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Relationships between the Stability, Circular Dichroism Spectrum and Type of Substitution in Inclusion Complexes Formed between Phenophthalein and Different Cyclodextrins

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Abstract. The perturbation of the three-site interaction of phenolphthalein on complexation with different cyclodextrins (CDs) has been investigated as a function of the type and degree of substitution of the CD. The UV-visible spectra are practically identical, while the circular dichroism spectra change dramatically both in intensities and signs. Parallel to this, the stability constants are influenced not only by the size of the cavity but also by the substitution of its rims, particularly by that of the primary hydroxyls. The two phenomena can be related to allow conclusions concerning the mode of inclusion.

Key words: Cyclodextrins, phenolphthalein, inclusion complexes, induced circular dichroism, stability constants.

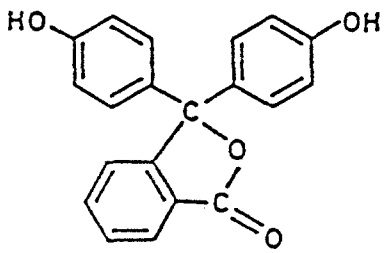
1. Introduction

Basic cyclodextrins (CDs) consisting of six (α-), seven (β-), or eight (γ-CD) D-(+)-glucopyranose units linked by α(1,4) bonds have been known for a relatively long time [1]. During the past 15 years several derivatives have also been prepared mainly by the substitution of the secondary (2, 3) or primary (6) hydroxyls of the glucose units, in several cases in the 2,6 variation [1]. In some respects these derivatives may have more favourable properties (e.g. solubility) compared to the parent compounds.

Several efforts have been made to understand and predict the inclusion complex stabilities of differently substituted and unsubstituted CDs [1], and even more efforts to explain the intensity and sign of the induced circular dichroism (ICD) in the absorption bands of achiral guest molecules [2–4]. The experimental and theoretical study of methylated phenols included in β-CD [5] showed that β-CD is largely asymmetric and even its shape fluctuates. Most data describing the changes in ICD as a results of changing the CDs have been reported for complexes with azo dyes [6, 7].

The ICD spectra of CD inclusion complexes have been investigated primarily with respect to the guests and the relationship with the stability of the complex

formed has not been extensively studied. Our aim was to investigate the relationships between the stabilities, the ICD spectra and the structures of inclusion complexes formed in aqueous solution with different CDs. Phenolphthalein (PP) (**1**) was chosen as guest since its acid–base properties, its absorption spectra as a function of pH, as well as its interaction, or more exactly its molecular recognition by β-CD, have been investigated in detail [8] using ICD as one of the techniques.



Phenolphthalein (**1**).

2. Experimental

Most of the CDs such as α-, β- and γ-CD, 2,6-dimethyl- and 2,3,6-trimethyl-β-CD (DM-β CD and TM-β CD), and an extensive collection of 2-hydroxypropyl-β-CDs (HP-β CD) were from Cyclolab Ltd. (Hungary). To complete the last group, a special series of HP-α , HP-β and HP-γ CDs were purchased from Aldrich Co., in which the glucose units were substituted on average by 0.6 —CH_2—CH(OH)—CH_3 groups (i.e. the average degree of substitution (DS) was 3.6 for HP-α CD; 4.2 for HP-β CD and 4.8 for HP-γ CD). Hydroxypropylation adds further chirality centres to the molecule, the specific rotations increases nearly parallel with the increasing DS value [9].

All the other materials were of analytical grade and were used without further purification, except for phenolphthalein (PP) which was twice recrystallized from an ethanol – water mixture.

The absorption spectra were recorded on a Perkin-Elmer Lambda 15 spectrophotometer and the stability constants of the complexes measured as published elsewhere [9]. All systems could be characterized by formation of 1 : 1 inclusion complexes and the equilibrium constants (K = [PP.CD]/[PP][CD]) calculated are summarized (in the form of their log values, together with the standard error) in Table I.

It should be emphasized that all of the absorption spectra investigated were identical and the characteristic band of PP (pH = 10.5, λ = 550 nm) always disappeared in the presence of excess of any CDs [8, 10].

ICD spectra were measured using a Jobin-Yvon Dichrograph Mark VI. The concentration of PP was (1.5–20) $\times$ 10^{-5} M, and the CDs were in relatively large excess to allow practically full complexation wherever possible. No ICD bands

Table I. Stabilities of 1 : 1 PP–CD complexes at pH = 10.5 and $T = 25$ °C.

Host	log K	Note
α-CD	0.64 ± 0.21	–
β-CD	4.38 ± 0.03	–
γ-CD	3.27 ± 0.02	–
HP-α CD	1.08 ± 0.17	DS = 3.6
HP-β CD	4.09 ± 0.01	DS = 4.2
HP-β CD	4.09 ± 0.02	DS = 6.0
HP-β CD	3.95 ± 0.02	DS = 10.0
HP-β CD	3.75 ± 0.02	DS = 12.0
HP-β CD	3.67 ± 0.02	DS = 14.0
HP-γ CD	3.36 ± 0.02	DS = 4.8
DM-β CD	3.17 ± 0.03	"DS" = 14.0
TM-β CD	1.08 ± 0.10	pH = 10.5
TM-β CD	2.53 ± 0.12	pH = 6.2

were observed in the visible range. The temperature was 25 ± 1 °C during the investigation. Some representative ICD spectra are presented in Figures 1–3.

3. Results and Discussion

Based on the relationship between the UV and ICD spectra of the PP–β-CD complex and their resemblances to those of phenol [3, 8, 11], together with the disappearance of the very characteristic absorption band of PP (in alkaline solution) at 550 nm, the structure of the very stable complex was explained by a three-site interaction. In the inclusion complex: (i) one of the phenolic rings of PP is included in the cavity of CD; (ii) its phenolate (or phenolic, or quinoidal) oxygen forms a hydrogen bond with the primary hydroxyls of CD; and (iii) the secondary OH-groups of the other rim interact with the carboxylate substituent and the central (methane or carbenium type) carbon atom, respectively. The interactions result in an $sp^2 \rightarrow sp^3$ change at the central carbon atom (resulting in the disappearance of the characteristic colour) [8].

As Figure 1 shows, the ICD spectra of PP–α- and –γ-CD complexes are similar to that of β-CD (the first band at longer wavelength is negative, the second one has a positive sign), demonstrating the similarity of the interactions. The relatively high intensities of the bands in the spectrum of the α-CD inclusion complex (in spite of the fact that its stability – see Table I – is very low) support the assumption, that the intensities in the ICD spectra are influenced first of all by the tightness of the inclusion [3]. (We have to mention that, because of the small and therefore less accurate stability constant, the concentration of the complex could be calculated less accurately, and so the possible error in the calculated $\Delta\mathcal{E}$ values can be relatively

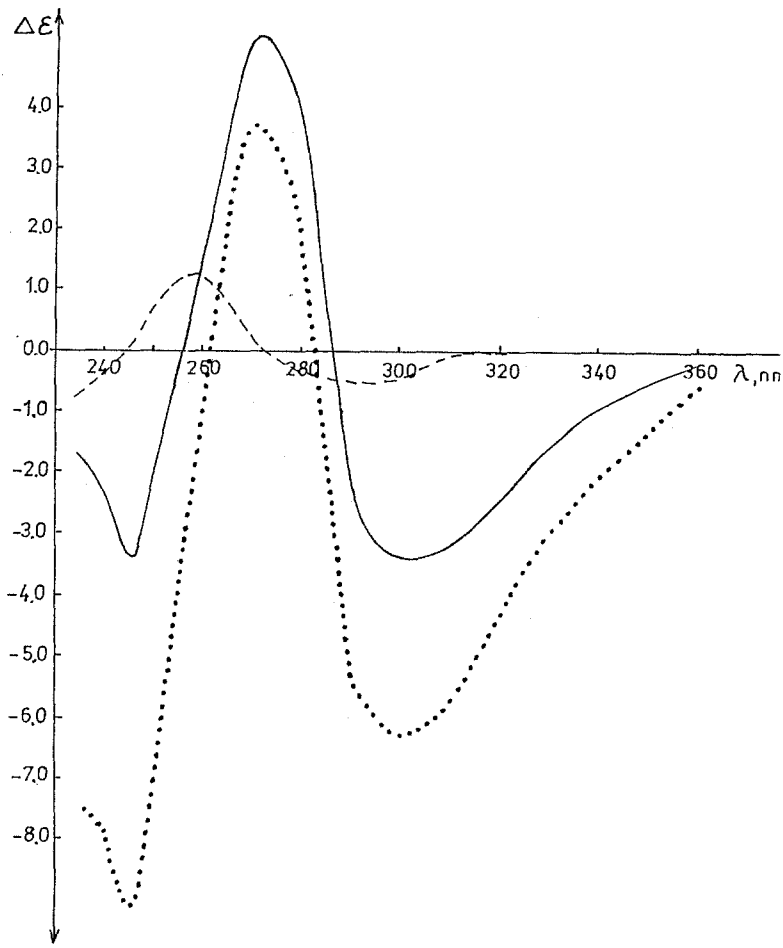


Figure 1. ICD spectra of PP-CD inclusion complexes (Concentrations are about 1.5×10^{-5} M; $T = 25$ °C; pH = 10.5; · · ·: α-CD; ——: β-CD; – –: γ-CD).

high.) This fit is worse in the case of the PP–γ-CD complex (though the stability is higher because the H bonds (ii) and (iii) are promoted) resulting in low intensities (and a small blue shift).

Two types of HP–CD derivatives were investigated: (i) those with comparable DS values (i.e. the DS of every glucose unit is 0.6), and (ii) samples with different DS values bearing substituents mainly on the secondary rim (which were prepared in one batch of low alkalinity [9]). (The note in Table I refers mainly to these values.)

As Table I shows, the stability constant of PP inclusion complexes seems to be increased by the hydroxypropylation of α- and γ-CDs, while the stabilities are systematically decreased by increasing the DS value of HP-β-CD samples. (No

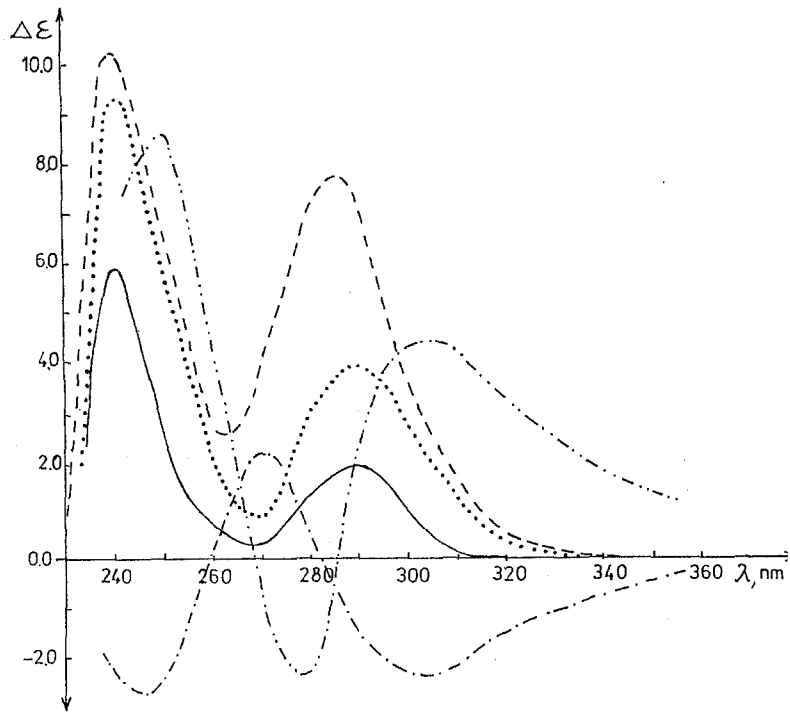


Figure 2. ICD spectra of PP-HP CD (DS of every glucose units is 0.6) inclusion complexes (Conditions are as above; · · ·: α at pH = 6.2; ——: β at pH = 6.2; —·—: β at 10.5; – – –: γ at pH = 6.2; —··—: γ at pH = 10.5).

systematic study has been published about the correlation between the stability of PP inclusion complexes and the DS value of HP-α or HP–γ CDs. It is possible that a maximum exists as in the case of the HP–β CD complexes at DS<1.5 [12].) In spite of the differences in stabilities, the UV-visible spectra of all PP–HP-CD inclusion complexes were practically identical. The first – unexpected – consequence of HP substitution was that the ICD spectrum of the HP–α-CD (DS = 3.6) complex became unmeasurable at pH = 10.5 (proving that the lack of an ICD spectrum does not mean the nonexistence of an inclusion complex), therefore the ICD spectra of all representative samples have also been recorded at lower pH (see Figure 2). These spectra are surprisingly similar to each other but of opposite sign compared to that of the unsubstituted β-CD complex [8], and even HP–γ-CD itself seems to include the PP tightly, which can be understood only if the H-bonded participation of HP groups is also assumed.

The ICD spectra of the HP–β-CD complexes at pH = 10.5 are similar to that of the unsubstituted β-CD complex and the decrease of complex stability constants is closely correlated with the DS value when the substitution is "regular", i.e. it takes place first of all on the rim of the secondary hydroxyls. (If the substitution is 'irregular', i.e. it is first of all on the primary (6) side, the deviation is more marked

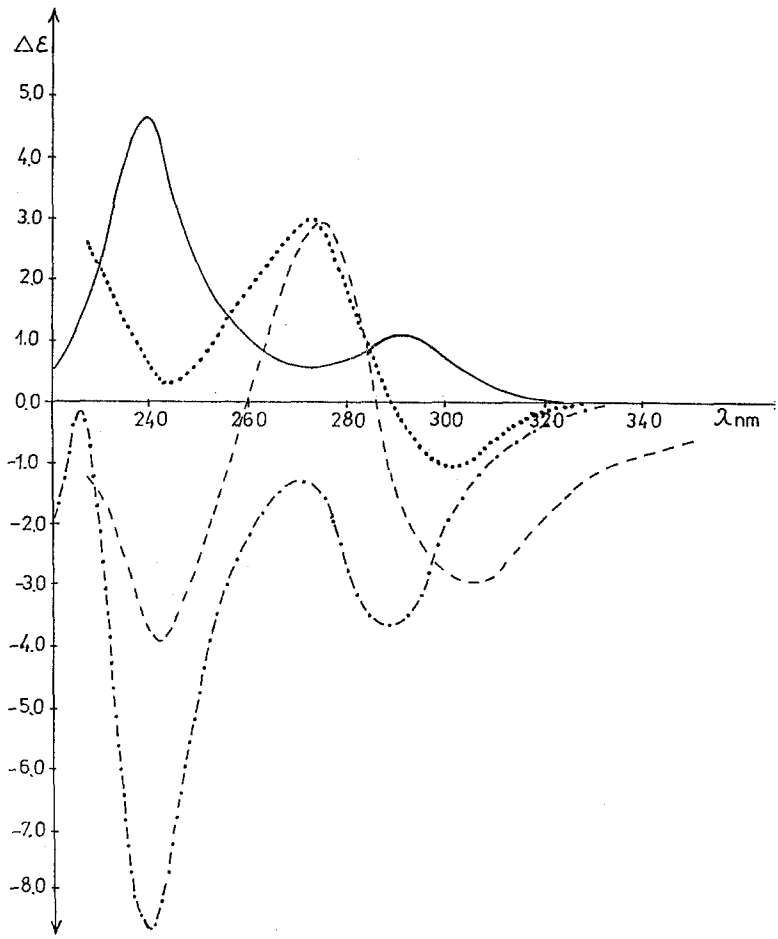


Figure 3. ICD spectra of PP-DM- and TM-β CD inclusion complexes. (Conditions are as above; ——: -DM- at pH = 6.2; — —: -DM- at pH = 10.5; —·—: -TM- at pH = 6.2; · · ·: -TM- at pH = 10.5.)

and the K values are smaller [9, 10].) These effects can be coupled to the steric disturbance of interaction (ii).

The ICD spectrum of the HP-γ-CD complex at pH = 10.5 (see Figure 2) differs significantly from all of the other spectra: the signs of the (red-shifted) maxima are opposite to those of HP–β-CD. (It should be mentioned that the structure of ICD spectra measured at pH = 10.5 below λ = 250 is not discussed as several authors do in the literature [5] because of the splitting of overlapping bands [3].)

Similar changes have been mentioned in discussing the ICD spectra of different azobenzene derivatives complexed by different CDs [6,7]. In the case of azo dyes, the explanation by assuming a split-type $\pi \rightarrow \pi^*$ transition band caused by dimer

formation (and inclusion) was one of the possibilities [7], but the effect of steric hindrances as the cause of the negative splitting seems to be more probable [6].

In our case, the assumption made by Kodaka [4] based on the coupled oscillator theory can be accepted, where the sign of the ICD is influenced not only by the direction but also by the depth of inclusion of the chromophore [4]. Substitution of CD OH groups may cause increased steric hindrance (especially in neutral solution where PP exists in its lactone form) and provide modified possibilities for H bonding. So we can assume that in the case of the larger γ-CD the hydroxypropylation may fix the PP in an inclined position. Both the increasing stability of this inclusion complex, the change in the sign of its ICD bands and their relative intensities can be explained this way.

We believe that this explanation is valid for the rather complex picture of the ICD spectra of PP–DM- and –TM-β-CDs (as a function of pH, see Figure 3). The UV–visible spectra are again identical to that of the parent complex, but the stabilities (see Table I) are rather different. (The difference between the stability constants of neutral and doubly deprotonated PP with TM-β CD is good though indirect proof of the H-bonded (ii) interaction.) In the case of DM-β CD the asymmetric contribution of the CD OH groups dominates while the most symmetrical TM-β CD resembles qualitatively the unsubstituted β-CD.

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