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Evaluation of complex forming ability of hydroxypropyl-β-cyclodextrins

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Abstract

The complex forming ability of hydroxypropyl-β-cyclodextrins (HP-β-CDs) is highly influenced by the distribution of substituents and the average degree of substitution (DS), both the size of the cavity and the reactivity of CDs are altered when the hydroxyl groups are substituted. On the other hand, the guests themselves influence these interactions by their sizes and configurations. In the present study, 9 HP-β-CDs with different substitution patterns and DS, which have been investigated by the reductive-cleavage method and methylation analysis, were chosen. The interactions among HP-β-CDs and phenolphthalein (as a model for 'larger spheriform' guests) or *p*-methyl red (as a model for 'smaller linear' guests) were studied for determining the complex forming ability of HP-β-CDs. The results indicated that, compared with parent β-CD, HP-β-CDs have a lower ability to form inclusion complexes with the 'larger spheriform' guest molecules. With regard to the 'smaller linear' guest molecules, HP-β-CDs have a higher complex forming ability, especially the low DS value (<6.5) HP-β-CDs which have a ratio of DS (2 + 3) to DS (6) close to 1.

Keywords: Hydroxypropyl-β-cyclodextrin; Substitution pattern; Inclusion complex; Phenolphthalein; *p*-Methyl red; Stability constant

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of several D-glucose units linked by α-(1,4) bonds. This cyclic configuration provides a hydrophobic internal cavity and gives the CDs a truncated cone shape. Many hydroxyl groups are situated on the edges of the ring which make the CDs both lipophilic and soluble in water. As a result, CDs are able to form inclusion complexes with a wide variety of hydrophobic compounds, and thus change the physical–chemical properties of the guest molecules (Del Valle, 2004; Dodziuk, 2002; Szejtli, 1998).

The most common parent CDs are α-, β-, γ-CD with the corresponding number of glucose units ($\alpha = 6$, $\beta = 7$, $\gamma = 8$). β-CD is the most accessible, the lowest-priced and generally the most useful, but β-CDs are not very soluble in water due to the strong hydrogen bond between HO-2 and O-3 (Saenger, 1980).

Hydroxylpropyl-β-cyclodextrin (HP-β-CD), a hydroxyalkyl derivative, is an alternative to α-, β- and γ-CD, with improved water solubility (Uekama, Hirayama, & Irie, 1998) and may be slightly more toxicologically benign (Sarah & Robert, 2005). As the first approved CD derivatives by FDA, HP-β-CDs have widely applications in food, agriculture and the pharmaceutical field (Hedges, 1998; Schneiderman & Stalcup, 2000; Singh, Sharma, & Banerjee, 2002; Szente & Szejtli, 2004). HP-β-CDs are prepared by reacting β-CD with propylene oxide in alkaline aqueous solutions. The high alkali concentration favours alkylation at O-6, while the low alkali concentration favours alkylation at O-2 (Pitha & Rao, 1990). The products are always substituted randomly when it comes to distribution among the different glucose units. In addition, the ratio of reactants, reaction time and the temperature affect the average degree of substitution (DS). It follows that the composition of the HP-β-CD samples show high variability, which is

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also reflected in the chemical and physical properties (Rao, Fales, & Pitha, 1990; Szente & Szejtli, 1999).

The effect of DS on the inclusion forming ability has been studied by different authors researching this field. Müller and Brauns (1986) reported that the DS of mixtures of HP-β-CD derivatives has a large influence on the complexing abilities. A low degree of substitution is preferable, since these derivatives show the best complexing properties. Rao and Pitha (1992) found the complex forming ability of HP-β-CDs first increases and then decreases with increasing DS. A clear conclusion has not been reached due to the amorphism of HP-β-CDs. Moreover, the substitution pattern affects the stability of inclusion complexes too. It seems that both the DS and the substitution pattern influence the stereospecificity of HP-β-CDs (Buvári-Barcza & Barcza, 1999; Loukas, Vraka, & Gregoriadis, 1996). However, there are very few studies focusing on the effect of the substitution pattern of HP-β-CDs on the formation of inclusion complexes.

On the other hand, the stability of inclusion complexes is also influenced by the sizes and configurations of the guests. Different guest molecules have different abilities to fit into the CD cavity. A recent evaluation found the stability constant value of 38 drugs/HP-β-CD complexes to vary considerably from $34\ M^{-1}$ to $150{,}000\ M^{-1}$ (Loftsson, Hreinsdóttir, & Másson, 2005). Therefore the appropriate model guests have to be chosen to study the issue. Phenolphthalein (PP) and *p*-methyl red (MR) may be the ideal choices (Fig. 1) since they are aromatic compounds, which are typical guests for inclusion, and the changes in absorbance make it easy to calculate the quantity. PP was chosen for modelling the 'large spheriform' guest, as only a part of its molecule is included during the complex formation (Buvári, Barcza, & Kajtár, 1988). MR served as a suitable 'small linear' molecule model since it can enter the CD cavity rather easily and the fitting is loose (Tawarah & Khouri, 1993; Tawarah & Khouri, 2000).

Preparations of HP-β-CD with low DS value (<8) were found to have optimal solubilisation properties for guests and these preparations could also be transformed into non-hygroscopic powders (Pitha, Milecki, Fales, Pannell, & Uekama, 1986). The HP-β-CDs used in this study had been investigated by the reductive-cleavage method and methylation analysis, the DS value of the HP-β-CD samples were well distributed between 0 and 9. The formation and stability of inclusion complexes formed between HP-β-CDs and PP or MR were investigated using two spectrophotometric methods. Comparing the trends in the stability constants for different hosts and guests, some new and more general conclusions were drawn.

2. Materials and methods

2.1. Materials

HP-β-CD samples were prepared in our laboratory by reacting β-CD with propylene oxide under two different base concentrations [group A (samples 1–4) under 18%; group B (samples 5–8) under 5%], except one (sample 9) which was purchased from Wako Pure Chemical Industries, Ltd. (Chuoku, Osaka, Japan). The substitution pattern and DS of the HP-β-CD samples had been investigated by a reductive-cleavage method and methylation analysis (Ciucanu & Kerek, 1984), Table 1 shows the result. All other materials were of analytical grade and used without future purification. The water used was deionized.

2.2. Determination of stability constant

The stability constants of CD–PP inclusions were determined by spectrophotometry using a UV1201 instrument (Rayleigh, Chaoyang, Beijing, China). Stock solutions of an average molar concentration of about 1.2×10^{-3} were prepared from β-CD and each HP-β-CD sample, a solution of PP (0.375 mM, 2.5 mL) was added to each stock solution (2 mL), sodium carbonate (0.04 M, 2.5 mL) was then added and the volume brought to 25 mL by addition of water. The obtained mixtures were stirred (500 rpm) at constant temperature (30 °C) until equilibrium (72 h). The absorbance was scanned from 200 nm to 850 nm to determine the formation of an inclusion complex and the characteristic absorption wavelength. At the characteristic absorption wavelength, absorbance was measured to determine the amount of PP in solutions since the complexed form is colourless (Buvári et al., 1988). With known concentrations of PP ([PP]) and the total concentrations (c_{PP} and c_{CD}), the stability constants can be calculated from the given Eqs. (1)–(3)

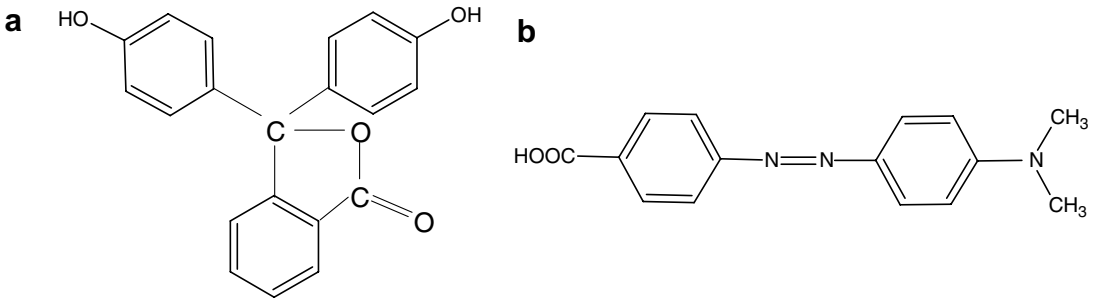

Fig. 1. Structural formulas of phenolphthalein (a) and *p*-methyl red (b).

Table 1
Distribution of substituents and the respective relative DS value of hydroxyl groups in HP-β-CDs

Position substituted	Samples (mole %)								
	Group A				Group B				
	1	2	3	4	5	6	7	8	9
–	67.4	58.8	41.4	20.1	67.3	49.5	37.9	18.3	48.2
3	0.83	2.81	6.31	4.45	2.57	1.67	1.50	1.83	2.09
2	5.77	9.75	13.1	12.2	17.0	24.5	21.3	29.4	20.5
6	12.8	13.9	14.1	20.5	1.09	3.58	2.35	4.15	3.67
23	0.59	1.17		1.25	3.85	10.8	15.8	18.6	11.0
36	1.22	2.69	1.81	6.78	6.16	3.89	8.31	6.01	6.63
22′					0.61	1.74	1.60	2.19	2.02
26	11.4	10.4	22.8	31.2	0.73	2.03	5.45	13.2	2.72
66′			0.49	1.83	0.74	1.22	2.02	1.77	1.62
236		0.49		1.51		0.49	1.57	1.86	0.71
22′6						0.59	2.16	2.65	0.86
266′				0.12					
Unsubstituted	67.4	58.8	41.4	20.1	67.3	49.5	37.9	18.3	48.2
∑Monosubstituted	19.4	26.5	33.5	37.2	20.6	29.8	25.1	35.4	26.3
∑Disubstituted	13.2	14.2	25.1	41.1	12.1	19.6	33.2	41.8	24.0
∑Trisubstituted	0	0.49	0	1.63	0	1.08	3.73	4.51	1.57
DS(3)[a]	0.18	0.50	0.57	0.98	0.88	1.18	1.90	1.98	1.43
DS(2)	1.24	1.53	2.51	3.24	1.59	2.97	3.61	5.10	2.85
DS(2 + 3)	1.43	2.03	3.08	4.22	2.47	4.15	5.51	7.08	4.28
DS(6)	1.78	1.92	2.78	4.48	0.66	0.91	1.67	2.20	1.25
DS(2 + 3)/DS(6)	0.80	1.05	1.11	0.94	3.73	4.55	3.30	3.22	3.43
Molecular DS[b]	3.21	3.95	5.77	8.68	3.14	5.06	7.19	9.27	5.53

[a] Average degree of substitution of O-3 position, i.e., average number of substituted O-3 hydroxyls of a HP-β-CD molecule.
[b] Average number of substituted hydroxyls per HP-β-CD molecule.

$$K = [\mathrm{PP} - \mathrm{CD}]/([\mathrm{PP}] \times [\mathrm{CD}]) \quad (1)$$

$$c_{\mathrm{PP}} = [\mathrm{PP}] + [\mathrm{PP} - \mathrm{CD}] \quad (2)$$

$$c_{\mathrm{CD}} = [\mathrm{CD}] + [\mathrm{PP} - \mathrm{CD}] \quad (3)$$

The stability constants of CD–MR inclusion complexes were determined by the classic phase-solubility procedure using the same spectrophotometer. Excess amounts of MR were added to 25 mL aqueous solutions containing increasing concentration of HP-β-CD or β-CD in the 0.01–0.10 mM range, at pH 2.4 (by adding 0.04 M HCl). The obtained suspensions were treated as for the PP mixtures and spectrometrically assayed for guest concentrations at their characteristic absorption wavelength. The apparent 1:1 stability constants were calculated from the straight line portion of the phase-solubility diagrams according to the Higuchi–Connors equation (Eq. (4))

$$K = \frac{\text{slope}}{\text{intercept} \times (1 - \text{slope})} \quad (4)$$

Each test was repeated at least three times.

3. Results and discussion

Table 1 shows the configuration information of the HP-β-CD samples used in the test. The distribution of HP-substituents was generally similar to the information reported previously (Mischnick, 1989; Pitha & Rao, 1990), which states that the substitution pattern depends on the alkali concentration used in the preparation. Weak alkali conditions favoured alkylation on the more acidic C-2 hydroxyls, whereas strong alkali favoured alkylation on the more accessible C-6 hydroxyls. The substitution pattern of sample 9 is similar to that of group B samples. With both alkaline conditions used in this experiment, the molar ratio of total disubstituted glucosyl residues (∑Disubstituted) increases markedly along with increasing molecular DS values, while the unsubstituted glucosyl residues decrease notablely. The amount of trisubstituted compound was found to be low in the investigated samples. The DS values of the three different positions also give some useful information on the configuration of each sample. In group A, the DS value for O-6 [DS(6)] is the highest, but the O-2 and O-3 positions also have a lot of substituents, the ratio of DS (2 + 3) to DS (6) is close to 1. The distribution of substituents on the primary and secondary hydroxyl groups is even, because the three free hydroxyl groups are all active under strong alkali condition and the O-6 position gets a little more opportunity to be substituted due to steric hindrance. In group B, however, the substituents are concentrated on O-2. The DS value of the secondary hydroxyl groups is about three times that of the primary groups. It is similar to the hydroxyethylation of cellulose, where O-2 is the most acidic position and weak alkali favours alkylation at O-2. The above analyses about the configuration characteristics of each HP-β-CD sample may help to explain the difference in complex forming ability.

UV–Vis absorption spectra of PP, HP-β-CD and their complex, scanned from 200 nm to 850 nm, are shown in Fig. 2. The absorbance of HP-β-CD is close to zero in the scanned range, therefore HP-β-CD has no effect on the absorption of PP. In alkaline solution, PP is considered to exist in the pink anion form, the whole PP anion forms a big conjugated system which has a characteristic absorption peak at 550 nm. When HP-β-CD was added to the PP solution, some of the PP anions formed inclusion complexes with the HP-β-CD and became colourless lactones, resulting in a reduction in the intensity of the absorption peak at 550 nm (Buvári et al., 1988). Some of the absorption peaks were weakened or disappeared, confirming the formation of the PP–HP-β-CD inclusion complexes. The concentration of PP in solution can be determined by measuring the absorbance at 550 nm. The stability constants, K_{PP}, of the complexes of PP with HP-β-CD were calculated using the given equation (Eqs. (1)–(3)).

Fig. 3 shows the stability constants of the complexes of PP with different HP-β-CD sample groups. The graph of group A is similar to that of group B, that is, along with the enhancement of the DS value, the complex forming ability of the HP-β-CDs decreased, giving a similar result to that given by Buvári-Barcza and Barcza (1999). In addition, some new information was found here: The K value of groups A and B are similar, suggesting that, for 'large' guests, the effect of the substitution pattern on complex stabilities is subordinate. The decrease in K value is minimal when the DS value is under 3, but decrease rapidly when the DS value is above 3. The reason may be that an increase in substitution of the secondary hydroxyl groups leads to increased steric hindrance, affecting the formation of the complex. The K_{PP} value of sample 9 is slightly higher than that of other samples with the same DS value in groups A and B. The complex forming ability of HP-β-CD to PP is lower than that of the parent β-CD. HP-β-CDs are not suited to carry 'large spheriform' organic molecules. However low DS value (<3) HP-β-CDs could be used to form inclusion complexes with the 'large spheriform' guests, which can be carried by parent β-CD, considering their high water solubility. The solubility of the guests can be highly improved under a similar complex forming ability to the parent β-CD.

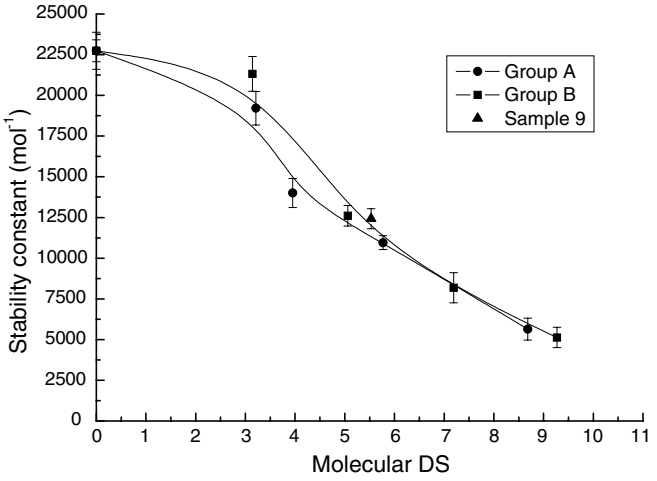

Fig. 3. Stability constant of PP inclusion complexes formed with the parent β-CD and HP-β-CDs.

In Fig. 4, the UV–Vis absorption spectra of MR, HP-β-CD and their complex is shown. The absorption spectrum of HP-β-CD is very close to the base line, similar to that described in Fig. 2. In acidic solution, MR is considered to be in the cationic protonated form (Tawarah & Khouri, 1992). The absorption spectrum of MR shows two main absorption maxima at 277 and 520 nm. The absorption at 520 nm is attributed to the whole molecular conjugated system and at 277 nm to the n–π^* transition of the carboxylate group. The addition of HP-β-CD to an aqueous solution of the MR cation resulted in a reduction in the intensity, and a red shift, of the absorption at 277 nm, confirming the formation of MR–HP-β-CD complexes. However, the intensity of the absorption at 520 nm showed no change. The solubility of MR in HP-β-CD aqueous solution can be determined by measuring the absorbance at 520 nm.

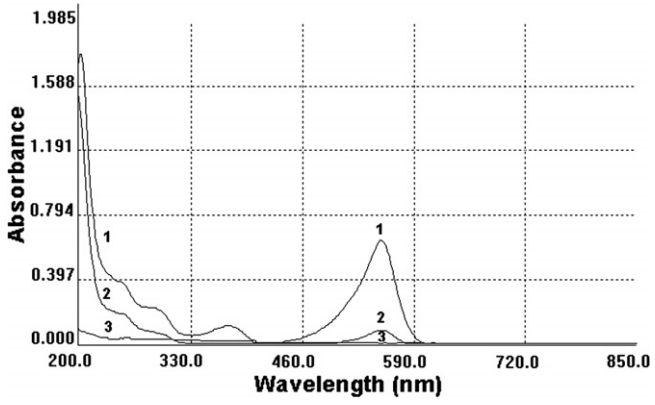

Fig. 2. UV–Vis absorption spectra of PP (**1**), HP-β-CD (**3**) and the PP–HP-β-CD inclusion complex (**2**).

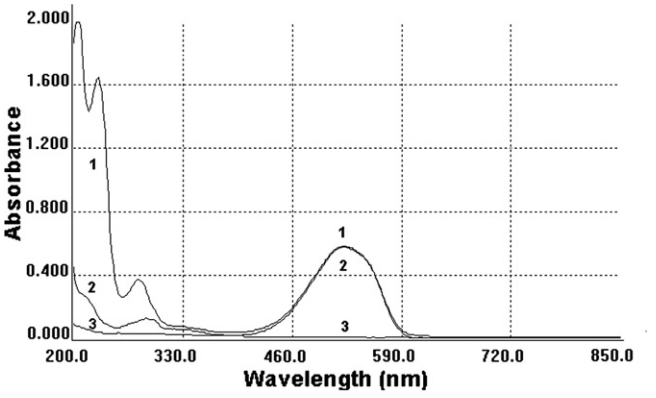

Fig. 4. UV–Vis absorption spectra of MR (**1**), HP-β-CD (**3**) and the MR–HP-β-CD inclusion complex (**2**).

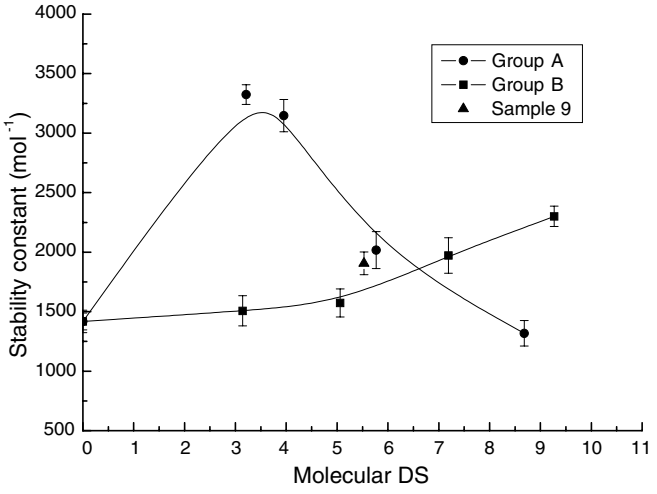

Fig. 5. Stability constant of MR inclusion complexes formed with the parent β-CD and HP-β-CDs.

The stability constants, K_{MR}, of the complexes of MR with HP-β-CD were calculated using Eq. (4) and are shown in Fig. 5. The K_{MR} value of group A rises at first and then falls with the peak value observed when the DS value is about 3.5. The K_{MR} value of group B rises slowly, and exceeds group A at around DS = 6.5. When the DS value is under 6.5, the K_{MR} of group A samples is bigger than of group B samples. This shows that the hindrance of group A samples is less than of group B, because there is less substitution of the secondary hydroxyl groups [DS(2 + 3)] of group A samples than of group B samples under similar DS values (Table 1). When the DS value is above 6.5, the K_{MR} of group A samples is smaller than for group B samples, a possible reason could be the increase of substitution on the secondary hydroxyl groups extends the cavity and thus improves the complex forming ability. The K_{MR} of sample 9 is between A and B, the complex forming ability of sample 9 with MR is lower than group A samples. Altogether, HP-β-CDs have a higher ability to form inclusion complexes with the 'small linear' guests than the parent β-CD, especially the low DS value (<6.5) HP-β-CD whose ratio of DS (2 + 3) to DS (6) is close to 1.

4. Conclusions

The complex forming ability of HP-β-CDs is not only determined by the DS and the substitution pattern but is also related to the character of the guest molecules. Compared with parent β-CD, HP-β-CDs have lower ability to form inclusion complexes with the 'large spheriform' guest molecules. However low DS value (<3) HP-β-CDs should be used to form inclusion complexes with the 'larger' guests, which can be carried by parent β-CD, considering their high water solubility. With regard to the 'smaller linear' guest molecules, HP-β-CDs have higher ability to form inclusion complexes than does the parent β-CD, especially the low DS value (<6.5) HP-β-CDs whose ratio of DS (2 + 3) to DS (6) is close to 1, and the sample with the highest complex forming ability had a DS value is about 3.5.

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