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Thermochimica Acta 259 (1995) 187–198

thermochimica
acta

Beta-cyclodextrin/citric acid complexation equilibrium: thermodynamic study. Apparent solubility of βCD in aqueous solutions of citric acid

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Received 22 November 1994; accepted 14 January 1995

Abstract

The thermodynamic parameters (K, ΔH, ΔG, ΔS) for the complexation equilibrium of citric acid with beta-cyclodextrin (βCD) in aqueous solution (1:1 stoichiometric reaction) have been determined by calorimetry at 298 and 308 K.

The apparent solubility and the percentage of complexed βCB are calculated for various citric acid concentrations (from 0 to 1 $\mathrm{mol\,l^{-1}}$).

Keywords: Beta-cyclodextrin; Calorimetry; Citric acid; Complexation; Solubility

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1. Introduction

Many studies on beta-cyclodextrin (βCD) complex formation equilibria have led to the determination of equilibrium constants and associated thermodynamic parameters such as the free enthalpy, enthalpy and entropy.

As reported by Saenger [1] and Clarke et al. [2] (and references cited therein), many of these results were obtained using a variety of techniques such as solubility, polarography, potentiometry and spectroscopic methods. These methods do not appear to be suitable for all compounds. Microcalorimetry is an important way to measure directly the thermodynamic properties of complex formation.

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SSDI 0040-6031(95)02260-0

The use of two different calorimetric techniques allows verification of the hypothesis and correlation of the thermodynamic data obtained.

2. Materials

Commercially available citric acid (Aldrich) of analytical grade was used. Its purity is higher than 99%.

Beta-cyclodextrin was supplied by Roquette under reference βCD-16. The hydration ratio, as measured by thermogravimetry, was found to be 13.6%.

3. Thermodynamic study

3.1. Apparatus

3.1.1. Flow microcalorimeter

An LKB 10700-1 differential calorimeter is used in the dynamic flow-mix mode [3]. The injection of the aqueous solutions of reagents is carried out by a peristaltic pump whose flow rate have previously been standardized. The reproducibility of the electric calibrations is better than 98%. The calorimeter is standardized using the THAM (Tris-(hydroxymethyl)-aminomethane) reaction with 0.1M HCl. The result is in agreement with the reference value [4].

3.1.2. Isoperibolic calorimeter

The heats of dissolution are determined at 298 K using an isoperibolic calorimeter built after the model of Sunner and Wadsö [5]. Beta-cyclodextrin powder is contained in a 1 cm^3 PTFE vessel which is opened into a 100 cm^3 cell.

3.2. Analysis of the calorimetric measurements

3.2.1. Treatment of flow calorimetric data: determination of K, ΔH, ΔG and ΔS

Assuming the 1:1 stoichiometric reaction, $\beta\text{CD} + \text{S} \rightleftharpoons \beta\text{CDS}$, the complex formation constant is given by

$$K = [\beta\text{CDS}]/[\beta\text{CD}][\text{S}] = 1/K_\text{D} \tag{1}$$

where K_D is the dissociation constant of the complex.

Before mixing in the calorimeter, the concentrations of the βCD and substrate aqueous solutions are $[\beta\text{CD}]'_0$ and $[\text{S}]'_0$. Just after mixing and before complexation takes place, their respective concentrations are $[\beta\text{CD}]_0$ and $[\text{S}]_0$ because of the dilution effect. After the reaction has occurred, the concentrations of the three components are [βCDS], [βCD] and [S]. Knowing that the flow rate of the βCD solution is d_1 and that of the substrate solution is d_2, the total flow rate in the cell is

$d = d_1 + d_2$ and the following equations can be obtained

$$[\beta\mathrm{CD}]_0 = [\beta\mathrm{CD}]'_0\, d_1/d \tag{2}$$

$$[\mathrm{S}]_0 = [\mathrm{S}]'_0\, d_2/d \tag{3}$$

Because of the material balances of the components

$$[\beta\mathrm{CD}]_0 = [\beta\mathrm{CD}] + [\beta\mathrm{CDS}] \tag{4}$$

$$[\mathrm{S}]_0 = [\mathrm{S}] + [\beta\mathrm{CDS}] \tag{5}$$

If m represents the quantity of complex formed during one second, then

$$[\beta\mathrm{CDS}] = m/d \tag{6}$$

Considering all these equations, the dissociation constant of the complex can be expressed as

$$K_\mathrm{D} = \frac{1}{K} = \frac{([\beta\mathrm{CD}]_0 - (m/d))\,([\mathrm{S}]_0 - (m/d))}{m/d} \tag{7}$$

If Q is the thermal effect of the complex formation ($Q = -\Delta H$), the heat flow rate P, directly measured by experiment while complexation occurs is given by $P = mQ$. Then if m is replaced by P/Q, K_D becomes

$$K_\mathrm{D} = [\beta\mathrm{CD}]_0\,[\mathrm{S}]_0\,\frac{Qd}{P} - [\beta\mathrm{CD}]_0 - [\mathrm{S}]_0 + \frac{P}{Q}\frac{1}{d} \tag{8}$$

Eq. (8) has two unknown parameters, K_D and Q. From experimental values of P measured for various concentrations $[\beta\mathrm{CD}]_0$ and $[\mathrm{S}]_0$, different expressions of Eq. (8) can be written that allow the calculation of K_D and Q at the experiment temperature. The resulting system of equations can be solved either mathematically or graphically. Then, the changes of free enthalpy and of entropy can be obtained from Eqs. (9) and (10)

$$\Delta G^T = -RT \ln K \tag{9}$$

$$\Delta S^T = \frac{\Delta H^T - \Delta G^T}{T} \tag{10}$$

Mathematical method. Eq. (8) can be expressed as: $K_\mathrm{D} = aQ - b + c/Q$, where $a = [\beta\mathrm{CD}]_0[\mathrm{S}]_0\,(d/P)$, $b = [\beta\mathrm{CD}]_0 + [\mathrm{S}]_0$, and $c = P/d$ are three known variables, while Q and K_D are constants.

The use of the least-squares method [6] leads to the following two equations

$$\frac{1}{K} = \frac{1}{n}\left(Q\sum a_i - \sum b_i + \frac{\sum c_i}{Q}\right) \tag{11}$$

$$\frac{1}{K} = \frac{Q^3\sum a_i - Q^2\sum a_i b_i + \sum b_i c_i - (\sum c_i/Q)}{Q^2\sum a_i - \sum c_i} \tag{12}$$

where n is the number of experimental values measured while varying the concentrations $[\beta\mathrm{CD}]_0$ and $[\mathrm{S}]_0$. Combining Eqs. (11) and (12) establishes a biquadratic equation in Q

$$Q^4\left(n\sum a_i^2-\left(\sum a_i\right)^2\right)-Q^3\left(n\sum a_ib_i-\sum a_i\sum b_i\right)+Q\left(n\sum b_ic_i-\sum b_i\sum c_i\right)$$
$$-\left(n\sum c_i^2-\left(\sum c_i\right)^2\right)=0 \tag{13}$$

Eq. (13) is solved using the classic method. The four roots are selected and the more reasonable Q value is kept. The formation constant K can then be calculated from Eq. (11) or Eq. (12).

Graphical method. The arbitrary choice of various Q values for each different Eq. (8) gives a bundle of curves that should intersect at one point. This point represents the value of the dissociation constant K_D at the temperature of the experiment.

This graphical method has two main advantages: it is easy to carry out and it leads directly to the uncertainties in K_D and Q.

3.2.2. *Treatment of data from isoperibolic calorimetry*

The calculation of the enthalpy change in the formation of a βCD–citric acid complex is carried out from the measurements of the enthalpies of dissolution of anhydrous βCD in solutions containing various amounts of citric acid. Let us hypothesize that when βCD is introduced into an aqueous solution of citric acid, the dissolution occurs before complexation takes place.

From a thermodynamic point of view, the calculations are carried out using the cycle shown in scheme 1.

$$\begin{tikzcd}
\beta\mathrm{CD}_{\mathrm{anhydrous}}+\mathrm{AC}_{\mathrm{solution}} \arrow[rr, shift left, "Q_{\mathrm{exp}}"] \arrow[rr, leftarrow, shift right] \arrow[dr, "\Delta H_{\mathrm{dissolution}}"'] & & (\beta\mathrm{CD}/\mathrm{AC})_{\mathrm{solution}} \\
& \beta\mathrm{CD}_{\mathrm{solution}}+\mathrm{AC}_{\mathrm{solution}} \arrow[ur, shift left] \arrow[ur, leftarrow, shift right, "Q_{\mathrm{complexation}}"'] &
\end{tikzcd}$$

$Q_{\mathrm{complexation}}=\alpha\,\Delta H_{\mathrm{complexation}}$, where α is the degree of advance of the reaction. So, for each citric acid concentration, the enthalpy of the complex formation reaction can easily be calculated from the enthalpy of dissolution of anhydrous βCD in water and from the thermal effect measured during the experiment.

$$\Delta H_{\mathrm{complexation}}=Q_{\mathrm{complexation}}/\alpha=(Q_{\mathrm{exp}}-\Delta H_{\mathrm{dissolution}})/\alpha$$

3.3. *Results*

3.3.1. *Flow calorimetry*

Aqueous solutions of βCD, on the one hand, and of citric acid, on the other, are introduced into the mixing cell with the same flow rate, $d_1=d_2=2.06\times10^{-6}\,\mathrm{l/s^{-1}}$.

The concentration of βCD solution is constant while the concentration of the citric acid solutions is variable.

Two series of experiments have been carried out, at 298 and at 308 K. Table 1 gives the heat flow rate P measured at each citric acid concentration and at the two temperatures. The concentration values take the dilution effect of mixing into account.

3.3.2. Graphical solution

Figs. 1 and 2 respectively present the bundles of curves giving dissociation constant K_D versus the absolute value of the heat of reaction at 298 and 308 K. Each straight line is obtained for one particular concentration of citric acid. The limits of the area where the curves intersect define the most probable values of $K_D = 1/K$ and of Q. The results obtained from Figs. 1 and 2 are given in Table 2.

3.3.3. Mathematical solution

Mathematical treatment of the $K_D = f(Q)$ equations leads to more precise values of K and ΔH. The results are presented in Table 3.

The complexation of citric acid by βCD is exothermic. The decrease in the equilibrium constant K for the complex formation when the temperature increases is logical. In spite of only a slight increase in temperature, the enthalpies of the reactions at 298 and 308 K are appreciably different. This result is probably the consequence of a change in the hydration state of βCD in solution between these temperatures. Thus, the energetic state is not the same at the two different temperatures.

3.3.4. Isoperibolic calorimetry

Samples of about 150 mg of anhydrous βCD are dissolved in 100 ml of aqueous solutions of citric acid at 298 K. The concentration of βCD is then about 1.3×10^{-3} mol l^{-1}. The concentrations of the solutions of citric acid vary between 0.1 and 1.1 mol l^{-1}, so that the amount of citric acid is always much higher than that of βCD. Table 4 presents the values obtained for the thermal effect (Q_{exp}) measured during

Table 1
Heat flow rate P measured from flow calorimetry at 298 and 308 K, while mixing a solution of βCD with a solution of citric acid. (The concentration values take the dilution effect of mixing into account)

[βCD]/(mol l^{-1})	[Citric acid]/(mol l^{-1})	P at 298 K/μW	P at 308 K/μW
5×10^{-3}	0.049	186	168
5×10^{-3}	0.061	210	192
5×10^{-3}	0.077	232	216
5×10^{-3}	0.096	252	240
5×10^{-3}	0.120	281	268
5×10^{-3}	0.150	301	292

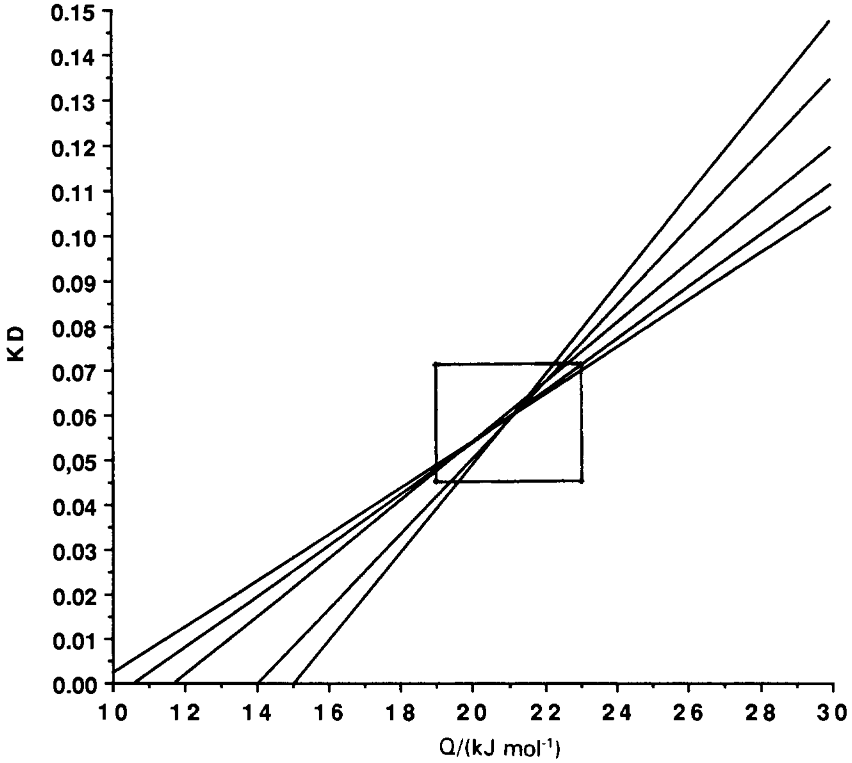

Fig. 1 Graphical solution of the system of equations $K_D = f(Q)$ at 298 K. The limits of the area where the straight lines obtained for each particular citric acid concentration intersect define the values of K_D and Q and their ranges of incertainties.

dissolution of anhydrous βCD in solutions containing variable amounts of citric acid. The thermal effects for complexation ($Q_{\mathrm{complexation}}$) are calculated using the method previously described. The enthalpy change corresponding to the dissolution of anhydrous βCD in water at 298 K used for the calculation is $\Delta H_{\mathrm{dissolution}} = -75.0$ J per g of anhydrous βCD. This value was obtained in our laboratory from isoperibolic calorimetry measurements.

The variation of $Q_{\mathrm{complexation}}$ versus citric acid concentration is proportional to the quantity of complex formed. Above a citric acid concentration of 0.5 mol l^{-1}, the equilibrium limit is reached. Taking into account the concentration ratio (citric acid)/βCD, the average thermal effect corresponding to this limit is -22 J per g of anhydrous βCD (-25 kJ mol^{-1}). This value approximately represents the complexation enthalpy. To compare these results with those obtained from flow calorimetry at 298 K, we have calculated the values of $Q_{\mathrm{complexation}}$ (called Q_{calc}) from the constant $K = 16.4$ and from the enthalpy of the complex formation $\Delta H = -18.7$ J per g of anhydrous βCD (-21.2 kJ mol^{-1}).

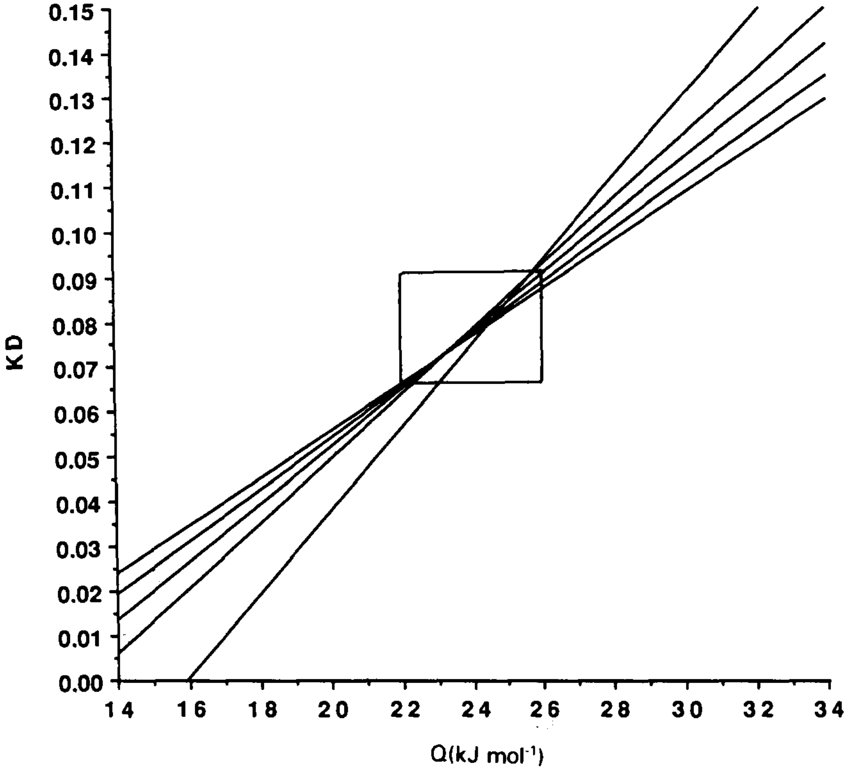

Fig. 2. Graphical solution of the system of equations $K_D = f(Q)$ at 308 K. The limits of the area where the straight lines obtained for each particular citric acid concentration intersect define the values of K_D and Q and their ranges of incertainties.

Table 2

Complexation of citric acid by βCD: ranges of uncertainties for the thermal effect $Q = -\Delta H$ and for the equilibrium constant $K = 1/K_D$ (graphical determination from flow calorimetric data)

T/K	Q_{min}/(kJ mol^{-1})	Q_{max}/(kJ mol^{-1})	$K_{D_{min}}$	$K_{D_{max}}$	K_{min}	K_{max}
298	19	23	0.071	0.045	14	22
308	22	26	0.091	0.066	11	15

3.3.5. *Principle of calculation of* Q_{calc}

Whatever the citric acid concentration, when equilibrium is reached the following equations can be expressed

$$\frac{[\beta\text{CD–AC}]_{eq}}{[\beta\text{CD}]_{eq}[\text{AC}]_{eq}} = K \tag{14}$$

$$[\text{AC}]_{initial} = [\text{AC}]_{eq} + [\beta\text{CD–AC}]_{eq} \approx [\text{AC}]_{eq} \tag{15}$$

Table 3

Complexation of citric acid by βCD: equilibrium constant values and changes of thermodynamic parameters at 298 and 308 K. Results calculated from flow calorimetric data by mathematical treatment of the $K_D = f(Q)$ equations

Temperature/K	K	$\Delta H/(\mathrm{kJ\,mol^{-1}})$	$\Delta G/(\mathrm{kJ\,mol^{-1}})$	$\Delta S/(\mathrm{J\,mol^{-1}\,K^{-1}})$
298	16.4	−21.2	−6.9	−47.9
308	12.4	−24.6	−6.4	−58.9

Table 4

Thermal effect (Q_{exp}) measured when samples of anhydrous βCD, of about 150 mg, are solved at 298 K in 100 ml of aqueous solutions of citric acid and calculated thermal effect of the complexation ($Q_{complexation}$). Results from isoperibolic calorimetry

[Citric acid]/(mol l^{-1})	0	0.1	0.2	0.3	0.5	0.9	1.1
Q_{exp}/(J per g βCD_{anh})	−75.0	−86.6	−91.6	−93.7	−97.1	−97.0	−96.5
$Q_{complexation}$/(J per g βCD_{anh})	0	−11.6	−16.6	−18.7	−22.1	−22.0	−21.5

Taking into account the low concentrations of βCD used in isoperibolic calorimetry experiments, the amount of complexed citric acid is negligible in view of the initial amount of citric acid.

From Eqs. (14) and (15), it is possible to calculate the ratio $R_{(\beta CD-AC)}$ of complex formed for 1g of anhydrous βCD in a solution of citric acid

$$R_{(\beta CD-AC)} = \frac{[\beta CD\text{–}AC]_{eq}}{[\beta CD]_{initial}} = \frac{K[AC]}{1 + K[AC]}$$

The released heat is proportional to the amount of complex formed

$$Q_{calc} = R_{(\beta CD-AC)}\,\Delta H \tag{16}$$

The ratio of complex and the calculated heat of complexation Q_{calc}, when the concentrations of the citric acid solutions vary from 0 to 1 mol l^{-1}, are given in Table 5.

The variations of Q_{calc} and of $Q_{complexation}$ versus citric acid concentration are plotted in Fig. 3.

Table 5

Complexation of citric acid by βCD. Percentage of formed complex $R_{(\beta CD-AC)}$ and calculated thermal effect of the reaction (Q_{calc}). Results from flow calorimetry

[Citric acid]/(mol l^{-1})	0.05	0.1	0.2	0.3	0.5	0.7	0.9	1	1.1
$R_{(\beta CD-AC)}$/%	45.0	62.2	76.6	83.1	89.1	92.0	93.6	94.2	94.7
Q_{calc}/(J per g βCD_{anh})	−8.4	−11.6	−14.3	−15.5	−16.6	−17.2	−17.5	−17.6	−17.7

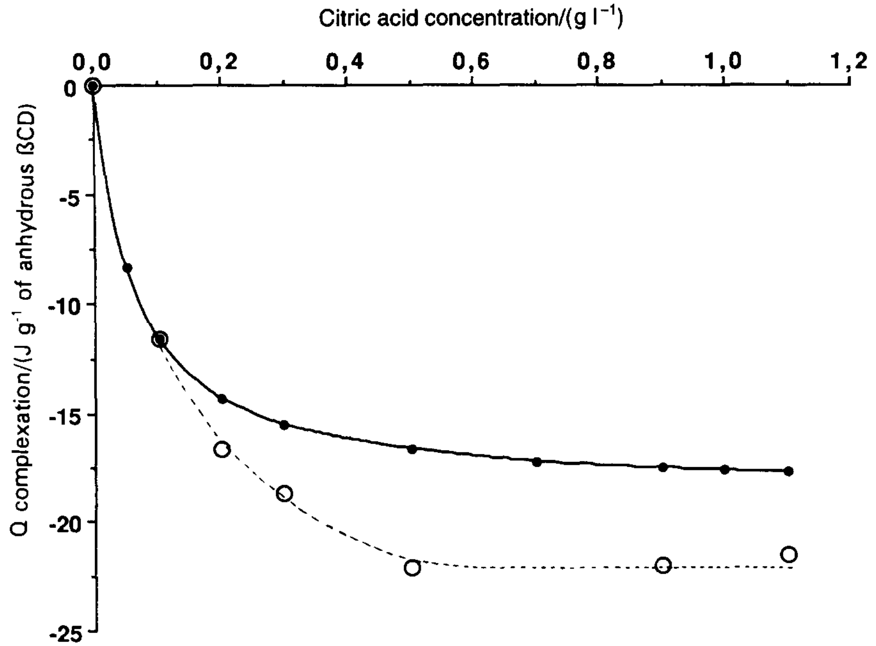

Fig. 3. Comparison of the results obtained from flow calorimetry (●) and from isoperibolic calorimetry (○) in the determination of the thermal effect of the complexation for various citric acid concentrations.

The comparative analysis of the curves enables our hypothesis to be discussed. The shapes of these curves are very similar in the concentration range studied and the progress of the reaction of complexation observed by isoperibolic calorimetry is identical with the degree of advance of the complexation calculated from the flow calorimetry data. The average difference observed in the heats of reaction corresponding to the two cases when equilibrium is reached, is about 4 J per g of anhydrous βCD. This is significant but can probably be explained by the different hydration states of βCD.

In our hypothesis concerning the experiments in isoperibolic calorimetry, we assume that the first stage of the reaction consists of a complete dissolution of βCD. The second stage is the complexation. This means that βCD undergoes total hydration before it reacts with citric acid to form the complex. However, the results above seem to indicate that the first stage is not complete, i.e. the hydration state of βCD is not complete when complexation takes place.

4. Apparent solubility of beta-cyclodextrin in citric acid aqueous solutions at 298 K

4.1. Method

The maximum solubilities of βCD in various citric acid solutions (concentrations from 0 to 1 mol l^{-1}) are obtained using a gravimetric technique at 298 K. Taking into account the successive addition of mass within saturation of the solutions, the absolute uncertainty is about 0.5 g l^{-1} for the measured apparent solubilities S (g l^{-1}). The results are given in Table 6.

4.2. *Development*

Using the results obtained from the first part of this work, we develop the calculation of the real βCD solubility in aqueous citric acid solutions. Knowing the value of the constant for the equilibrium

$$\beta\mathrm{CD} + \mathrm{AC} \rightleftharpoons \beta\mathrm{CD{-}AC}$$

it is possible to calculate the amount of complex formed. Neglecting the very small dissociation of citric acid, the following equation can be written

$$[\mathrm{AC}]_{\mathrm{initial}} = [\mathrm{AC}]_{\mathrm{eq}} + [\beta\mathrm{CD{-}AC}]_{\mathrm{eq}} \approx [\mathrm{AC}]_{\mathrm{eq}} \tag{17}$$

If S is the apparent measured solubility of βCD

$$S = [\beta\mathrm{CD}]_{\mathrm{eq}} + [\beta\mathrm{CD{-}AC}]_{\mathrm{eq}} \tag{18}$$

The equilibrium constant is

$$K = [\beta\mathrm{CD{-}AC}]_{\mathrm{eq}}/([\beta\mathrm{CD}]_{\mathrm{eq}}[\mathrm{AC}]_{\mathrm{eq}}) \tag{19}$$

The combination of these three relations leads to a quadratic equation

$$K[\beta\mathrm{CD{-}AC}]_{\mathrm{eq}}^2 - (1 + KS + K[\mathrm{AC}]_{\mathrm{initial}})[\beta\mathrm{CD{-}AC}]_{\mathrm{eq}} + KS[\mathrm{AC}]_{\mathrm{initial}} = 0 \tag{20}$$

The roots ($[\beta\mathrm{CD{-}AC}]_{\mathrm{eq}}$) are calculated with the value $K = 16.4$ (at 298 K) for each concentration of citric acid.

The real concentrations of "free" βCD are deduced from Eq. (18) and summarized in Table 6. All the results are given in g of anhydrous βCD per l because of the high molar weight of βCD.

Fig. 4 shows values for the apparent solubility of βCD (S), for the concentration of complex and for the concentration of "free" βCD, versus citric acid concentration.

Table 6
Study of the increase of βCD solubility in aqueous citric acid solutions. Comparison of the apparent solubility (S) with the calculated amounts of free and complexed βCD

[Citric acid]/(g l^{-1})	0	10.0	25.0	50.0	70.0	100.3	140.1	192.0
Measured solubility S/(g $\beta\mathrm{CD}_{\mathrm{anh}}$ l^{-1})	20.1	32.9	50.9	83.5	109.8	140.9	182.6	223.3
Complexed βCD/(g $\beta\mathrm{CD}_{\mathrm{anh}}$ l^{-1})	0	13.1	31.8	64.2	90.5	122.8	165.4	207.8
Free βCD/(g $\beta\mathrm{CD}_{\mathrm{anh}}$ l^{-1})	20.1	19.8	19.1	19.3	19.3	18.1	17.2	15.5

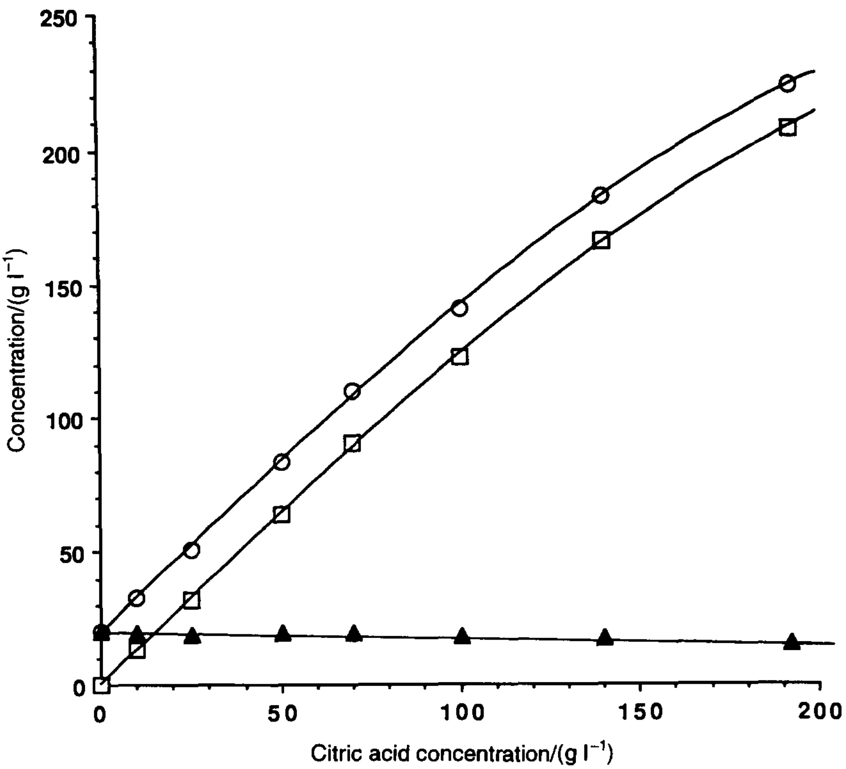

Fig. 4. Study of the βCD solubility in aqueous citric acid solutions: ○, apparent solubility (S); □, concentration of complexed βCD; ▲, concentration of uncomplexed βCD (free βCD).

5. Discussion

The use of βCD is often limited by its low solubility in water (approximately 18.5 g of anhydrous βCD in 1 l). This solubility can be significantly enhanced with the help of citric acid. For example, 223 g of anhydrous βCD are dissolved in 1 l of citric acid (1 mol l^{-1}), but in such a solution, 93% of the βCD is complexed and the solubility expressed above is, of course, an apparent solubility. The data in Table 6 show that the concentration of "free" βCD remains very close to the aqueous solubility of hydrated βCD, at any citric acid concentration.

6. Conclusions

Calculations of the equilibrium constant for the formation of the βCD–citric acid complex were based on a 1 : 1 binding model. The correlation of the calorimetric results confirms the suitability of this model.

At 298 and 308 K, both ΔH and ΔS have negative values, but the enthalpy–entropy compensation, which is common for binding processes in aqueous solutions [7], is such that ΔG is almost invariant with temperature.

From these results, we cannot describe this process as "enthalpy- or entropy-driven", but the large negative values for ΔS at 298 K probably indicate an important transfer of initially bound water molecules from βCD cavity to bulk water.

However, the few differences between some values obtained by flow calorimetry and by isoperibolic calorimetry can be attributed to the hydration state of βCD, which is evidently not the same. In the first case, βCD is already solved in water when it is introduced in the reaction cell, while it is anhydrous in the second case. As discussed above, the hydration state is not necessarily the same even if the two reactions occurred in water medium. The equilibrium constant explains the increase of the βCD solubility in aqueous solutions of citric acid. Under particular conditions, it would be interesting to use such concentrated aqueous βCD solutions.

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