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Use of charged and neutral cyclodextrins in capillary zone electrophoresis: enantiomeric resolution of some 2-hydroxy acids

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ABSTRACT

Enantiomers of racemic 2-hydroxy acids, namely 2-phenyllactic, 3-phenyllactic, mandelic, *m*-hydroxymandelic, *p*-hydroxymandelic and 3,4-dihydroxymandelic acid, were resolved by capillary zone electrophoresis. The separation was achieved by using the background electrolyte with addition of cyclodextrins. The effects of the type of cyclodextrin, the pH of the electrolyte and the shape of analyte compounds on the migration time and resolution were studied. Good resolution was obtained with background electrolytes in the pH range 5–7, supplemented with 2-hydroxypropyl, 6^{A}-methylamino- and $6^{A},6^{D}$-dimethylamino-β-cyclodextrin.

INTRODUCTION

The resolution of enantiomers in racemic mixtures of hydroxy acids is an interesting field of application in analytical chemistry, especially in pharmaceutical analysis and metabolism studies [1].

Gas chromatography (GC) and liquid chromatography (LC) have so far been used for the resolution of 2-hydroxy acid enantiomers [2–5].

In LC the resolution is obtained by using chiral compounds either in the stationary phase or in the mobile phase, *e.g.*, metal complexes with chiral amino acids, cyclodextrins or triacetylcellulose.

Capillary zone electrophoresis (CZE) is a recent method that allows rapid separations to be obtained with high efficiency and high resolution of compounds with different effective electrophoretic mobilities [6]. When the electrophoretic analysis of a racemic mixture is carried out in an achiral background electrolyte (BGE), the two enantiomers are usually not resolved because they have the same mobilities. Hence the use of a suitable chiral additive in the background electrolyte is required in order to

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interact selectively with enantiomers to give a difference in their mobilities and hence allow their separation. This can be done, *e.g.*, by using some equilibria with chiral additives by forming diastereoisomeric complexes with the analytes. Among several mechanisms used in CZE, inclusion complexation seems to be interesting for the resolution of several classes of enantiomers.

Cyclodextrins (CDs) have already been successfully used for these purposes, added either to the BGE or to the gel or bonded to the capillary walls [7–11]. Recent developments in the electrophoretic separation of optical isomers can be found elsewhere [12–14]. CDs are chiral, natural oligosaccharides with a shape similar to a truncated cone with a relatively hydrophobic cavity able to form inclusion complexes with analytes (aromatic groups are preferred). The outside of the CD is more hydrophilic [15]. The enantioselectivity arises from the chiral carbon of the glucose units in the CDs. The formation of inclusion complexes between enantiomers and CDs is strongly influenced not only by the hydrophobic interaction in the cavity but also by bonding between the hydroxyl groups (or other substituents) on the rim of CDs and substituent groups of the asymmetric centre of the analytes.

This paper describes a continuation of our studies on chiral separations in CZE by using CDs as chiral additives to the BGE [16–19]. This work was aimed at an experimental study of the effects of the type and concentration of the CD and the pH of the BGE on the migration time and the resolution of DL-2-phenyllactic, DL-3-phenyllactic, DL-mandelic, DL-*m*-hydroxymandelic, DL-*p*-hydroxymandelic and DL-3,4-dihydroxymandelic acid. We selected β-CD and some modified β-CDs, namely, heptakis(2,6-di-O-methyl)-, 2-hydroxypropyl, 6^A-methylamino- and 6^A,6^D-dimethylamino-β-cyclodextrin for electrophoretic experiments. The modified CDs can provide greater effectiveness in enantiomeric resolution because they possess different properties with respect to the parent compounds, *e.g.*, the solubility can be higher, the cavity can be deeper (dimethyl-β-CD) or the presence of charged groups can stabilize the inclusion complex etc.

EXPERIMENTAL

Sodium dihydrogenphosphate, phosphoric acid, sodium acetate, acetic acid and L- and D-mandelic acid (MA) were purchased from Carlo Erba (Milan, Italy), DL-3-phenyllactic acid (3-PhL), L-3-phenyllactic acid, DL-*m*-hydroxymandelic acid (*m*-MA), DL-*p*-hydroxymandelic acid (*p*-MA), DL-3,4-dihydroxymandelic acid (3,4-di-MA) and β-cyclodextrin from Sigma (St. Louis, MO, USA), DL-atrolactic acid hemihydrate (2-PhL) from Fluka (Buchs, Switzerland) and heptakis(2,6-di-O-methyl)-β-cyclodextrin (di-Me-β-CD) and 2-hydroxypropyl-β-cyclodextrin (2-PRO-β-CD) from Chinoin (Budapest, Hungary). 6^A-Methylamino-β-cyclodextrin (6-NH-β-CD) was synthesized through tosylated intermediates [20] with subsequent substitution of the tosyl groups by methylamine [21]. 6^A,6^D-Dimethylamino-β-cyclodextrin (6-di-MeNH-β-CD) was prepared by the reaction of methylamine [21] with 6^A,6^D-(biphenyl-4,4′-disulphonyl)-β-cyclodextrin, obtained by a regioselective capping procedure in accordance with ref. 22. Analytical data for the aminated cyclodextrins are presented elsewhere [23]. The formulae of the racemic acids, their abbreviations and the symbols used in the figures are shown in Fig. 1.

Apparatus

A Bio-Rad HPE 100 apparatus (Bio-Rad Labs., Richmond, CA, USA) equipped with an

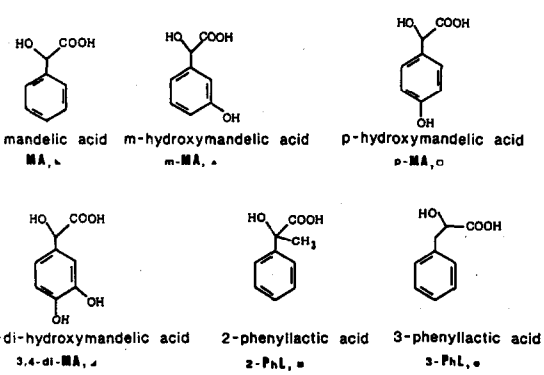

Fig. 1. Structures of mandelic acid and derivatives.

on-column UV detector operated at 206 nm was used for electrophoretic experiments. The high-voltage power supply was operated in either a constant-voltage or constant-current mode. The electrophoretic analyses were performed in a capillary (20 cm × 0.025 mm I.D.) with a coated inner wall mounted in a cartridge (Bio-Rad Labs.). This capillary showed negligible electroosmosis. Samples were injected by the electromigration method at constant voltage. The electropherograms were recorded by using a Model 2210 line recorder (LKB, Bromma, Sweden).

Background electrolyte (BGE)

Stock solutions 0.1 *M* sodium dihydrogenphosphate titrated with either phosphoric acid or sodium hydroxide at pH 6 and 7 were used for electrophoretic experiments. For the separations at pH 5, 0.1 *M* sodium acetate titrated with acetic acid was used. The appropriate amount of a selected CD was added to the stock solutions before the experiments.

The resolution (R) of the enantiomers was calculated by using the following equation:

$$R = \frac{2(t_L - t_D)}{w_L + w_D} \quad (1)$$

where t is the migration time, w the width of the peak at the baseline and L and D represent the two enantiomers.

RESULTS AND DISCUSSION

Considering the requirement that the acids in question (see Fig. 1) should be sufficiently dissociated and considering the data from ref. 24, we selected a BGE in the pH range 5–7 for electrophoretic experiments. Under these conditions all the analyte compounds migrated anodically. In the absence of CDs the migration times of the analytes were short (less than 5 min) and, as expected, the racemic mixtures were not resolved. When enantiomers of the same compound have to be separated it is necessary to modify selectively their mobility and this can be done, *e.g.*, by using some equilibria with chiral additives by forming diastereoisomeric complexes with analytes. Among several mechanisms used in CZE, inclusion complexation seems to be interesting for the resolution of several classes of enantiomers.

Fig. 2 shows the effect of the concentration of β-CD added to the BGE at pH 5 on the migration time of the analyte compounds. By increasing the amount of CD in the BGE the migration time of all the analytes increased. The effect was most evident for 3-phenyllactic and 2-phenyllactic acid. The resolution of enantiomers was obtained only for 3-PhL ($R = 0.5$) and 2-PhL ($R = 0.8$). By performing similar electrophoretic experiments with the BGE at pH 6 and 7 an increase in the migration times with increasing amount of CD was observed in all instances, but no resolution of enantiomers was obtained.

The increase in the migration times of the acids investigated when CD was added to the BGE shows that inclusion complexation takes place. The stronger the complexation, the larger is the increase in migration time.

By using di-Me-β-CD as the additive to BGE (up to 80 m*M*), the migration time of all the racemic compounds was increased in proportion to the amount of CD added, but no resolution was observed in any instance. The effect was greater for the two phenyllactic acids than the other compounds, probably owing to the forma-

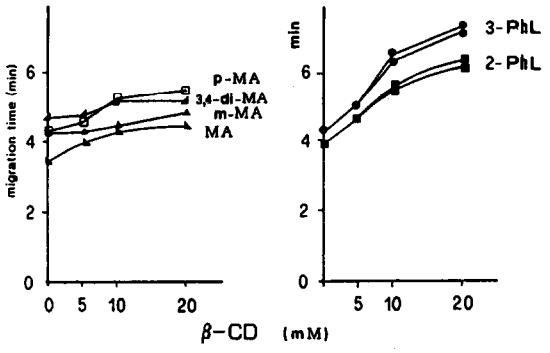

Fig. 2. Effect of the amount of β-cyclodextrin (β-CD) added to the BGE at pH 5 on the migration times of 2-hydroxy acids. Electrophoresis, 12 μA (constant), 7 kV; sampling, electromigration at 8 kV, 8 s.

tion of inclusion complexes with higher stability constants; the effect was more evident when the BGE at pH 7 was used.

When we used 2-PRO-β-CD as an additive to the BGE, the migration time of all the compounds increased when the amount of CD increased in all experiments. Fig. 3 shows the effect of the amount of modified CD on the migration times of the compounds when the BGE at pH 6 was used. It is clear that the differences in the migration times of the separated enantiomers are relatively high.

Concerning the enantiomeric resolution, none was observed for *m*-MA or 3,4-di-MA at any pH. MA and *p*-MA were resolved poorly and only when the BGE at pH 6 was used ($R = 0.5$ at 80 m*M* 2-PRO-β-CD). For 2-PhL good enantiomeric resolution was obtained at all pH values. For 3-PhL some enantiomeric resolution was also obtained at all pH values, but the resolution was not satisfactory (the best result obtained was $R = 0.5$ at pH 5 and 20 m*M* 2-PRO-β-CD). In spite of the greater inclusion complexation of 3-PhL than 2-PhL, the former showed a higher degree of resolution, which was strongly related to the concentration of chiral additive (at pH 6, $R = 0.9$ and 1.1 with 20 and 80 m*M* 2-PRO-β-CD, respectively). The higher enantiomeric resolution of 2-PhL than that obtained for 3-PhL can be explained by considering their structures. The chiral carbon is in a different position, α- and β- for 2- and 3-PhL, respectively, and also 2-PhL possesses a methyl group that can interact with the hydroxypropyl substituent of the CD.

Further experiments were carried out with a charged type of CD, namely 6^A-methylamino-β-CD. Fig. 4 shows the effect of the amount of this CD on the migration times of the 2-hydroxy acids at pH 5.

Generally, all the compounds moved anodically with a reduced velocity when the CD content was increased. 3-PhL showed higher complexation than 2-PhL. The complexation order for mandelic acid and its derivatives was *p*-MA > MA > 3,4-di-MA > *m*-MA. 3,4-Di-MA and *m*-MA are less complexed than the other compounds owing to the hindering effect of OH groups on the aromatic ring. The enantiomeric resolution of all compounds under investigation was obtained in a broad range of added concentrations of this CD, and, it was pH dependent.

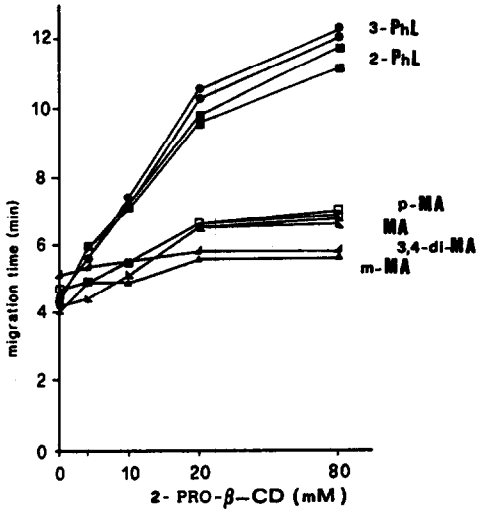

Fig. 3. Effect of 2-hydroxypropyl-β-cyclodextrin (2-PRO-β-CD) concentration on the migration times of 2-hydroxy acids. BGE, pH 6; electrophoresis, 20 μA (constant), 6.5 kV; sampling, electromigration at 8 kV, 8 s.

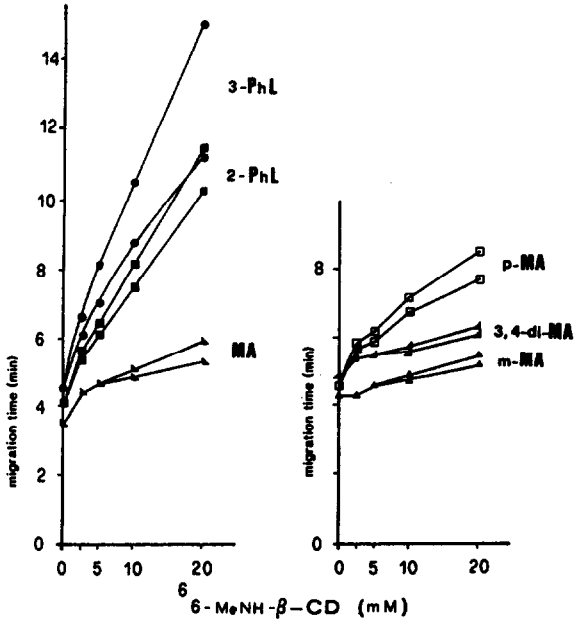

Fig. 4. Effect of 6^A-methylamino-β-cyclodextrin (6-MeNH-β-CD) on the migration times of 2-hydroxy acids at pH 5.

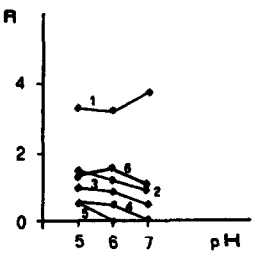

Fig. 5. Effect of the pH on the resolution (R) of 2-hydroxy acids: 1 = 3-PhL; 2 = MA; 3 = *p*-MA; 4 = *m*-MA; 5 = 3,4-di-MA; 6 = 2-PhL. BGE containing 10 m*M* 6^A-methylamino-β-cyclodextrin.

Fig. 5 depicts this effect and shows plots of R *versus* pH for racemic compounds analysed in presence of 10 m*M* 6^A-methylamino-β-CD. By increasing the pH of the BGE the resolution improved for 3-PhL, for 2-PhL it increased at pH 6 and decreased at pH 7 and for MA and its derivatives the resolution decreased.

The results indicate that the presence of the methylamino substituent on the β-CD ring is very important for improving the complexation and the resolution of racemic mixtures. Inclusion complexation seems to be the main mechanism involved during the electrophoretic process and the complex stability and chiral discrimination are improved by the electrostatic interactions between amino (β-CD) and carboxylic (analyte) groups.

In order to verify the effect of the methylamino substituent, which brings one positive charge to the CD, on the resolution of hydroxy acids, experiments were also performed (at pH 5) with a disubstituted methylamino-β-CD (6^A,6^D-dimethylamino-β-CD), which has two positive charge. Table I gives the results. Obviously, the migration time and the resolution increased on increasing the amount of CD added. The effect was stronger for 2- and 3-PhL than for the other compounds. When 5 m*M* 6^A,6^D-dimethylamino-β-CD was used, 2-PhL and 3-PhL gave very broad peaks, probably owing to the slow kinetics of the complexation.

TABLE I

EFFECT OF THE CONCENTRATION OF 6^A,6^D-DIMETHYLAMINO-β-CYCLODEXTRIN ON THE MIGRATION TIMES AND RESOLUTION OF 2-HYDROXY ACIDS

t_m = Migration time (min); R = resolution.

2-Hydroxy acid	6^A,6^D-Dimethylamino-β-CD (m*M*)									
	0.0		0.5		1.0		2.5		5.0	
	t_m	R	t_m	R	t_m	R	t_m	R	t_m	R
MA	3.5	–	3.7	0.5	4.1	1.0	4.7	2.0	6.3	3.2
			3.8		4.4		5.3		7.6	
m-MA	4.3	–	4.1	–	4.2	0.5	4.4	1.1	5.3	3.4
					4.3		4.7		6.0	
p-MA	4.4	–	4.4	0.8	4.8	1.4	5.7	2.8	9.5	4.0
			4.6		5.3		6.9		13.5	
3,4-Di-MA	4.7	–	4.3	–	4.6	0.5	4.7	1.0	5.6	2.0
					4.7		4.9		6.1	
2-PhL	4.0	–	4.5	1.0	5.3	1.3	8.2	2.3	n.m.[a]	n.m.
			4.8		6.0		10.5			
3-PhL	4.3	–	4.6	1.6	5.8	3.5	8.8	3.2	n.m.	n.m.
			5.5		6.3		15.3			

[a] n.m. = Not measured.

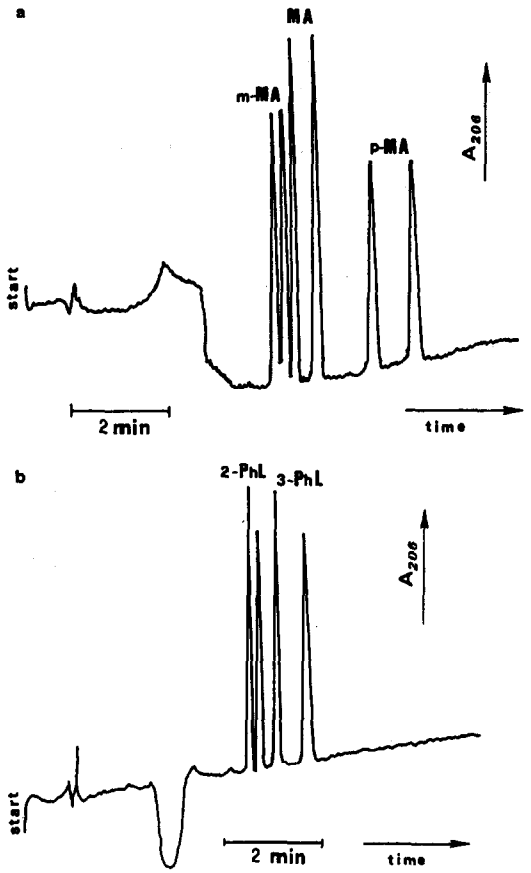

Fig. 6. Chiral resolution of 2-hydroxy acids by CZE. Capillary column, 20 cm × 0.025 mm I.D., coated; BGE, 0.1 *M* acetate buffer (pH 5) containing 6^A-methylamino-β-cyclodextrin at (a) 20 and (b) 5 m*M*; electrophoresis, 7 kV (constant), 15 μA; sampling, electromigration at 8 kV, 8 s; 10^{-5} *M* of each racemic compound.

As an example, Fig. 6a and b show the resolution of a mixture of the racemic 2-hydroxy acids into their enantiomers when the BGE at pH 5 was supplemented with 5 and 20 m*M* 6^A-methylamino-β-CD, respectively. The migration order was verified by spiking the racemic mixture with pure standards (L- and D-mandelic acid, L-phenyllactic acid). In all instances when β-CD and modified CDs were used, the L-isomer moved more slowly than the D-isomer.

CONCLUSIONS

It has been demonstrated that CZE can be successfully used for the enantiomeric resolution of racemic mixtures of 2-hydroxy acids when modified CDs serve as the chiral additive to the BGE and thus selective retardation is involved owing to an inclusion complexation mechanism. Among the various cyclodextrins tested, modified β-CD (di-Me-β-CD, 2-PRO-β-CD, 6^A-MeNH-β-CD and 6^A,6^D-di-MeNH-β-CD) seem to be the most useful for the enantiomeric resolution of this type of compound. The chiral discrimination is influenced by the type of CD, its concentration and the pH of the BGE.

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