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Use of MDL 63 246 (Hepta-Tyr) antibiotic in capillary zone electrophoresis
II. Chiral resolution of α-hydroxy acids

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Abstract

The glycopeptide antibiotic MDL 63 246 (Hepta-Tyr), belonging to the teicoplanin family, has been adopted for the separation of several α-hydroxy acid enantiomers by capillary zone electrophoresis (CZE). A polyacrylamide coated capillary was employed for the electrophoretic runs in order to suppress/minimize both the electroosmotic flow (EOF) and the antibiotic adsorption. The experiments were carried out using a background electrolyte (BGE) (aqueous–organic buffer) in the pH range 4–6 that allowed the analytes to reach the detector, as negatively charged species, in a relatively short time while the antibiotic moved in the opposite direction (positively charged). The chiral selector, dissolved in the BGE at relatively low concentration, filled only part of the capillary (partial filling-counter current method) allowing the detection of analytes with good sensitivity and short analysis time (5–8 min). Experimental parameters influencing the enantioresolution such as antibiotic concentration, buffer pH, organic modifier type and concentration and capillary temperature were investigated. Hepta-Tyr antibiotic exhibited a high enantiorecognition capability towards mandelic acid and their hydroxy and chloro derivatives even at very low concentration (1 mg/ml) using a background electrolyte (BGE) at pH 4 containing 20% (v/v) of methanol. On the other hand 2- and 3-phenyllactic acids were baseline resolved in their enantiomers with the same BGE containing acetonitrile and 4 mg/ml of chiral selector.

Keywords: Enantiomer separation; Chiral selectors; MDL 63 246; Hepta-Tyr; Antibiotics; Mandelic acids; Phenyllactic acids; Hydroxymandelic acids

1. Introduction

The separation of chiral compounds is an important subject of research in several fields such as biological, environmental, clinical, pharmaceutical, etc. It is well known that two enantiomers of the same compound may exhibit a different biological or pharmacological activity, e.g., the *l*-isomer of isoproterenol is 50 times as powerful as the *d*-isomer in circulatory activity in man [1].

Analytical methods so far used for the enantiomers separation include high-performance liquid chromatography (HPLC), gas chromatography (GC), thin-layer chromatography (TLC) and recently capillary electrophoresis (CE) [2–8].

In the last decade CE has been shown to be a powerful tool for chiral analysis due to its characteristics, e.g. very high resolution capability, short analysis time, use of minute amounts of both sample and buffer, etc.

Several strategies have been applied in chiral CE analysis [9], however, the most simple and rapid

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separation method is the direct one where the chiral selector is simply added to the background electrolyte (BGE) prior to the electrophoretic run. A wide number of chiral selectors have been successfully used in CE for chiral separations including cyclodextrins or their derivatives, chiral crown-ethers, chiral micelles, proteins, antibiotics, etc. [6–8,10].

Macrocyclic antibiotics such as vancomycin, ryfamycin, ristocetin, A82846B and teicoplanin have been used in CE for the enantioseparation of a wide number of racemic compounds (charged and/or uncharged) [11–18]. All these macrocyclic antibiotics contain several asymmetric centres with a number of functional groups responsible for their chiral recognition capability. Due to the presence of ionogenic groups in their structure they can be either positively or negatively charged as well as uncharged depending on the buffer pH. The enantioresolution mechanism is based on charge–charge, hydrogen, dipole–dipole, hydrophobic, $\pi-\pi$ interactions. Furthermore, steric repulsion has to be considered in the enantioresolution process.

Although macrocyclic antibiotics have been shown to be excellent chiral selectors in CE, they exhibited some drawbacks such as strong absorption in the UV wavelengths and adsorption on the capillary wall. The use of coated capillaries with either counter current [11,18] or partial filling-counter current methods [13,17,19,20] can be successfully applied for method optimization in chiral analysis by CE. With both methods the detector path is chiral selector-free when the analytes are recorded.

Recently we reported the use of a new macrocyclic antibiotic, Hepta-Tyr MDL 63 246, belonging to the teicoplanin's family as a chiral selector for the enantiomers resolution of compounds of pharmaceutical and environmental interest by CE [20]. In this paper we further investigated the enantiorecognition capability of Hepta-Tyr antibiotic towards α-hydroxy acid derivatives. The electrophoretic experiments were carried out in a polyacrylamide coated capillary using the partial filling-counter current method. The effect of chiral selector concentration, pH of the BGE, organic modifier type and concentration as well as column temperature on chiral resolution of racemic mandelic acid (MA), *m*-hydroxymandelic acid (*m*-OH-MA), *p*-hydroxymandelic acid (*p*-OH-MA), 3,4-dihydroxymandelic acid (3,4-di-OH-MA), 4-chloromandelic acid (4-Cl-MA), 3-phenyllactic acid (3-PhL) and 2-phenyllactic acid (2-PhL) were investigated.

2. Experimental

2.1. Chemical and reagents

Acetic acid and boric acid were purchased from Carlo Erba (Milan, Italy). Methanol (MeOH), *n*-propanol (*n*-PRO), 2-propanol (2-PRO), acetone (ACT) and acetonitrile (ACN), all of analytical grade, were from BDH (Poole, UK). The glycopeptide antibiotic MDL 63 246 (Hepta-Tyr) was synthesized in the Lepetit Research Center (Gerenzano, Varese, Italy) [21] and purified by isoelectric focusing [22]. MA, *m*-OH-MA, 3,4-di-OH-MA and D- and L-3-phenyllactic acids were purchased from Sigma (St. Louis, MO, USA). Racemic *p*-OH-MA was from Ega Chemie (Steinheim, Albuch, Germany). Racemic 2-PhL, 4-Cl-MA, phosphoric acid (85%, w/w) and sodium hydroxide were from Fluka (Buchs, Switzerland).

2.2. Instrumentation and procedures

Electrophoretic experiments were performed with an automated BioFocus 3000 Capillary Electrophoresis Apparatus (Bio-Rad Labs., Hercules, CA, USA) equipped with a multi-wavelength variable UV detector, operating at 206 nm, using either untreated or polyacrylamide coated capillaries (for the coating procedure see Ref. [23]). The polyacrylamide coated capillary was 35 cm (30.5 effective length)×50 μm I.D., while the untreated capillary was 50 cm (45.5 cm, effective length)×50 μm I.D. (Composite Metal Services LTD, Hallow, UK). The capillaries were inserted into the user assembler cartridge (Bio-Rad) after removing the polyimide layer (0.5 cm) with sulfuric acid (>100°C). The temperature of both cartridge and carousel was kept at 25°C, if not stated otherwise. Samples were injected by pressure ($34.475 \cdot 10^{-3}$ MPa, 2 s) at the anodic or cathodic end when uncoated or coated capillary were used, respectively.

A stock solution (A) 150 m*M* (equal concentration

of acetic, phosphoric and boric acid, 50 m*M* each) was prepared. Solution A was titrated with 0.1 *M* sodium hydroxide to the desired pH (range 3–10) and diluted with water to the final concentration of 75 m*M* Britton Robinson buffer (B.R.B.). The running background electrolyte was a mixture of 75 m*M* B.R.B.–organic modifier (80:20, v/v), if not stated otherwise.

For the partial filling-counter current method the capillary was rinsed with 75 m*M* B.R.B.–ACN (60:40, v/v) for 100 s followed by the running buffer (80 s). The next step was the introduction of the running buffer containing Hepta-Tyr at low pressure in order to control the filling process of the capillary up to the detector path and then the sample injected. The applied voltage was −20 or +20 kV with polyacrylamide coated or untreated capillary, respectively.

Standard analytes were dissolved in MeOH (stock solutions of 10^{-3} *M*) and diluted with 7.5 m*M* B.R.B. to the final concentration $1\cdot10^{-4}$–$2.5\cdot10^{-5}$ *M* prior to the injection.

3. Results and discussion

MDL 63 246 Hepta-Tyr antibiotic is a semisynthetic compound related to a class of amides of 34-de(acetylglucosamynil)-34-deoxy teicoplanin recently synthesized [21] and purified by isoelectric focusing [22]. This new antibiotic exhibits a unique activity, not shown by vancomycin and teicoplanin, against *Enterococcus fecalis* and *E. faecium* bacteria strains. Due to the presence of several asymmetric centres in its chemical structure, MDL 63 246 can exhibit enantiorecognition activity. For its chemical structure see Fig. 1 reported in Ref. [20].

As recently described by Bossi et al. [22] the

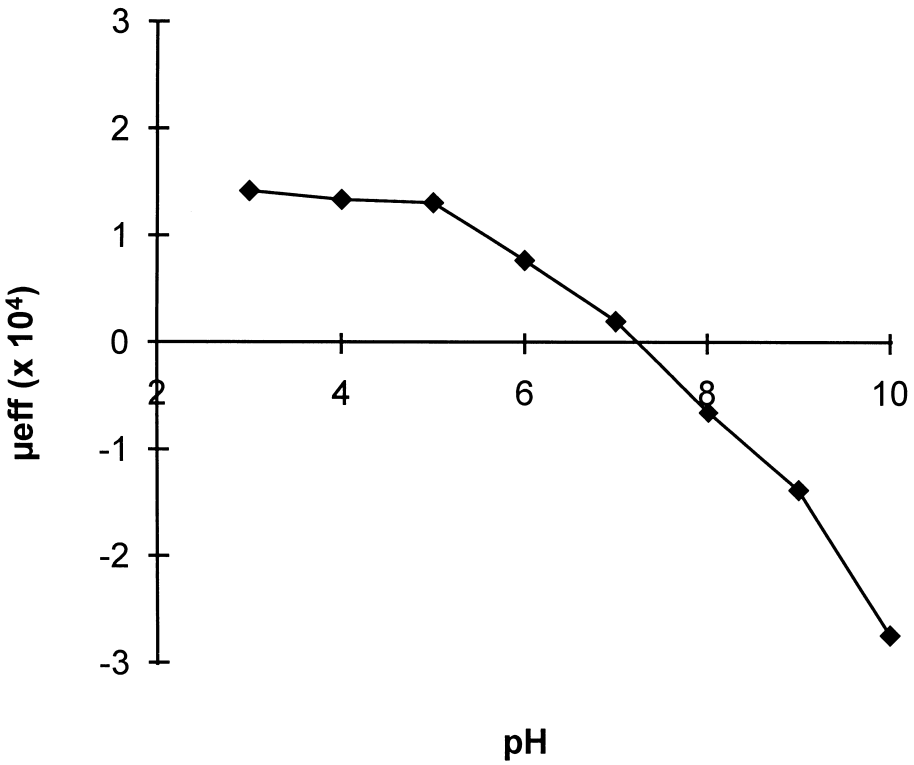


Fig. 1. Effect of buffer pH on effective mobility of Hepta-Tyr antibiotic. Capillary, fused-silica 50 (45.5 effective length) cm×50 μm I.D.; background electrolyte, 75 m*M* B.R.B. pH range (3–10)–ACN (80:20, v/v); applied voltage +20 kV; injection, $34.475\cdot10^{-3}$ MPa, 2 s of 0.01 mg/ml of Hepta-Tyr.

antibiotic exhibited an isoelectric point of 8.26 and poor solubility in aqueous solvents depending on buffer composition and pH. Another drawback on using Hepta-Tyr in chiral CE analysis is the strong absorbance at low wavelengths [20,22].

Electrophoretic experiments were carried out in untreated fused-silica capillary in order to measure the effective mobility of Hepta-Tyr using different BGEs (75 m*M* B.R.B.–ACN, 80:20, v/v) at pH in the range 3–10. Fig. 1 shows the effect of buffer pH on effective mobility of the studied glycopeptide antibiotic. At the lowest pH the chiral selector (C.S.) was positively charged and its mobility decreased when increasing the pH. The zero charge was recorded at pH=7.3. At pH>p*I* the antibiotic was moving as negatively charged and its effective mobility increased by raising the pH of the BGE. The discrepancy between the electrophoretic titration curve, leading to an apparent p*I* of 7.3, and the isoelectric focusing in immobilized pH gradients data (IEF-IPG), resulting in an isoelectric point (p*I*) of 8.3 (p*I* value also corroborated by titration data) are most probably due to the fact that the former gives an 'isoelectric point', whereas the latter technique gives an isoionic point, i.e. a true p*I* in an ion-free environment [24]. In other words, in conventional electrophoresis, the analyte (antibiotic) p*I*, as measured by extrapolation to zero mobility, might greatly diverge from the true isoprotic (or isoionic) point in cases in which there is substantial binding of the analyte (antibiotic) to the buffering ion in the BGE.

Based on our previously published results [20] we selected the BGE containing ACN for further investigation of Hepta-Tyr as the C.S. for the separation of several α-hydroxy acid derivatives (for their chemical structure see Fig. 2). In this aqueous–organic buffer we observed the highest solubility of the C.S. (up to 6 mg/ml).

Fig. 3a and b show the chiral separation of MA using the aqueous–organic BGE at pH 4 containing 0.5 and 2 mg/ml of chiral selector, respectively filling the whole capillary as well as the electrode compartments. Good enantiomeric resolution was achieved in both cases, however both the UV peak's response and the baseline signal were not satisfactory for CE analysis at the operating wavelength. Thus, the partial filling-counter current (PF-CC) method was applied in order to achieve the highest sensitivity operating at 206 nm.

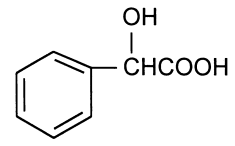
The PF-CC method was first introduced by Valtcheva et al. [25] by employing cellobiohydrolase I as the C.S. for the CE chiral separation of several drugs. Furthermore, the method was also applied in CE when using glycopeptide antibiotics. The enantioresolution capability of Hepta-Tyr was studied in polyacrylamide coated capillaries in order to suppress/minimize the electroosmotic flow (EOF). Several operational parameters were studied such as Hepta-Tyr antibiotic concentration, pH of the BGE, organic modifier type and concentration and capillary temperature in order to optimize the chiral resolution of the studied α-hydroxy acid derivatives.

3.1. Effect of antibiotic concentration

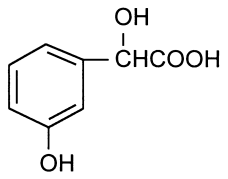
On the basis of our experience and preliminary experiments [20], the 75 m*M* of B.R.B. pH 4 containing 20% (v/v) ACN was selected as the BGE for studying the effect of Hepta-Tyr antibiotic concentration (in the range 0.5–4 mg/ml) on hydroxy acids migration time (t_m) and chiral resolution (R_s) using the PF-CC method. The selected aqueous–organic buffer allowed the migration of analytes as negatively charged ions in a short time (4.2–5.3 min) and provided good solubility of the chiral selector (up to 6 mg/ml). Additional experiments were carried out for 2-PhL and 3-PhL at 6 mg/ml of antibiotic.

Table 1 shows the effect of chiral selector concentration on migration time and resolution (R_s) of studied compounds.

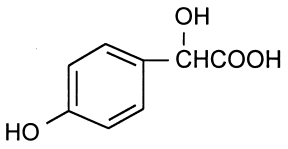
The addition of a very low amount of chiral selector to the BGE (0.5 mg/ml) induced the chiral discrimination of all the studied racemic analytes except for 3-PhL and 2-PhL enantiomers which started to be resolved at 4 and 2 mg/ml of Hepta-Tyr antibiotic, respectively. The increase of the C.S. concentration in the range 0.5–6 mg/ml provoked a general increase of R_s and the maximum of resolution was achieved at 4 mg/ml for MA, *m*-OH-MA, *p*-OH-MA, 3,4-di-OH-MA and 4-Cl-MA and 6 mg/ml for 3-PhL and 2-PhL. From the above reported results it can be remarked that raising the chiral selector concentration a slight increase of migration time is experienced for almost all the studied compounds. This effect is in accord with the general behavior of macrocyclic antibiotics in CE



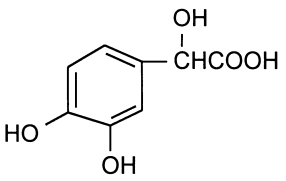
mandelic acid
(MA)



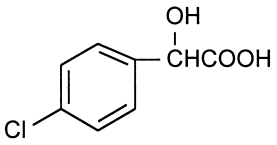
m-hydroxymandelic acid
(m-OH-MA)



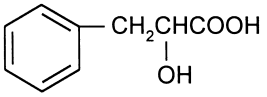
p-hydrohymandelic acid
(p-OH-MA)



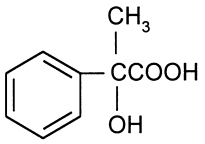
3,4-dihydroxymandelic acid
(3,4-di-OH-MA)



4-Cl-mandelic acid
(4-Cl-MA)



3-phenyllactic acid
(3-PhL)



2 -phenyllactic acid
(2-PhL)

Fig. 2. Chemical structures of studied racemic analytes.

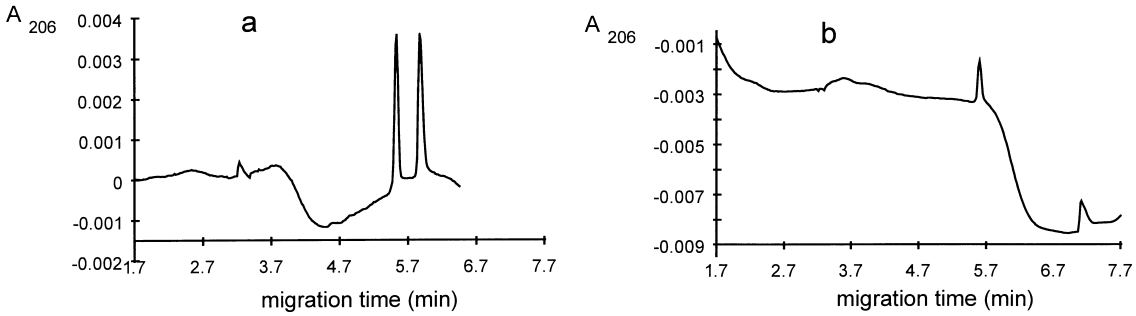


Fig. 3. Electropherograms of the chiral separation of 3,4-di-OH-MA filling the whole capillary with the BGE containing the chiral selector (a) 0.5 mg/ml, (b) 2 mg/ml (the chiral selector was also present in the electrode compartments). Capillary, polyacrylamide coated 35 (30.5 effective length) cm×50 μm I.D.; background electrolyte, 75 m*M* B.R.B. pH 4–ACN (80:20, v/v); 20 kV, 19 μA; injection $34.475 \cdot 10^{-3}$ MPa, 2 s of $2 \cdot 10^{-5}$ *M* of sample.

that exhibit very high stereoselectivity without strongly affecting the analysis time [20,26,27].

The enantiorecognition capability of the studied chiral selector towards the analytes (at 4 mg/ml) was as follows: *p*-OH-MA>4-Cl-MA>3,4-di-OH-MA> MA>*m*-OH-MA>2-PhL>3-PhL. From the data shown it can be remarked that the distance of the phenyl group from the carboxylic one as well as the position of the substituent hydroxyl on the aromatic ring played a key role in the enantioresolution of studied analytes (3-PhL exhibited the lowest resolution).

As an example Fig. 4 shows the electropherograms of the chiral separation of racemic MA obtained at different concentrations of Hepta-Tyr antibiotic.

Table 1
Effect of Hepta-Tyr antibiotic concentration on migration time and resolution (R_s) of α-hydroxy acid enantiomers[a]

Compound	Antibiotic concentration (mg/ml)									
	0	0.5		1.0		2.0		4.0		6.0	
	t_m[b]	t_m	R_s	t_m	R_s	t_m	R_s	t_m	R_s	t_m	R_s
MA	4.20	4.17	0.76	4.23	2.66	4.29	3.08	4.47	5.61		
		4.23		4.52		4.62		5.29			
m-OH-MA	4.64	4.62	0.10	4.80	1.39	4.76	2.23	4.87	4.73		
		4.67		4.97		5.01		5.35			
p-OH-MA	5.01	4.94	1.25	5.09	3.45	5.03	5.27	5.08	12.10		
		5.10		5.69		6.07		8.28			
3,4-di-OH-MA	5.32	5.28	0.61	5.32	2.00	5.26	3.31	5.45	6.96		
		5.34		5.54		5.65		6.68			
4-Cl-MA	4.38	4.33	1.02	4.41	3.22	4.56	5.92	4.88	8.51		
		4.42		4.75		5.26		6.70			
3-PhL	5.12	5.15	0	5.18	0	5.08	0	5.21	1.02	5.35	1.23
								5.33		5.50	
2-Ph-L	4.56	4.56	0	4.53	0	4.54	0.69	4.72	1.64	5.18	2.54
						4.61		4.88		5.62	

[a] Capillary, polyacrylamide coated 35 (31.5, effective length)×50 μm I.D.; background electrolyte, 75 m*M* B.R.B. pH 4–ACN (80:20, v/v) and the appropriate amount of chiral selector; applied voltage −20 kV, 18.6–22.4 μA; injection $34.475 \cdot 10^{-3}$ MPa, 2 s of $1 \cdot 10^{-4}$–$5 \cdot 10^{-5}$ *M* of racemic samples; PF-CC, $34.475 \cdot 10^{-3}$ MPa, 33–35 s.

[b] t_m, min.

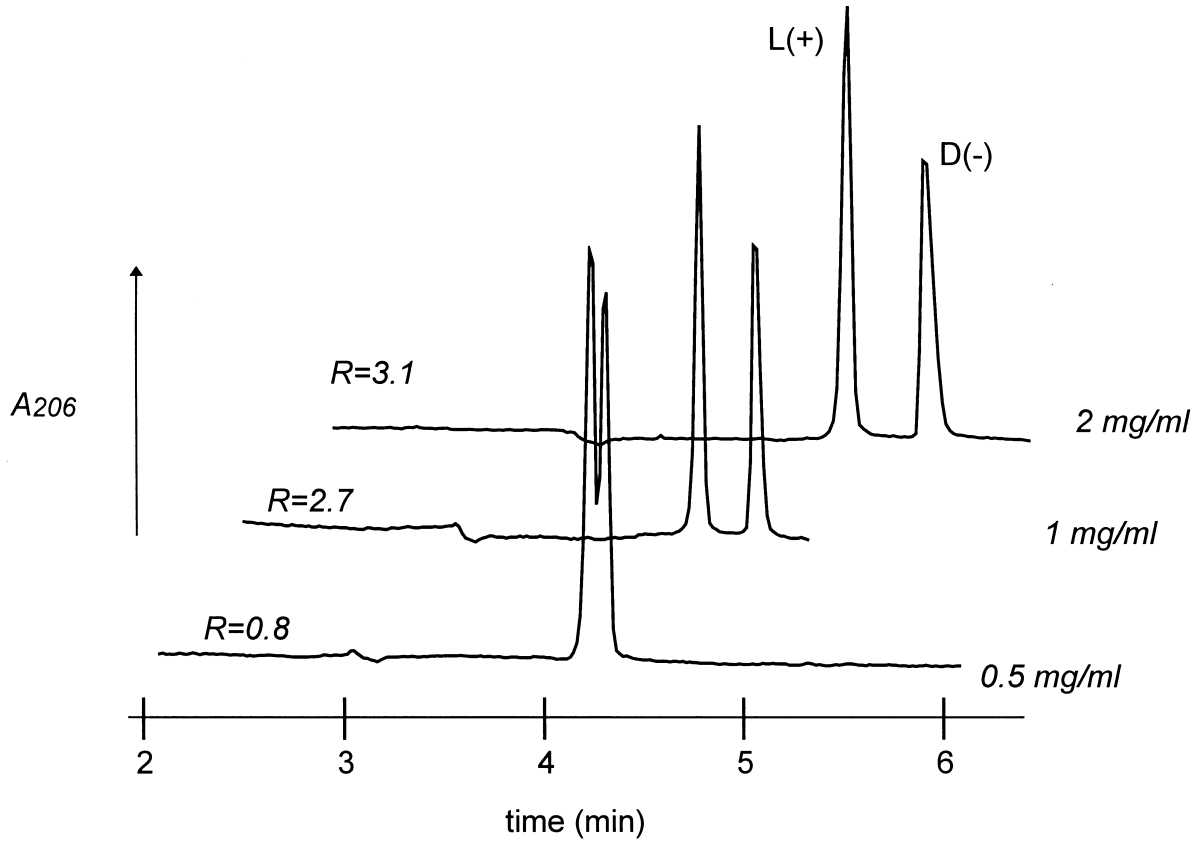


Fig. 4. Electropherograms of the enantiomers separation of racemic mandelic acid obtained at different concentrations of Hepta-Tyr antibiotic. Partial filling-counter current method ($34.475 \cdot 10^{-3}$ MPa, 35 s) (only the effective length of the capillary contained the chiral selector); applied voltage, −20 kV, 19 μA; injection, $34.475 \cdot 10^{-3}$ MPa, 2 s of $2.5 \cdot 10^{-5}$ *M* of MA. For other experimental conditions, see Fig. 3.

3.2. Effect of buffer pH

The pH of the BGE is a very important parameter to be studied when chiral CE analysis using macrocyclic antibiotics has to be carried out. In fact pH change influences the charge of both analytes and chiral selector and thus their electrophoretic mobilities. Furthermore, the electrostatic interactions (analyte–antibiotic), the stability as well as the solubility of Hepta-Tyr antibiotic are also influenced.

The effect of the buffer pH was studied using the BGE (aqueous–20% ACN) containing 2 mg/ml of Hepta-Tyr antibiotic where satisfactory chiral resolution was achieved for almost all studied compounds at pH 4.

Fig. 5 shows the effect of the pH of the BGE on resolution of the studied compounds. The increase of the pH caused a general decrease of enantiomers resolution; 3-PhL was not resolved at all in their enantiomers at any pH, 2-PhL showed a partial resolution only at pH 4. The best resolutions were obtained for mandelic acid and its derivatives at pH 4 where Hepta-Tyr antibiotic had the highest positive charge and analytes were partly or completely dissociated. It has been reported that in order to achieve good enantioresolution in CE it is necessary to maximize the differences of mobility between free analyte and analyte–chiral selector complex [28].

As an example Fig. 6 shows the enantiomers resolution of 3,4-di-OH-MA at different pH values

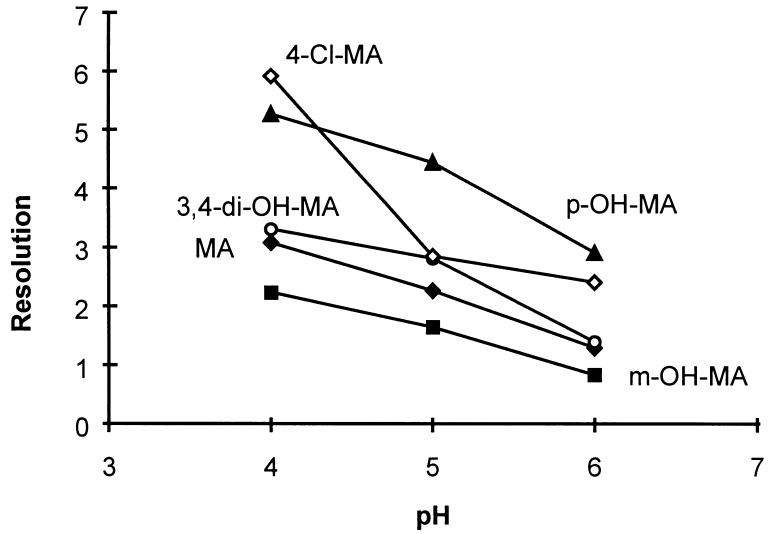


Fig. 5. Effect of buffer pH on migration time and resolution of studied α-hydroxy acids. Background electrolyte, 75 m*M* B.R.B. pH 4–ACN (80:20, v/v) and 2 mg/ml of Hepta-Tyr antibiotic, applied voltage −20 kV, 18.8–37.8 μA. For other experimental conditions, see text.

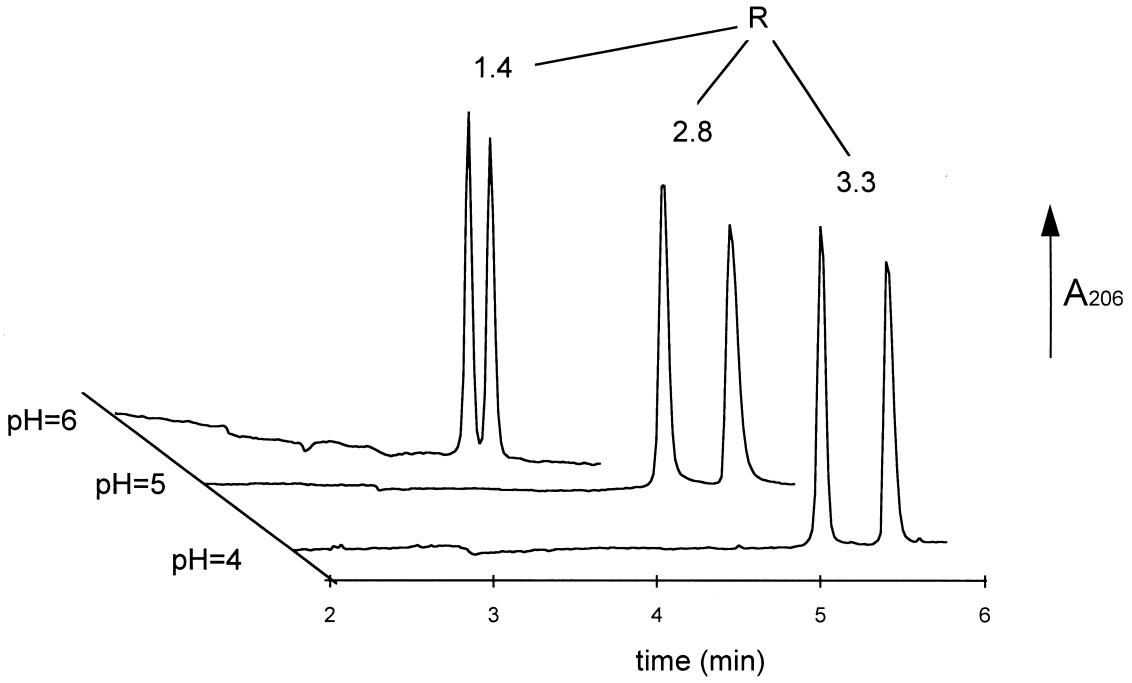


Fig. 6. Electropherograms of the enantiomers separation of racemic 3,4-di-OH-MA at different buffer pHs containing 2 mg/ml of Hepta-Tyr antibiotic. For experimental conditions, see Fig. 5.

using B.R.B.–ACN (80:20) and 2 mg/ml of Hepta-Tyr antibiotic.

3.3. Effect of capillary temperature.

The effect of capillary temperature on enantiomers resolution of racemic α-hydroxy acids was studied in the range 15–35°C by performing the electrophoretic runs with 75 m*M* B.R.B pH 4–ACN (80:20) and 1 mg/ml of chiral selector. Fig. 7a shows the effect of capillary temperature on resolution of studied enantiomers.

The increase of capillary temperature caused a general decrease of migration time (results not

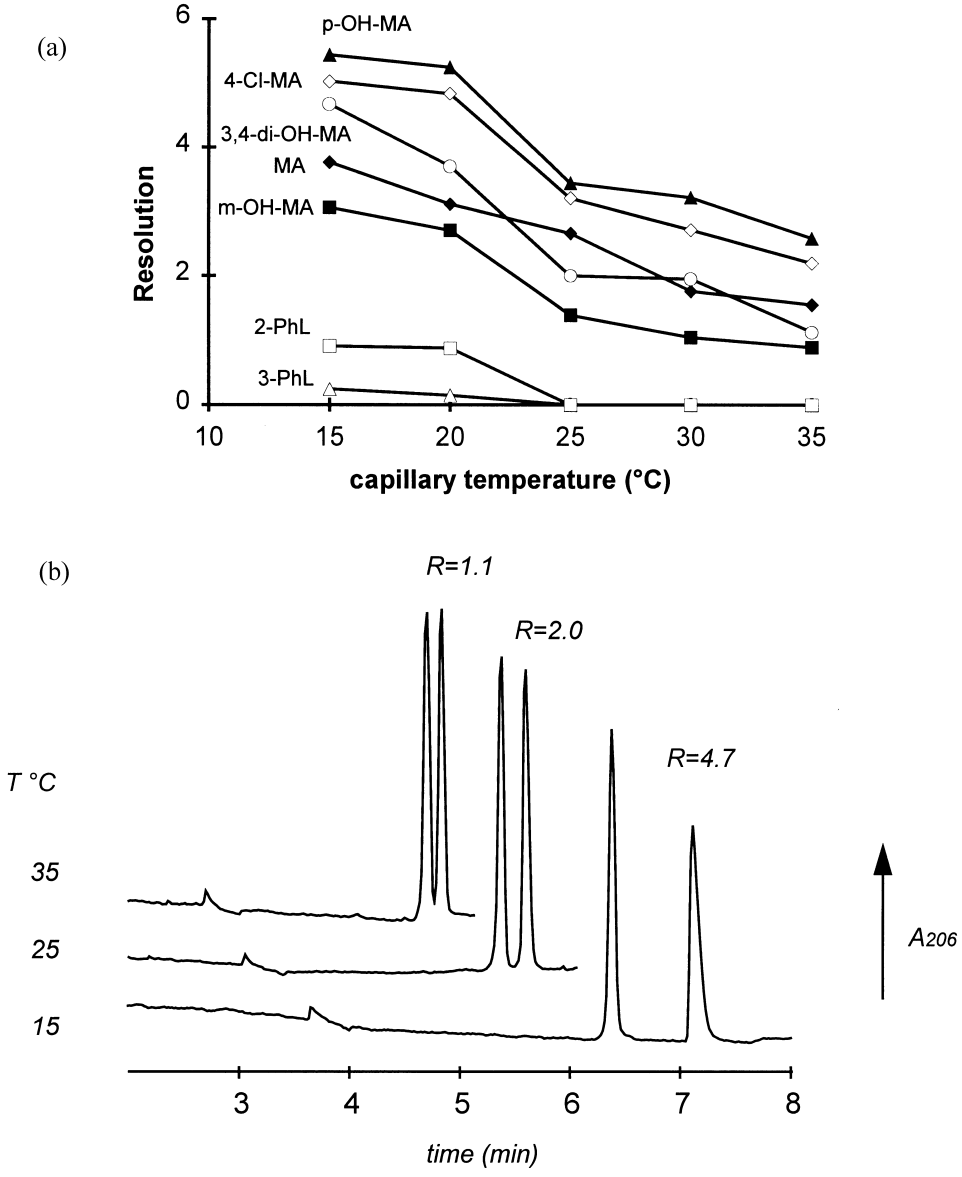


Fig. 7. (a) Effect of capillary temperature on resolution (R_s) of studied analytes. (b) Electropherograms of the enantiomers separation of racemic 3,4-di-OH-MA at different temperatures. Background electrolyte, 75 m*M* B.R.B pH 4–ACN (80:20, v/v) and 1 mg/ml of Hepta-Tyr antibiotic; PF-CC, $34.475 \cdot 10^{-3}$ MPa, 29–41 s. For other experimental conditions, see text and Table 1.

reported) and a decrease of resolution. The resolution of 3-PhL and 2-PhL was achieved at 15 and 20°C but was completely lost at higher temperatures. The decrease of resolution for MA, 4-Cl-MA, *m*-OH-MA and *p*-OH-MA was remarkable between 20 and 25°C. The electropherograms of the enantiomers separation of 3,4-di-OH-MA at different temperatures are depicted in Fig. 7b.

The experimental data confirm the importance of capillary temperature for the optimization of the chiral separation CE method. In fact several physicochemical properties, involved in the separation process, can be modified, e.g. viscosity of the BGE, stability constants (analytes–chiral selector) etc.

3.4. Effect of organic modifier

The use of an organic additive to the BGE can have strong influence on enantiorecognition depending on the antibiotic as well as on analyte type. The organic solvent can influence several parameters such as buffer viscosity, analyte and chiral selector mobility, analyte–chiral selector interaction. Furthermore, the presence of the organic modifier seems to enhance the charge–charge interaction involved in the enantioseparation mechanism when using macrocyclic antibiotics [26].

The effect of organic modifier on enantioresolution was carried out performing the electrophoretic runs with 75 m*M* B.R.B. at pH 4 containing 1 mg/ml of Hepta-Tyr antibiotic and 20% (v/v) of either acetone, or MeOH or *n*-propanol or isopropanol.

Table 2 illustrates the effect of organic solvent type on chiral resolution of the studied α-hydroxy acid compounds.

By comparing the results obtained using ACN and other organic solvents it can be observed that the solvent type plays a very important role in enantioresolution of all studied analytes. In fact both 3-PhL and 2-PhL (not resolved with ACN and 1 mg/ml of chiral selector) were stereoselectively discriminated using either MeOH or *n*-PRO or 2-PRO; while with acetone only 2-PhL was partly resolved.

The chiral resolution of all studied analytes improved enormously when using either MeOH or *n*-PRO instead of ACN. In 2-PRO and *n*-PRO solvents, relatively high migration times were observed probably due to both increased viscosity and stronger interactions with the chiral selector. Satisfactory results were achieved using acetone, obtain-

Table 2
Effect of organic solvent type on migration time and resolution (R_s) of α-hydroxyacid enantiomers[a,b]

Compound	Organic solvent (20%, v/v)									
	Acetonitrile		Acetone		Methanol		*n*-Propanol		2-Propanol	
	t_m[c]	R_s	t_m	R_s	t_m	R_s	t_m	R_s	t_m	R_s
MA	4.23	2.66	5.02	3.25	5.39	6.16	6.93	5.77	7.13	3.05
	4.52		5.40		6.11		8.21		8.25	
m-OH-MA	4.80	1.39	5.56	2.59	5.95	4.82	7.79	5.18	8.04	4.21
	4.97		5.83		6.45		8.49		8.84	
p-OH-MA	5.09	3.45	5.58	4.80	6.25	4.95	8.14	6.46	8.43	5.35
	5.69		6.68		8.07		11.19		11.70	
3,4-di-OH-MA	5.36	1.68	6.29	3.31	6.42	5.12	8.69	6.19	9.48	5.85
	5.53		6.68		7.22		9.89		10.86	
4-Cl-MA	4.53	1.88	5.05	3.33	5.40	4.69	7.36	5.50	7.72	5.52
	4.74		5.53		6.32		8.93		9.35	
3-PhL	5.18	0	5.98	0	6.21	0.65	8.32	0.93	8.63	0.82
					6.28		8.44		8.75	
2-Ph-L	4.53	0	5.25	0.64	5.55	0.97	7.35	1.77	7.64	1.53
			5.31		5.63		7.55		7.98	

[a] PF-CC, $34.475 \cdot 10^{-3}$ MPa, 41–64 s; applied voltage −20 kV, 13–20 μA; Hepta-Tyr antibiotic, 1 mg/ml.
[b] For other experimental conditions, see Table 1.
[c] t_m, min.

ing baseline chiral resolution for MA, *m*-OH-MA, *p*-OH-MA, 3,4-di-OH-MA and 4-Cl-MA. From the above reported results (see Table 2) it seems that MeOH is particularly useful for the enantiomeric separation of α-hydroxy acids, at pH 4, because it allows to achieve good chiral resolution in a reasonable time. We further investigated the effect of Hepta-Tyr antibiotic added to the BGE containing 20% (v/v) of MeOH in the concentration range 0.1–1 mg/ml.

Increasing the chiral selector concentration a general increase of resolution was recorded; 3-PhL and 2-PhL were resolved only at the highest concentration of antibiotic. Baseline chiral resolution was achieved at 0.5 mg/ml of chiral selector for the other studied analytes (R_s=2.14, 1.89, 2.98, 2.18 and 2.47 for MA, *m*-OH-MA, *p*-OH-MA, 3,4-di-OH-MA and 4-Cl-MA, respectively).

The excellent enantiorecognition capability of Hepta-Tyr antibiotic dissolved in aqueous–MeOH buffer, is documented by the enantiomers separation of MA reported in Fig. 8. As can be observed in this figure, although the chiral selector allowed a very high value of resolution, broader peaks (second migrating enantiomer) were recorded probably due to the strong interaction selector–selectand.

Considering the results obtained we further investigated the effect of MeOH concentration (10–60%, v/v) on chiral resolution using the BGE at pH 4 and 0.5 mg/ml of antibiotic. For these experiments the final concentration of B.R.B. was 75 m*M* and only the MeOH concentration was changed.

As can be observed in Table 3, the increase of MeOH concentration caused a general increase of migration time probably due to the change of the viscosity as well as to the interactions with the complexing antibiotic. A maximum of resolution was recorded at 30% of MeOH for *m*-OH-MA, 3,4-di-OH-MA, while the optimum MeOH concentration for MA, *p*-OH-MA and 2-PhL was found at 40%. 2-PhL was poorly resolved at 40 and 50% of MeOH.

The effect of ACN concentration was studied in the range 10–50% with 75 m*M* of B.R.B. pH 4 and 1 mg/ml of chiral selector. The experiments (results not shown) revealed that 3-PhL and 2-PhL were not resolved in their enantiomers at any concentration of

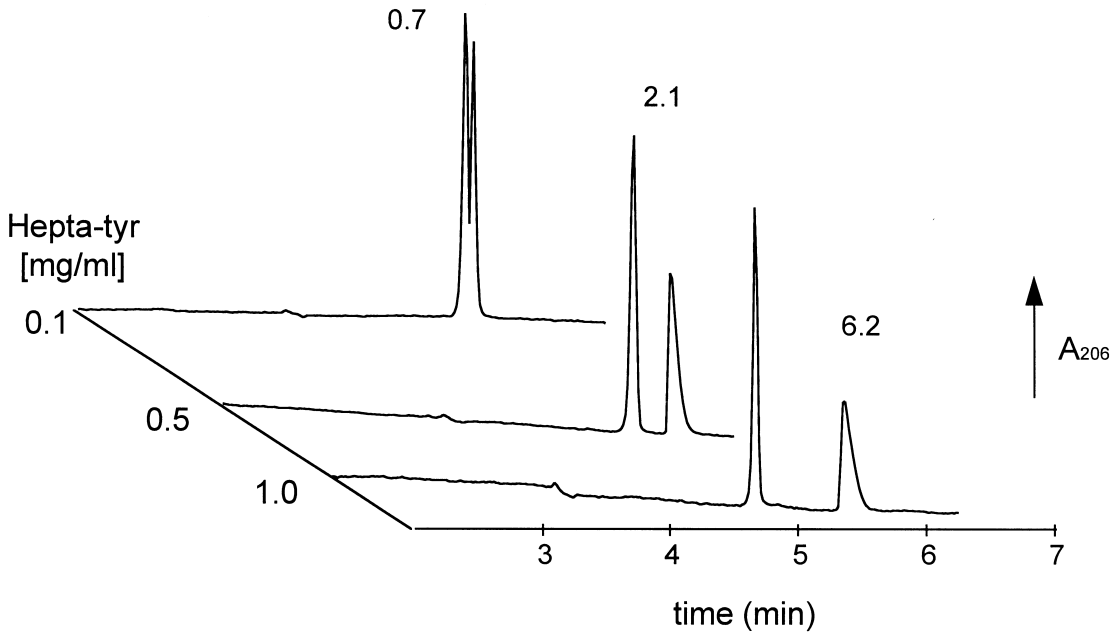


Fig. 8. Electropherograms of the enantiomers separation of racemic MA in aqueous–MeOH buffer (75 m*M* B.R.B. pH 4–MeOH, 80:20). PF-CC, $34.475 \cdot 10^{-3}$ MPa, 46–52 s; applied voltage, −20 kV, 16–17 μA. For other experimental conditions, see text. Values in figure indicate resolution.

Table 3
Effect of methanol concentration on migration time and resolution of α-hydroxy acid enantiomers using Hepta-Tyr antibiotic as the chiral selector[a,b]

Compound	Methanol concentration (%, v/v)											
	10		20		30		40		50		60	
	t_m[c]	R_s	t_m	R_s	t_m	R_s	t_m	R_s	t_m	R_s	t_m	R_s
MA	4.65	1.89	5.40	2.30	6.52	3.03	7.18	3.07	7.53	2.92	7.58	2.22
	4.87		5.68		6.94		7.72		8.06		8.09	
m-OH-MA	4.95	1.61	5.95	1.89	7.25	2.66	7.90	2.35	8.53	2.27	8.81	1.71
	5.10		6.17		7.52		8.24		8.85		9.14	
p-OH-MA	5.34	2.48	6.24	2.81	7.53	3.23	8.14	3.50	8.90	3.30	9.37	2.51
	5.82		6.92		8.42		9.25		9.92		10.49	
3,4-di-OH-MA	5.54	2.32	6.35	2.77	8.50	3.25	8.96	3.00	9.55	2.44	10.02	2.32
	5.81		6.75		8.97		9.52		10.06		10.63	
4-Cl-MA	4.83	2.46	5.48	2.49	6.59	2.33	7.42	2.31	7.45	2.28	7.50	2.19
	5.20		5.88		7.05		7.96		7.94		7.98	
3-PhL	5.62	0	6.38	0	7.82	0	8.58	0.10	8.59	0.10	8.73	0
							8.62		8.65			
2-Ph-L	4.99	0	5.58	0	6.92	0.30	7.76	0.73	7.82	0.30	8.10	0
					6.98		7.85		7.95			

[a] Chiral selector concentration, 0.5 mg/ml; PF-CC, $34.475 \cdot 10^{-3}$ MPa, 43–58 s; applied voltage −20 kV, 9–20 μA.
[b] For other experimental conditions, see Table 1.
[c] t_m, min.

organic additive while MA, *m*-OH-MA, *p*-OH-MA, 3,4-di-OH-MA and 4-Cl-MA exhibited a maximum of resolution (baseline) when using 20% of ACN.

4. Conclusions

We studied the enantioresolution capability of the new MDL 63 246 Hepta-Tyr antibiotic towards several α-hydroxy acid compounds by capillary electrophoresis using an aqueous–organic buffer. Very good enantiomers resolution was obtained for analytes under study. It was shown that the chiral resolution was strongly influenced by the concentration of the chiral selector. Employing 20% of ACN, the highest resolutions were achieved at 4 and 6 mg/ml of Hepta-Tyr antibiotic. Resolution and migration times of analytes as well as solubility of the chiral selector were also influenced by the organic modifier type. Among the different organic solvents used methanol or *n*-propanol allowed to achieve the best chiral resolutions (with MeOH low chiral selector concentration can be employed, 0.5–1 mg/ml). However, longer migration times and lower efficiency of the most complexed enantiomers than those obtained when using acetonitrile were observed.

The capillary temperature played a very important role on α-hydroxy acids chiral resolution (studied in aqueous–acetonitrile buffer). In fact the best results were observed at the lowest capillary temperature used (15°C).

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