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Solubility Characteristics of β-Cyclodextrin Inclusion Complexes

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Abstract. Some inclusion complexes of β-cyclodextrin (cyclomaltoheptaose) have been investigated, particularly with respect to their solubility. The mathematical characterization of the equilibrated host-guest system containing both solid and solution phases is discussed (first of all those, which contain 1 : 1 or 1 : 1 + 2 : 1 species) and demonstrated by different examples.

Key words: Inclusion complexes, β-cyclodextrin, solubility, phase diagram.

1. Introduction

Both the solubility enhancement of the guest and the precipitation of the inclusion complex are of special importance in the chemistry of cyclodextrins. In all of the known and studied cases [1], the solubility is considerably increased and this phenomenon is often the first motive for the application of a cyclodextrin. Most data (and their proofs) of the early cyclodextrin literature were based on solubility experiments. Generally, the solubility of the guest has been measured as a function of the (total) cyclodextrin concentration and the stability of the inclusion complex was thought to be directly proportional to the slope [2]. Although the first contradictions could be solved rather easily [3], there are many unanswered problems, and sometimes the older ones are revived, too.

During the last decades, several data and experiences have been accumulated and also a more detailed computer analysis has become possible.

Based on these facts and on the interesting results measured with β-cyclodextrin benzoic acid systems [4], the reconciliation of some new and old data has been undertaken.

2. Experimental

β-Cyclodextrin of over 99% purity; salicylic, *o*- and *m*-toluic and 4-aminobenzoic acids were of analytical grade.

The interaction between β-cyclodextrin and the given acid was followed by titrating the dissolved (total) acid content of a cyclodextrin solution of known

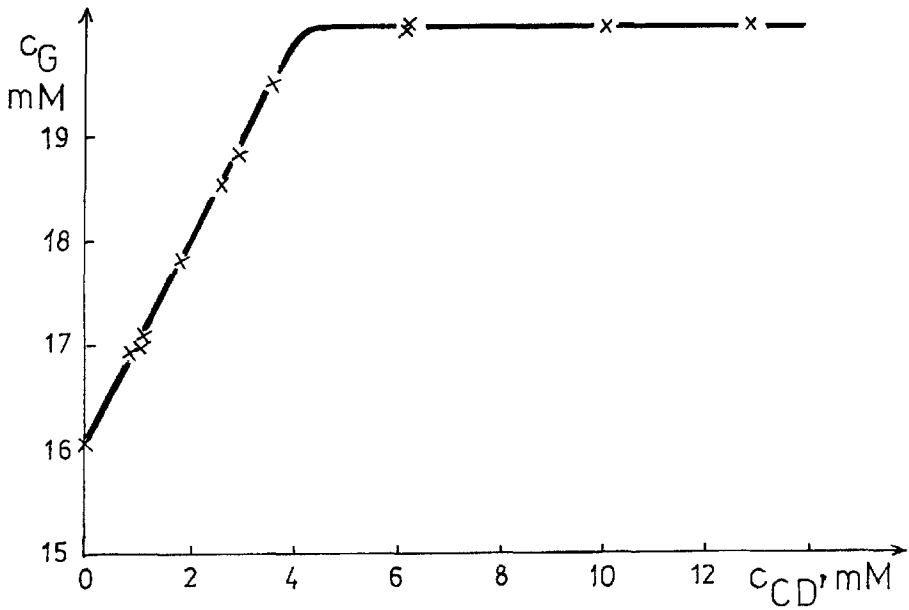

Figure 1. Solubility of salicylic acid as a function of β-cyclodextrin concentration (T = 25.0 ± 0.1 °C).

concentration after equilibration with the solid acid at constant (thermostatted) temperature.

The (solid) acid was always in excess, and we experienced that the time needed to reach real phase equilibrium depended not only on the size of the solid particles but on the amount of the residue. The quantity of the given acid necessary for saturation had been determined in preliminary tests and 120% of this amount was weighed in the experiments. This way the equilibrium was reached at 25 °C in 12–14 h. The time required to reach equilibrium is higher in the case of precipitate formation, i.e. when the solid phase contains both unreacted guest and its insoluble inclusion complex. Sometimes a whole week proved insufficient; therefore the conclusions drawn for the quantitative composition of the solid phase must be always regarded with reserve.

As a characteristic example, the results measured with salicylic acid are shown in Figure 1. Only the first, rising part of the isotherm was used for the quantitative evaluation, as in other, similar cases.

3. Background of the Calculation

It is only too obvious that the cyclodextrins can affect any property of another molecule (guest) when they interact with each other, in the general form:

$$nG + m\mathrm{CD} \rightleftharpoons (\mathrm{G})_n(\mathrm{CD})_m \tag{1}$$

where G stands for the guest and CD indicates the cyclodextrin.

The equilibrium can be characterized (at constant temperature) by the stability (formation) constant:

$$\beta_{nm} = \frac{[(G)_n(CD)_m]}{[G]^n[CD]^m} \tag{2}$$

As the total concentration of G (c_G) in the solution is equal to the sum of the equilibrium concentrations of the free and complexed guest:

$$c_G = [G] + \sum_n \sum_m n[(G)_n(CD)_m] \tag{3}$$

the relation can be expressed in a general form:

$$c_G = [G] + \sum_n \sum_m n\beta_{nm}[G]^n[CD]^m \tag{4}$$

and similarly:

$$c_{CD} = [CD] + \sum_n \sum_m m\beta_{nm}[G]^n[CD]^m. \tag{5}$$

In our special case, when G is present in the solid form (in the first part of the investigations), the following equilibrium also exists, depending only on the temperature:

$$G_{solid} \rightleftharpoons G_{dissolved} \tag{6}$$

It follows, that [G] in Equations (2), (4) and (5) must be constant, $[G]_0$, and remains constant as long as undissolved G can be found in the solid phase.

Thus Equation (4) can theoretically give a straight line as a function of c_{CD} (Equation (5)) when $m = 1$, i.e. the associate contains only one CD.

4. Results and Discussion

The most common case in cyclodextrin chemistry is the formation of a 1 : 1 inclusion complex (i.e. $m = n = 1$) [1], and neglecting the high n values characterizing the solvation type interactions of CD molecules [5], the value of n can be maximized to $n = 2$. A 3 : 1 salicylic acid – CD complex has been reported [6], thus the data measured with salicylic acid (Figure 1) have been analysed very carefully using Equations (4)–(6) extensively, as discussed in detail further on. The presence of a single associate with a 1 : 1 stoichiometry can be proved. The calculated stability constant can be found in Table I, together with those for other acids investigated.

The constants can be compared with that of benzoic acid [4], and only the value of m-toluic acid seems to be higher, supposedly because of increased hydrophobic

Table I. Stability constants of β-cyclodextrin inclusion complexes with some benzoic acid derivatives at 25.0 ± 0.1 °C

Guest	$\beta_{11} \pm 3\sigma$, M^{-1}	$[G]_0$, M
Benzoic acid*	$(7.94 \pm 0.12) \times 10^2$	2.75×10^{-2}
Salicylic acid	$(6.6 \pm 0.6) \times 10^2$	1.61×10^{-2}
o-Toluic acid	$(2.7 \pm 0.3) \times 10^2$	9.36×10^{-3}
m-Toluic acid	$(1.3 \pm 0.15) \times 10^3$	7.77×10^{-3}
p-Aminobenzoic acid	$(3.0 \pm 0.25) \times 10^2$	3.72×10^{-2}

* Ref. [4].

interaction and hydrogen bonding at the same time. Aminobenzoic acid is highly hydrated, while the substitution at the ortho position gives steric hindrance in the other two cases. We regard the difference between the stabilities of salicylic and *o*-toluic acids as a consequence of a secondary H-bond.

It is interesting that no sign of the parallel formation of 1 : 1 and 2 : 1 complexes was found from analysing the measured data. In this special case, Equation (5) takes the form

$$c_{CD} = [CD](1 + \beta_{11}[G]_0 + \beta_{21}[G]_0^2) \tag{7}$$

and substituting [CD] into Equation (4):

$$c_G = \frac{\beta_{11}[G]_0 + 2\beta_{21}[G]_0^2}{1 + \beta_{11}[G]_0 + \beta_{21}[G]_0^2} c_{CD} + [G]_0 \tag{8}$$

gives a linear relation between c_G and c_{CD}, where the first coefficient is the correct definition of the slope. Assuming that $\beta_{21} = 0$ (i.e. only a 1 : 1 associate is formed) Equation (8) also describes this most common case. (Similarly, we can suppose that $\beta_{11} = 0$, i.e. only a G_2·CD species exists, but no such proven case is known for the solution or for solid phase, either.)

It is obvious that the numerical value of the slope is determined by the ratio of the β_{11}, β_{21} and $[G]_0$ constants. (A slope less than 1.0 is no proof of the formation of a single 1 : 1 complex.) As $[G]_0$ can be measured and controlled separately, and the probability of the association of a second G to the G·CD species (i.e. the ratio between β_{11} and β_{21}/β_{11}) can be limited reasonably, there is some possibility to evaluate both the β_{11} and β_{21} constants based on solubility measurements using Equations (4), (5) and (6) in a computer program.

As the best example, the pioneering work of Pauli and Lach on a series of phenyl-substituted carboxylic acids [7] must be mentioned. Using their data, very good association constants can be computed (Table II), which compare rather well with the original ones, except that of 4-phenyl-butyric acid. The background of their 'infinite' note in this case was the contradiction between the slope (1.10)

Table II. Recalculated constants for β-cyclodextrin complexes with some phenyl-substituted carboxylic acids ($T \sim 30$ °C).

Acid	β_{11}	β_{11}^*	β_{21}	β_{21}^*
2-phenylacetic	2.3×10^1	(2.4×10^1)	–	–
3-phenylpropionic	2.8×10^2	2.0×10^2	–	–
4-phenylbutyric	6.5×10^2	'inf.'	1.1×10^4	–
5-phenylvaleric	9.7×10^2	–	2.5×10^5	1.1×10^5
Cinnamic	4.8×10^2	–	1.7×10^4	3.5×10^4

* Ref. [7].

Table III. Data concerning the β-cyclodextrin inclusion complexes with some phenyl-substituted carboxylic acids ($T \sim 30$ °C).

Acid	Slope of isotherms		$[G]_0$	Stoichiometry*
	measured*	calc**	corr.	
2-phenylacetic	0.77	0.77	1.44×10^{-1}	–
3-phenylpropionic	0.93	0.95	6.24×10^{-2}	1 : 1
4-phenylbutyric	1.10	1.09	1.40×10^{-2}	1 : 1
5-phenylvaleric	1.25	1.29	4.14×10^{-3}	2 : 1
Cinnamic	0.67	0.74	3.63×10^{-3}	2 : 1

* Ref. [7] (stoichiometry measured in the solid phase).
** Using Equation (8) and the β_{11}–β_{21} values (Table II).

and the stoichiometry (1 : 1) found in the isolated complex. Using the constants refined in computer calculations and the known $[G]_0$ values, the original slope of the isotherms can be recalculated (Table III), as expected, but the contrast between the assumed systems existing parallel in solid and solution phases is remarkable.

Before discussing this problem, the cases where $m > 1$ (Equation 1) have to be summarized briefly. Among the cyclodextrin complexes studied [1], the existence of G·$(CD)_2$ species can be detected when the guest molecule is large enough. Their formation seems to follow that of the 1 : 1 species, i.e. the systems are always complex:

$$c_G = [G] + \beta_{11}[G][CD] + \beta_{12}[G][CD]^2. \tag{9}$$

The shape of the curve characterized by Equation (9) depends on both the absolute and relative concentrations, and (as [CD] depends on c_{CD} differently, in a special combination of $[G]_0$, β_{11} and β_{12} values) it may form a straight line, too, but either positive or negative deviations can be assumed and found. Practically the correct evaluation is hindered first of all by the accidental errors of measurements.

Cyclodextrin inclusion complexes of 1 : 3 stoichiometry have only been described in the special case of long fatty acids [8, 9] and even the extraordinary

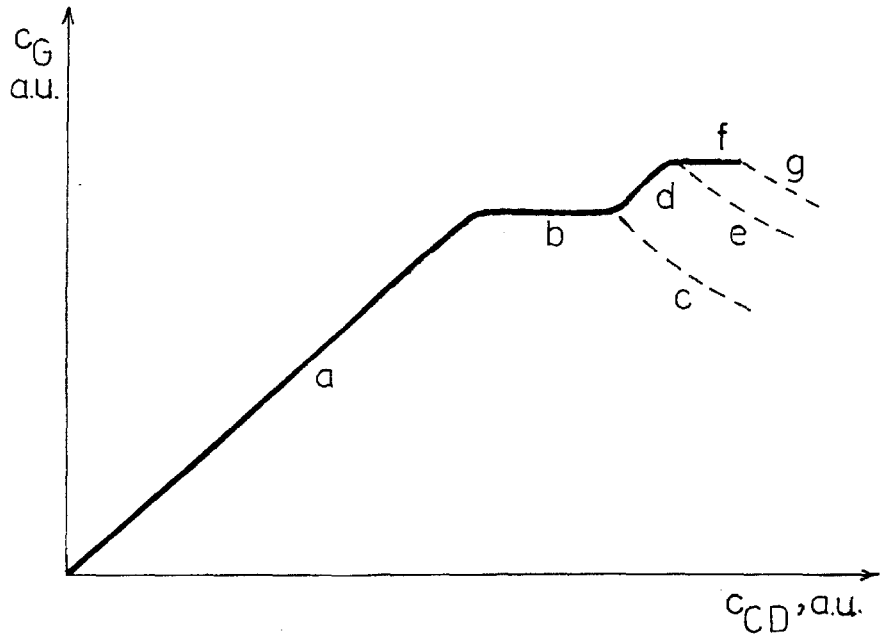

Figure 2. Different possibilities in solubility diagrams of cyclodextrin inclusion complexes (for explanation see the text).

system of saturated β-cyclodextrin solution (in the presence of inorganic salts [10]) can be characterized assuming different 1 : 1 and 1 : 2 (mixed) complexes. (When the polymeric ethylene glycols react with cyclodextrins [11], the stoichiometric ratio can be extremely high, but it may be a mere multiplication of the simple interactions.)

5. Solubility Diagrams

The graphical representation of solubility investigations (Figure 2) can be traced back to Equation (8), which seems to be valid in this form as long as the solid (equilibrated) phase contains undissolved G only (Figure 2a).

When any inclusion complex formed reaches its saturation limit, it starts to be precipitated, i.e. the solid phase will contain both G and $(G)_n(CD)_m$. The consequence is that all of the concentrations (not only [G] but also $[(G)_n(CD)_m]$ and therefore [CD] as well as c_G) become constant in the solution (Figure 2b). Considering the possible chemical interactions, a lower solubility may be assigned to 2 : 1 associates as found for 5-phenylvaleric and cinnamic acid complexes [7], but other $(G)_n(CD)_m$ species may theoretically also have the lowest solubility. Cyclodextrin inclusion complexes having apparently no strict stoichiometry may also be precipitated, first of all when the different species are built up by different interactions. A mixed (1 : 1 and 2 : 1) stoichiometry must be assumed in the case

of 4-phenylbutyric acid [7] similar to the β-cyclodextrin–benzoic acid interaction, where the precipitate has a definite G to CD ratio of 1.05 : 1.00 [4]. As confirmed, the first benzoic acid molecule is included into the cavity of the cyclodextrin while the second one is linked by hydrogen bonds to the hydroxyls of cyclodextrin, forming an 'outer sphere' type complex.

Generally, the constant part b in Figure 2 lasts as long as the solid phase contains any undissolved G. Unfortunately, to reach a real equilibrium in this part of investigations takes a great deal of time and the data measured may contain misleading errors. When all of the solid guest is dissolved, c_G will decrease slowly to the level of the solubility of the less soluble inclusion complex (Figure 2c).

But a second possibility exists theoretically after the dissolution of the solid guest, when it forms inclusion complexes with a higher m/n ratio than in the solid phase (precipitate). E.g. the solid phase consists of G_2·CD precipitate but exists as a G·CD complex of appropriate stability, too – or similarly a G·CD – G·$(CD)_2$ variation. In this case, we have noticed a second increasing part (Figure 2d), followed by the slow decrease of c_G (Figure 2e).

Furthermore, a further possibility could not be excluded: the solubility of the assumed second inclusion complex is also limited. In this case a second plateau (Figure 2f) may be formed before the descending part (Figure 2g). It seems that the excellent work of Connors and Rosanske [12] presents just such an experimental example for this very interesting and rare interaction.

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