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Hydroxypropyl-β-Cyclodextrins: Correlation between the Stability of Their Inclusion Complexes with Phenolphthalein and the Degree of Substitution*

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Abstract. The stability constants of the inclusion complexes between 2-hydroxypropyl-β-cyclodextrin and phenolphthalein have been found to decrease with increasing degree of substitution; the hydroxypropylation of the primary rim has a relatively higher effect. The change of specific rotations seems to support this finding. The decrease can be explained by the disturbance of the three-site interaction characteristic for the molecular recognition of phenolphthalein by cyclodextrins.

Key words: Hydroxypropyl cyclodextrins, inclusion complexes, stability constants, three-site interaction.

1. Introduction

2-Hydroxypropyl-β-cyclodextrin has been claimed to be the 'right' cyclodextrin derivative by two experts [1] and the statement can be supported by several facts: data characterizing its solubility, solubilization and toxicology/safety properties are excellent.

There are still some problems to be solved; first of all the dependence of physico-chemical properties on the average degree of substitution (DS). This characteristic value does not mean the average number of substituted hydroxyl groups in cyclodextrin, but rather the average number of hydroxypropyl units per cyclodextrin unit. It follows that the DS value gives information neither on the position (2, 3 or 6) and type (primary: 6, or secondary: 2, 3) of the substituted hydroxyl group nor on the kind of substituent (monomeric 2-hydroxypropyl or oligomeric: $-[CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-O]_n-H$ group).

The DS value can be measured by different methods, such as soft ionization mass spectrometry [2], NMR spectroscopy and gas chromatography [1] and rather large differences can exist depending on the methods used [1, 3]. From the methods

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used plasma desorption and fast atom bombardment mass spectrometry give the possibility to measure the amounts of differently substituted species: their distribution around the average DS value is symmetrical and 'the number of isomers' increases with increasing DS value [1, 2, 4].

In the case where DS = 6.2, the ratio of derivatives containing one, two, three and even four hydroxypropyl units per glucose unit (the last case must mean an oligomeric substituent) can be simplified as 2 : 2 : 1 : 1 [4], but not all substituents are located on primary hydroxyls [4] as shown by more detailed studies [3, 5, 6].

Hydroxypropyl-β-cyclodextrins are produced by a condensation of β-cyclodextrin with propylene oxide in alkaline solution; the DS value of the product depends on the ratio of reactants, reaction time and temperature [1–4].

Experience shows that the hydroxypropylation of 0–2 (secondary) hydroxyls is favoured at relative low alkali hydroxide concentration ($\sim$ 0.5 M) [1, 5, 7] while high alkali hydroxide content ($\geq$ 10 M) really promotes the formation of 0–6 derivatives [1, 7]. Similarly, this last factor seems to be most responsible for the further hydroxypropylation of 2-hydroxypropyl groups [1, 4].

The variability of hydroxypropyl-β-cyclodextrin samples in chemical composition is reflected in the physical properties, particularly in the amorphousness of the preparates (which is considered to be very advantageous in pharmaceutical use) [1, 2, 4]. The solubility is enormously increased by increasing DS; while β-cyclodextrin itself (i.e. DS = 0.0) has a solubility of 1.8% (in water), the solubility of a sample with a DS value of 7 exceeds 50% [1, 3]. The trend of melting points is very interesting: they decrease parallel with increasing DS value [1].

The complexation behaviour of CD derivatives depends on several factors, among them on the kind of guest compound [8 and references therein]. As the production of 2-hydroxypropyl-β-cyclodextrin has no common codification, the assumption of possible differences among samples having similar DS values [1] is well established. Our aim was to investigate the connection between the stability of inclusion complexes and the DS value, using phenolphthalein as guest, using previously established methods [9].

2. Experimental

The 2-hydroxypropyl-β-cyclodextrin samples were from Cyclolab Ltd. (Hungary), prepared in different ways as summarized in Table I. All the other materials were of analytical grade and used without further purification. The absorption spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer and the absorbances measured with a Spectromom 195D at λ = 550 nm. Optical rotations were investigated using a Zeiss Polamat A multiwavelength polarimeter.

The stock solutions of 2-hydroxypropyl-β-cyclodextrin samples were 2×10^{-4} and 5×10^{-4} M, prepared from water-free materials by direct weighing. The solutions for spectrophotometric determination contained uniformly 3×10^{-5} M phenolphthalein, 2×10^{-2} M sodium carbonate (to stabilize the ionic strength and

TABLE I. Characteristics of the solid hydroxypropyl-β-cyclodextrin samples studied.

No. of sample	DS value	Preparation at	Legend used in Figs.
1	2.9		one batch
		low alkalinity	preparation
2	5.0		(•)
3	3.1	high alkalinity	(□)
4	3.9		similarly
5	4.4	high alkalinity	prepared
6	8.0		(▽)
7	4.0		one batch
8	8.0	low alkalinity	preparation
9	16.0		(+)
10	6.0		one batch
		low alkalinity	preparation
11	8.0		(○)
12	6.0		identical
13	10.0	low	preparative
14	12.0	alkalinity	conditions
15	14.0		(×)

pH = 10.5) and the following concentrations of given cyclodextrin: 0.0; 2×10^{-5}; 4×10^{-5}; 6×10^{-5}; 8×10^{-5}; 5×10^{-5}; 10^{-4}; 1.5×10^{-4}; 2×10^{-4} and 3×10^{-4} M. The temperature was 25 ± 1°C during the investigations.

The stability constants can be easily calculated using a desk computer [10]. The background to the calculation is based on the fact that the concentration of free phenolphthalein, [I] can be measured at $\lambda = 550$ nm directly, using the separately measured molar absorptivity. Knowing the total concentrations of both reactants (i.e. $[I]_T$ and $[CD]_T$), the equilibrium concentration of phenolphthalein and the equations of mass balances:

$$[I]_T = \sum \sum p K_{pq} [I]^p [CD]^q \quad \text{and}$$

$$[CD]_T = \sum \sum q K_{pq} [I]^p [CD]^q ,$$

where the definition of stability constants is as follows:

$$K_{pq} = \frac{[(I)_p (CD)_q]}{[I]^p [CD]^q} ,$$

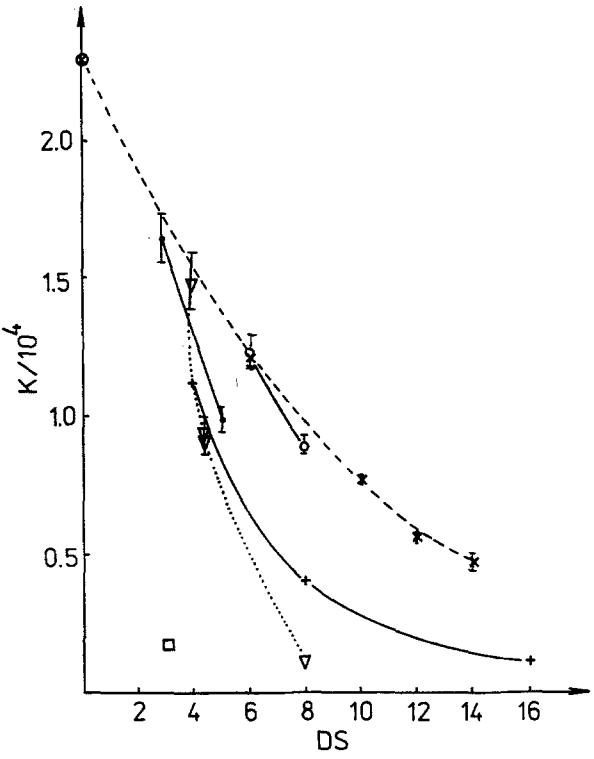

Fig. 1. The measured stability constants of phenolphthalein- hydroxypropyl-β-cyclodextrin complexes (at 25°C, $I = 0.04$) as a function of DS. (Lines show connection between samples of the same series, see also Table I.)

any association constant can be computed. (It should be noted that the general case (like the case discussed here) is that $p = q = 1$, i.e. only a 1 : 1 complex is formed.)

As the concentrations of the reactants and the inclusion complex are of the same order of magnitude, no graphical (or computerized graphical) solution [11] can be used.

The stability constants are represented in Figure 1 as a function of DS. Similarly the measured specific rotations (at $\lambda = 366$ nm) are collected as a function of DS in Figure 2.

3. Results and Discussion

The formation of inclusion complexes and their stabilities are of crucial importance. There are known phenomena (e.g. in the effect of solubilization property) which do not correlate linearly with increasing DS, therefore 2-hydroxypropyl-β-cyclodextrins of lower DS value (< 8) are recommended [2] and used [1]. Sometimes a reverse connection was observed. This points unambiguously to the

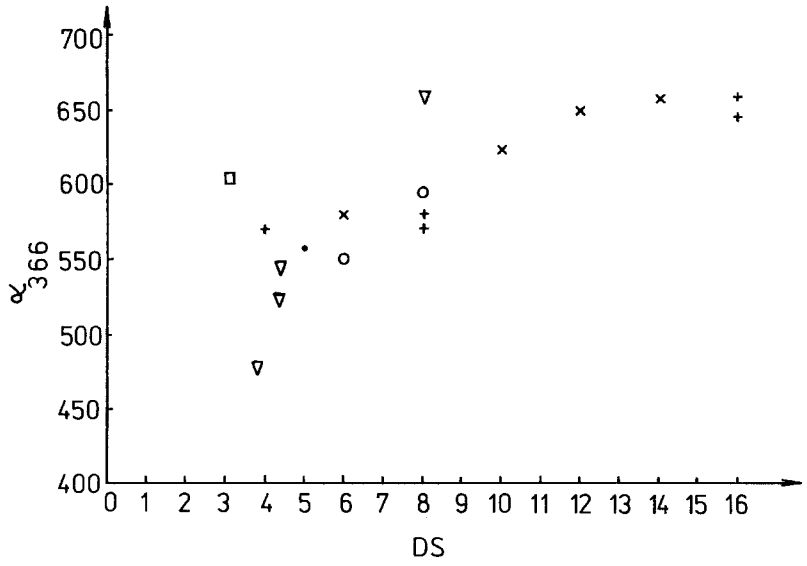

Fig. 2. Specific rotations (at $\lambda = 366$ nm, $t = 25°C$) of hydroxypropyl-β-cyclodextrin samples. (Legends are the same as in Figure 1 and Table I.)

decrease of stability constants with increase of DS, proved experimentally, just with phenolphthalein [3].

Apart from the problems of evaluation [11] as mentioned and from some discrepancies between the data of Refs. 3 and 7, the decreasing trend of stability constants with increasing DS (DS > 1.5) is well demonstrated [3] for samples of identical batches.

The results obtained with various samples of hydroxypropyl β-cyclodextrin (Figure 1) show that the stability constants generally decrease with increasing DS value but this general trend is valid within a series of samples of identical or at least of similar preparation only. This trend is emphasized in the figure by the lines connecting samples within the same series, showing clearly the fact that an identical average DS value does not necessarily mean identical complex forming ability. When identical average DS values cover different patterns of substitution (in the case of samples from different batches, because of perhaps slight deviations in preparation), this may result in significantly different stability constants of inclusion complexes with the same guest.

It is not surprising that the specific rotations increase parallel with the increasing DS values as shown in Figure 2, for hydroxypropylation adds further chirality centres to the molecule (in spite of the fact that generally racemic propylene oxide is used). No trends like those in Figure 1 could be detected although the deviation found with samples Nos. 3, 4 and 6 prove that this parameter is also strongly affected by the method of preparation.

The interaction between β-cyclodextrin and phenolphthalein was investigated in detail and a very stable three-site contact was found [9], which can be regarded as molecular recognition of phenolphthalein by cyclodextrin. In spite of the decreases, the stability constants measured are sufficiently high for the conclusion: the three-site interaction may also exist in 2-hydroxypropyl-β-cyclodextrin inclusion complexes.

We can conclude that one of the phenolic rings is included in the cavity as established formerly for β-cyclodextrin [9], and this interaction is only slightly weakened by the hydroxypropylation of the secondary hydroxy groups.

The second interaction among the secondary rim, the carboxylate substituent and the central (methane or carbenium-type) carbon atom must also be retained as this interaction is responsible for the disappearance of the red colour and the complexes of 2-hydroxypropyl-β-cyclodextrins (at pH = 10.5) are also colourless.

The third site of the molecular recognition is the hydrogen bonding between the primary hydroxyls of the other rim and the phenolate (or phenolic, or quinoidal) oxygen of the included phenolic ring. This interaction seems to be disturbed mainly, because the estimated higher substitution of 0–6 hydroxyls always evokes a higher decrease in stability constants in cases of identical DS values (Figure 1).

Further investigations of this system are intended and are in progress.

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