  $q$ [ $CS_2$ ]. If the heat effects of complex formation are  $Q_1$  and  $Q_2$  respectively, the total heat flow  $N$  can be expressed as follows

$$N = q([CS]Q_1 + [CS_2]Q_2) \quad (13)$$

From eqn. (11)

$$[CS_2] = \frac{[CS][S]_0 - [CS]^2}{K_2 + 1/2[CS]} \quad (14)$$

Consequently,

$$N = q\left([CS]Q_1 + \frac{[CS][S]_0 - [CS]^2}{K_2 + 1/2[CS]}Q_2\right) \quad (15)$$

Therefore, using eqns. (12) and (15), the values of the four constants  $K_1$ ,  $K_2$ ,  $Q_1$  and  $Q_2$  can be found from experimental data of heat flow measurements by the non-linear regression method, minimizing the sum  $(N_{\text{calc}} - N_{\text{expt}})^2$ . Of course, when solving the cubic equation (12), the real solution with the physical meaning must be found (the conditions  $[CS] > 0$  and  $[CS] < [C]_0$  should be fulfilled).

## EXPERIMENTAL

### *Materials and apparatus*

“Chinoin” cyclodextrins were used in the experiments.  $\alpha$ - and  $\gamma$ -CD were kindly given by Prof. E. László from Budapest Technical University. Ben-

zoic acid of chemically pure grade was used. All solutions were prepared in distilled water.

A LKB 2277 microcalorimeter (Bioactivity Monitor) was used in flow-mix mode at 30 °C. The solutions of CD and substrate (benzoic acid, BA) were pumped into the measuring cell using a LKB microperpex pump with equal flow rates ( $q_1 = q_2$ ) of about 10 ml h<sup>-1</sup>, and a total flow rate  $q$  about 20 ml h<sup>-1</sup>. The exact value of  $q$  was measured in all experiments. The heat flow was monitored with a LKB 2210 potentiometric recorder. Some details of the thermochemical method used have been published earlier [7].

## RESULTS AND DISCUSSION

Experiments have been carried out using the flow-mix cell of the calorimeter with water solutions of  $\alpha$ -CD,  $\gamma$ -CD and benzoic acid at different molar ratios of BA : CD. Two or three parallel measurements were made and

TABLE 1

Thermochemical study of  $\alpha$ - and  $\gamma$ -CD complexes with benzoic acid in water at 30 °C (flow rate 0.0054 ml s<sup>-1</sup> (19.4 ml h<sup>-1</sup>))

Concentration after dilution (M)		Molar ratio BA : CD	Heat flow $N$ ( $\mu$ W)		Calculated percentage CD in complexes		
[C] <sub>0</sub> (CD)	[S] <sub>0</sub> (BA)		Exptl.	Calc.	1 : 1	1 : 2	Total
$\alpha$ -CD + benzoic acid							
0.00129	0.009	6.98	208.5	208.3	88.0	—	88.0
0.00217	0.009	4.15	363.8	346.0	86.9	—	86.9
0.00257	0.009	3.50	405	407.1	86.4	—	86.4
0.00424	0.009	2.12	637.5	650.2	83.6	—	83.6
0.00514	0.009	1.75	772.5	770.8	81.8	—	81.8
0.01029	0.009	0.875	1261.2	1258.2	66.7	—	66.7
0.01029	0.00751	0.730	1087.5	1102.0	58.4	—	58.4
0.01029	0.0045	0.437	727.5	707.6	37.5	—	37.5
0.01029	0.00257	0.250	393.8	415.9	22.0	—	22.0
$\gamma$ -CD + benzoic acid							
0.00058	0.00895	15.43	36.6	33.7	36.8	29.0	65.8
0.00116	0.00895	7.72	70.2	65.4	36.8	28.0	64.8
0.001835	0.00895	4.88	93	96.0	35.2	25.8	61.0
0.002895	0.00895	3.09	150	146.7	36.0	24.7	60.7
0.00381	0.00895	2.35	180.9	184.1	35.9	23.3	59.2
0.00579	0.00895	1.55	244	248.4	35.2	20.2	55.4
0.01025	0.00895	0.873	331	330.8	32.3	14.1	46.7
0.01025	0.00448	0.437	126	121.2	20.5	4.0	24.5
0.01025	0.00224	0.218	45	45.5	10.9	1.1	12.0
0.00579	0.00448	0.773	94	97.2	24.2	6.4	30.8

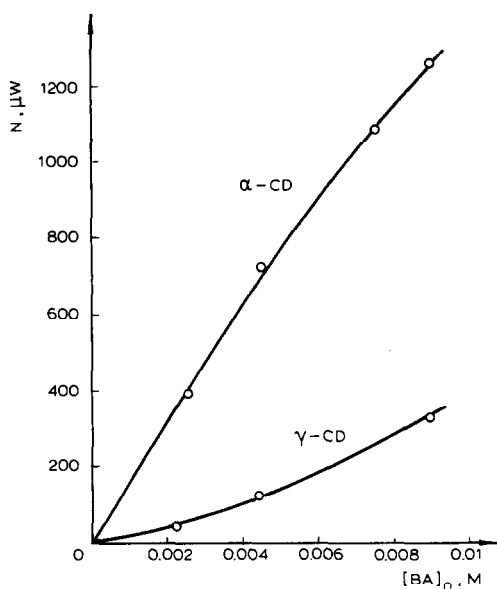


Fig. 1. Dependence of heat flow on concentration of benzoic acid in solution. Concentration of CD 0.0103 M.

the mean value of the heat flow was calculated. The results of the measurements are given in Table 1 and some of them are illustrated in Fig. 1. As can be seen on this figure, where CD is in excess the appearances of the curves for  $\alpha$ -CD and  $\gamma$ -CD are different.

The mathematical analysis of the experimental data prove that for  $\alpha$ -CD-BA complexes, the theoretical model I is reliable; the complex structure is 1 : 1. (This structure was also found earlier for  $\beta$ -CD-BA complexes [7]). For  $\gamma$ -CD-BA complexes, the results of heat flow measurements cannot be explained by the models which consider the formation of a single complex, either 1 : 1, 1 : 2, or 2 : 1. But using the theoretical model II described above, we have obtained satisfactory results. This suggests that, in water solutions, two molecular complexes between  $\gamma$ -CD and BA 1 : 1 and 1 : 2 are simultaneously formed.

The values of the dissociation constants ( $K_d$ ,  $K_1$ ,  $K_2$ ) and the  $\Delta H$  of complex formation were determined by computer, and were used to calculate the changes of excess Gibbs energy and entropy. All the results are summarized in Table 2. Our earlier results for  $\beta$ -CD complexes are included in this table.

In Fig. 2, the calculated distribution of CD forms for  $\alpha$ -/and  $\gamma$ -CD in steady-state conditions in solution is demonstrated (concentrations as in Fig. 1).

Binding of the first molecule of BA in the cavity of  $\gamma$ -CD causes a small change in enthalpy ( $-4.5 \text{ kJ mol}^{-1}$ ), but the subsequent formation of the

TABLE 2  
The values of thermodynamic parameters and dissociation constants for  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD complexes with benzoic acid at 30 °C

CD	Molar ratio CD:BA	Dissociation constant (M)	$\Delta G$ (kJ mol <sup>-1</sup> )	$\Delta H$ (kJ mol <sup>-1</sup> )	$\Delta S$ (J mol <sup>-1</sup> K <sup>-1</sup> )	(e.u.)		
$\alpha$	1:1	0.0011 ± 0.0001	-17.2 ± 0.2	-4.1 ± 0.1	-33.9 ± 2.0	-8.1 ± 0.5	-55 ± 5	-13 ± 1
$\beta$	1:1	0.0027 ± 0.0002	-14.9 ± 0.2	-3.6 ± 0.1	-15.0 ± 1.0	-3.6 ± 0.2	0	0
$\gamma$	1:1	0.0080 ± 0.0004	-12.1 ± 0.2	-2.9 ± 0.1	-4.5 ± 0.2	-1.1 ± 0.1	25 ± 2	6 ± 1
	1:2	0.0110 ± 0.0005	-11.4 ± 0.2	-2.7 ± 0.1	-31.5 ± 2.0	-7.5 ± 0.5	-66 ± 5	-16 ± 1

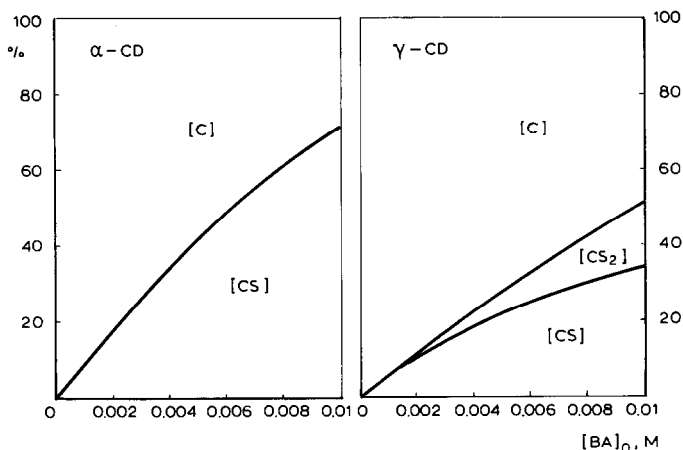


Fig. 2. Distribution of CD-forms in the system CD-BA-water.

1 : 2 complex leads to a significant change ( $-31.5 \text{ kJ mol}^{-1}$ ). And so the detected heat flow value corresponding to the complex formation between  $\gamma$ -CD and BA significantly increases at higher concentrations of benzoic acid in solution.

By comparing the dissociation constants and  $\Delta G$  values of  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD complexes we can conclude that the most stable is the 1 : 1 complex of BA with  $\alpha$ -CD; BA forms a fairly stable complex with  $\beta$ -CD. Relatively unstable inclusion compounds are formed by  $\gamma$ -CD with one or two molecules of BA as a guest.

As seen in Fig. 3, interesting regularities have been observed in the change of the thermodynamic parameters. The large changes of enthalpy are balanced by a noticeable decrease in entropy value. Hence, the differences of  $\Delta G$  values are relatively insignificant.

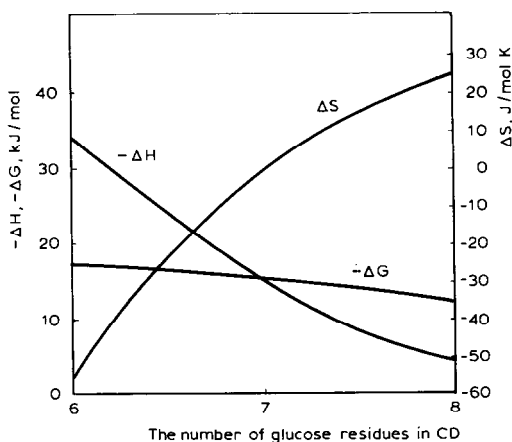


Fig. 3. Dependence of  $\Delta H$ ,  $\Delta G$  and  $\Delta S$  of the complex formation on the structure of CD.

The observed regularities may be explained by the different structure of the complexes and the differences in their binding forces. NMR should be used to further these investigations.

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