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Effect of the degree of substitution of (2-hydroxy)propyl-β-cyclodextrin on the enantioseparation of organic acids by capillary electrophoresis

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Abstract

Optical isomers of nine organic acids were separated by high-performance capillary electrophoresis using (2-hydroxy)propyl-β-cyclodextrins, with a degree of the substitution between 3.0 and 7.3 (2-hydroxy)propyl groups/cyclodextrin molecule. The degree of substitution has a significant influence on the resolution of the enantiomers and is therefore an important tool in the optimisation of chiral separations. Accordingly, a proper description of derivatized cyclodextrins should include the degree of substitution.

1. Introduction

The separation of enantiomers by capillary electrophoresis (CE) is a quickly growing field in analytical chemistry [1–3]. Micellar electrokinetic chromatography using chiral micelles, ligand-exchange mechanism and protein- or polysaccharide-type chiral selectors were successfully used for the separation of several optical isomers. However, the type of separation mostly utilised is based on inclusion complex formation, primarily with cyclodextrins (CDs) as chiral selectors.

CDs are cyclic oligosaccharides built up from D-(+)-glucopyranose units linked by α(1,4) bonds. The naturally occurring α-, β- and γ-CDs consist of 6, 7 and 8 glucose units, respectively. The glucose units form a torus with a rather hydrophobic cavity. The hydroxyl groups on the chiral carbon atoms 2 and 3 are on the wider rim of the torus. It is important to notice that these secondary hydroxyl groups cannot rotate; therefore, they provide an ideal site for chiral recognition. In contrast, the rotational primary hydroxyl groups on the narrower rim are less important for chiral recognition. Molecules can penetrate the cavity depending on their hydrophobicity, size and shape, and form an inclusion complex with the CD. Although many separation problems have been solved with the natural CDs, the application range and selectivity could be substantially enlarged by the use of synthetic derivatives. The CD derivatives often have better aqueous solubility than β-CD [4]. This can be explained by the "impure" nature of the CD derivatives. As they are mixtures of compounds with different degrees and patterns of substitu-

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tion the overall solubility can be better than that of the pure derivatives.

Heptakis(2,3,6-tri-O-methyl)-β-CD [5], heptakis(2,6-di-O-methyl)-β-CD [6], 2,6-dimethylated and 2,3,6-trimethylated α-CD [7], hydroxyethyl-β-CD, hydroxypropyl-β-CD [8] and glycosylated-α-CD [9] were successfully used for the separation of different enantiomers. In addition, the application of a series of charged CDs, e.g. mono-(6-β-aminoethylamino-6-deoxy)-β-CD [10], 6^A-methylamino- and 6^A,6^D-dimethylamino-β-CD [11], carboxymethylated, carboxyethylated β-CD [12] and carboxylated methylethyl-β-CD [13] for the separation of optical isomers has been reported. These CDs can be used in charged form for the separation of neutral or ionic enantiomers, but after proper adjustment of the pH they can be used in the uncharged form as well [12]. Most of the derivatized CDs successfully used in CE had already been used in HPLC, GC and TLC.

Some of the derivatized CDs are commercially available or can be synthesized with different degrees of substitution (DS). DS refers to the number of hydroxyl groups per CD molecule which were substituted by other functional groups. Most of the CD derivatives are mixtures of compounds with different degrees and patterns of substitution. Therefore the DS is usually an average value, and its deviation around the average is also important for the proper characterisation of a certain CD.

(2-Hydroxy)propyl-β-cyclodextrin (HP-β-CD) is commercially available with different DS values. This CD was suitable for the resolution of the enantiomers of a series of organic acids [11]. HP-β-CD was successfully used as chiral selector in TLC [14], HPLC [15] and GC [16]. Many important aspects of the use of HP-β-CD such as the DS, the chirality of the hydroxypropyl substituent, the effect of organic modifiers etc. was addressed in chromatographic applications [14–16]. However, in CE the influence of the DS on the enantioselectivity has not been studied in detail yet. In this paper the effect of the (2-hydroxy)propylation of β-CD on the chiral separation of mandelic acid and eight of its enantiomeric analogues is reported.

2. Experimental

2.1. Chemicals

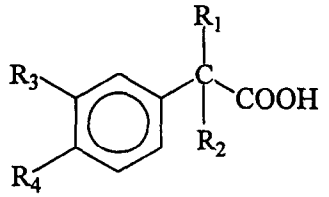
HP-β-CDs of four different DS values were purchased from Cyclolab (Budapest, Hungary). The hydroxypropyl substituents of the β-CD were racemic. β-CD was obtained from Fluka (Buchs, Switzerland). Some characteristic properties of the CDs are listed in Table 1. Mesityl oxide, DL-mandelic acid (MA), DL-3-hydroxymandelic acid (3HMA), DL-4-hydroxymandelic acid (4HMA), DL-2-phenyllactic acid (2PLA), DL-3-phenyllactic acid (3PLA), DL-2-phenylpropionic acid (2PPA), DL-α-methoxyphenylacetic acid (MPA), and DL-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) were purchased from Fluka. DL-3,4-Dihydroxymandelic acid (DMA) was from Aldrich (Steinheim, Germany). The structural formulae of the nine enantiomeric organic acids used in this study are shown in Fig. 1. Sodium hydrogenphosphate was obtained from Merck (Darmstadt, Germany). Deionised water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA). All the chemicals were at least of analytical grade and were used without further purification.

2.2. Apparatus

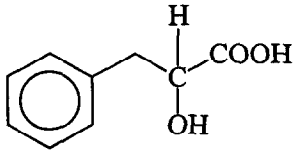
The CE experiments were carried out on a fully automated BioFocus 3000 system (Bio-Rad, Hercules, CA, USA). An uncoated fused-silica capillary 50 cm (45.4 cm effective length) × 50 μm I.D. was used throughout the study. The capillary was filled with the run buffer. The

Table 1
Characteristic properties of β-CD and HP-β-CDs

Average degree of substitution	Average molecular mass	Solubility in water (g/100 ml)
0	1135	1.85
3.0 (±5%)	1309	>33
4.3 (±5%)	1382	>33
6.3 (±5%)	1497	>33
7.3 (±5%)	1558	>33



	R_1	R_2	R_3	R_4
MA	-OH	-H	-H	-H
3HMA	-OH	-H	-OH	-H
4HMA	-OH	-H	-H	-OH
DMA	-OH	-H	-OH	-OH
2PLA	-OH	$-CH_3$	-H	-H
2PPA	$-CH_3$	-H	-H	-H
MPA	$-OCH_3$	-H	-H	-H
MTPA	$-OCH_3$	$-CF_3$	-H	-H



3PLA

Fig. 1. Structures of the enantiomeric organic acids investigated.

samples and the capillary were thermostated at 20°C. The samples were injected with 4 p.s.i.·s (1 p.s.i. = 6894.76 Pa). A constant voltage of 20 kV was applied. The analytes were detected at 206 nm.

2.3. Buffers and samples

HP-β-CD (0–80 m*M*) or 0–15 m*M* β-CD was dissolved in 50 m*M* phosphate buffer pH 7 or 8. These buffers were used as background electrolytes. The samples were dissolved in 5 m*M* phosphate buffer pH 8 in a concentration of 50 μg/ml. Mesityl oxide was used as an electroosmotic flow marker.

2.4. Calculations

The resolution (R_s) was calculated using the well known equation $R_s = 1.18(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the migration times and w_1 and w_2 are the widths of the first and the second peak at half of the peak heights. The width of poorly resolved peaks cannot be precisely measured at the half of the peak height. In such cases the resolution was expressed as $R' = 100H'/H$, where H is the height of the first peak and H' is the depth of the valley between the first and the second peak. According to this definition $R' = 100$ means baseline resolution.

3. Results and discussion

To separate the enantiomers of organic acids with uncharged CDs like β-CD or HP-β-CDs the analytes have to be in ionic form. At a pH of 7 or higher the selected acids are predominantly in charged form. Throughout the study an uncoated capillary was used. The anions were migrating electrophoretically opposite to the detector but the intense electroosmotic flow reversed the direction of the apparent mobility. The stronger electroosmotic flow at pH 8 resulted in shorter migration times than at pH 7. Although the resolution was better at the lower pH (data not shown) the effect of the DS was studied at pH 8. With this compromise the run times could be kept reasonably low. Our intention was to show the influence of the DS on the separation of a set of compounds rather than to find the optimum conditions for the enantiomer separation.

When underivatized β-CD was dissolved in the background electrolyte no or very poor ($R' < 10$) separation was achieved for most of the enantiomeric pairs up to the maximal concentration of 15 m*M*. However, there are two exceptions; MTPA ($R_s = 2.31$) and 2PPA ($R_s = 1.21$). The resolution is a strong function of the chiral selector concentration. Upon increasing the CD concentration the resolution passes through a maximum [17,18]. The low aqueous solubility of the β-CD sets the limit of the chiral selector concentration so low, that often the optimum cannot be reached or even no sign of chiral recognition can be observed. One of the strategies to overcome this problem is to dissolve urea in the buffer containing β-CD, which can enhance the solubility considerably [19].

Another approach is to derivatize the β-CD so that the solubility can be increased and the enantioselectivity can be altered.

All the nine pairs of enantiomers were resolved with at least one of the HP-β-CDs. However, the resolution was in some cases remarkably different for the same components when HP-β-CD with different *average* DS was used. The resolution values of the enantiomers obtained with HP-β-CDs having different DS values are listed in Table 2.

2PLA was the worst resolved compound. Some weak enantioseparation ($R' = 13.8$) was achieved with 80 m*M* HP-β-CD, DS = 7.3 but no chiral separation was possible with any of the other CDs. The resolution for 3PLA was better than for 2PLA. R' of the 3PLA enantiomers is substantially different when HP-β-CDs with different DS values are used (Fig. 2). The resolutions in the figure are expressed as R' because the R_s values for the weakly resolved peaks cannot be reliably calculated. However, the weak point of this expression is that R' stays constant after baseline separation was reached. In order to give proper information about the separation of both poorly and well resolved peaks Table 2 shows the resolution expressed as R_s but the figures indicate R' values.

Good resolution can be achieved for 2PPA with all the HP-β-CDs as shown in Fig. 3. All

Table 2
Resolution of the enantiomers of nine organic acids

DS	m*M*	R_s								
		MA	3HMA	4HMA	DMA	2PLA	3PLA	2PPA	MPA	MTPA
0	5	–[a]	–[a]	–[a]	–[a]	–[a]	–[b]	1.21	–[a]	2.31
	10	–[a]	–[a]	–[a]	–[a]	–[a]	–[b]	1.03	–[a]	2.11
	15	–[a]	–[a]	–[b]	–[a]	–[a]	–[b]	1.09	–[a]	2.05
3.0	10	–[a]	–[a]	–[a]	–[a]	–[a]	0.91	1.08	–[a]	–[b]
	20	–[b]	–[a]	–[a]	–[b]	–[a]	1.02	1.35	–[a]	0.86
	40	1.11	–[b]	–[a]	0.87	–[a]	1.01	1.48	0.75	0.82
	60	1.50	1.04	–[b]	1.28	–[a]	0.70	1.39	1.40	0.81
	80	1.55	1.14	1.49	n.d.	–[a]	–[b]	1.52	1.44	–[b]
4.3	10	–[a]	–[a]	–[b]	–[a]	–[a]	–[b]	0.84	–[a]	–[a]
	20	–[b]	–[b]	1.11	–[b]	–[a]	–[b]	1.32	–[b]	–[a]
	40	1.16	0.91	1.57	–[b]	–[a]	–[b]	1.56	0.96	–[a]
	60	1.49	1.46	2.21	0.91	–[a]	0.81	1.89	1.46	–[a]
	80	1.89	1.30	2.63	1.97	–[a]	0.91	2.02	2.21	–[a]
6.3	10	–[a]	–[a]	–[b]	–[a]	–[a]	–[b]	1.16	–[a]	–[b]
	20	–[b]	–[a]	1.00	–[a]	–[a]	0.91	1.60	–[b]	–[b]
	40	0.94	–[b]	1.36	–[b]	–[a]	0.86	1.74	0.93	–[b]
	60	1.67	0.89	1.25	0.89	–[a]	0.83	1.60	1.16	–[b]
	80	2.40	1.37	1.85	1.37	–[a]	0.89	2.05	1.72	–[b]
7.3	10	–[a]	–[a]	–[b]	–[a]	–[a]	–[b]	0.81	–[a]	–[b]
	20	–[b]	–[b]	1.33	–[b]	–[a]	0.89	1.37	–[b]	–[b]
	40	1.28	1.57	2.69	1.57	–[a]	1.04	1.70	1.98	–[b]
	60	1.89	1.69	1.14	1.69	–[b]	1.22	1.07	1.89	–[b]
	80	2.34	2.57	1.27	2.58	–[b]	1.17	0.93	2.21	–[b]

For experimental conditions see the text. n.d. = No data.
[a] No separation.
[b] Poor enantioseparation, the peaks were not resolved at the half of the peak height.

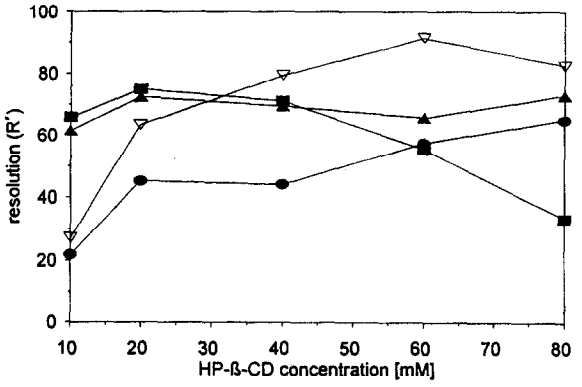


Fig. 2. Resolution (R') of the 3PLA enantiomers as a function of the HP-β-CD concentration. DS values: ■ = 3.0; ● = 4.3; ▲ = 6.3; ▽ = 7.3. See the text for experimental conditions.

the four HP-β-CDs can provide baseline resolution, but resolution versus CD concentration plots are different. When HP-β-CD, DS = 7.3 is used the resolution reaches a maximum at 40 mM. With the other HP-β-CDs the maximum resolution was not achieved in the concentration range studied. The greater the affinity of the enantiomers for the chiral selector the lower is the optimum CD concentration [17]. This means that the inclusion complex of 2PPA with HP-β-CD has the highest equilibrium constant at DS = 7.3.

MTPA was the only compound that was better resolved with β-CD than with any of the HP-β-CDs. The best resolution of MTPA with HP-β-CDs (R' = 57.5) was measured at DS = 3.0. At DS = 4.3 no enantioseparation was found. MTPA and 2PLA are both disubstituted on the α-carbon atom and have the worst resolution in the set of enantiomers studied. MPA differs from MTPA only at the α-carbon which is monosubstituted in MPA. This compound was well resolved with all the HP-β-CDs. The (2-hydroxy)propyl groups on the rim of the CD cavity may form a steric barrier for compounds bearing two substituents on the α-carbon. This effect can be seen in the case of MTPA, where the absolute value of the effective mobility decreases with a factor of 1.8 if 10 mM β-CD was dissolved in the phosphate buffer. This low CD concentration does not influence the electroosmotic flow considerably, so the reduced effective mobility is a consequence of the strong complexation with the β-CD. In contrast the decrease in absolute value of the effective mobility is only 1.2 if the concentration of HP-β-CD DS = 7.3 is increased from 0 to 10 mM. Fig. 4 shows the effective mobility of the MTPA and 2PLA as a function of the concentration of β-CD and HP-β-CD, DS = 7.3. The absolute value of the effective mobility is reduced significantly if β-CD is dissolved in the buffer. The decrease in the effective mobility is much less pronounced if HP-β-CD is used as a chiral selector. These findings may indicate that

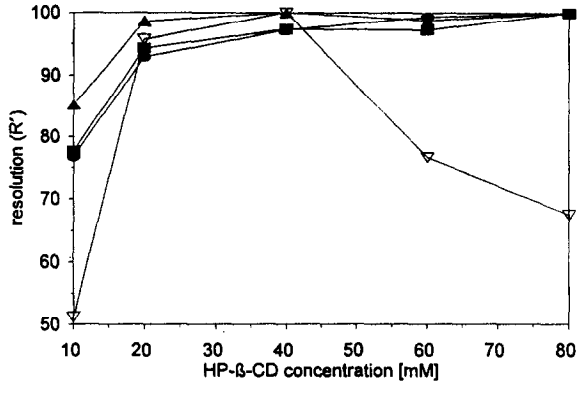


Fig. 3. Resolution (R') of the 2PPA enantiomers as a function of the HP-β-CD concentration. DS values: ■ = 3.0; ● = 4.3; ▲ = 6.3; ▽ = 7.3. See the text for experimental conditions.

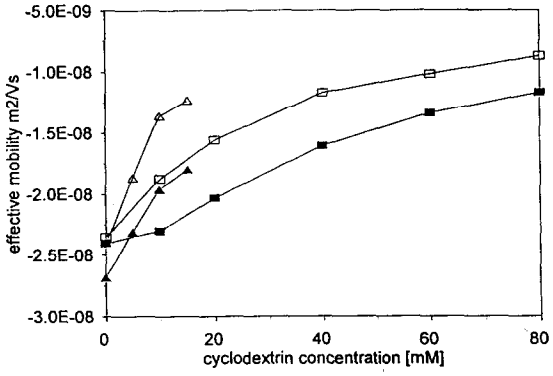


Fig. 4. The effect of β-CD and HP-β-CD (DS = 7.3) concentration on the effective mobility of 2PLA and MTPA. Symbols: ■ = 2PLA + HP-β-CD (DS = 7.3); ▲ = 2PLA + β-CD; □ = MTPA + HP-β-CD (DS = 7.3); △ = MTPA + β-CD. See the text for experimental conditions.

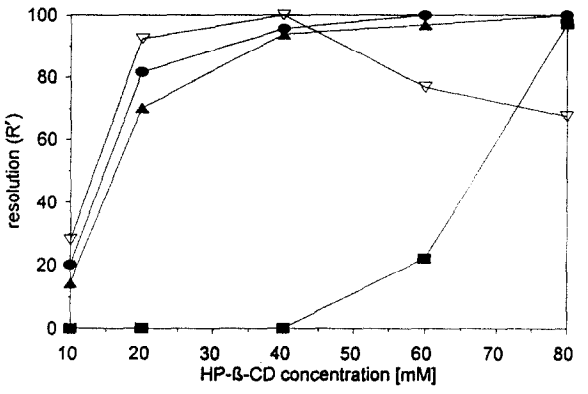


Fig. 5. Resolution (R') of the 4HMA enantiomers as a function of the HP-β-CD concentration. DS values: ■ = 3.0; ● = 4.3; ▲ = 6.3; ▽ = 7.3. See the text for experimental conditions.

for compounds disubstituted in the α-carbon atom, the inclusion complex formation is hindered. In addition to the less favoured formation of the complex the enantioselectivity of HP-β-CD may also be substantially different from that of β-CD.

MA and its analogues hydroxylated on the aromatic ring are well resolved with the HP-β-CDs. However, at different degrees of substitution the resolution of the same compound is sometimes very different. Fig. 5 shows how the resolution of 4HMA is effected by the concentration of different HP-β-CDs. 4HMA was the best resolved compound from the nine enantiomeric pairs investigated in this study. At 40 m*M* HP-β-CD, DS = 7.3 the resolution was as high as 2.69.

4. Conclusions

The enantioselectivity of derivatized CDs can be significantly influenced by changing the DS. The resolution of a certain pair of enantiomers can be very different if the DS of the chiral selector is different. In some cases the chiral recognition can be completely lacking at a DS value but using the same type of CD at another DS the separation of the enantiomers may be possible. These effects can be successfully used for the optimization of chiral separations. On the other hand, our results emphasise that proper description of the derivatized CDs should include the information about the DS.

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