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Separation of *cis*-β-lactam enantiomers by capillary electrophoresis using cyclodextrin derivatives

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ABSTRACT

Chiral separation of 19 pairs of *cis*-β-lactam (BL) stereoisomers of pharmacological importance was examined by capillary electrophoresis using cyclodextrin (CD) derivatives. In order to select the most effective conditions separating the highest number of stereoisomers of BLs, single carboxymethyl α-, β- and γ-, as well as sulfobutyl β-CD derivatives were applied. Additionally, carboxymethyl and sulfobutyl β-CD derivatives complemented with neutral β-CD derivatives as dual CD systems were tested. Both the composition and concentration of applied selectors and the pH of background electrolyte were selected. In single systems the structural characteristics of BLs and the complex forming affinity were correlated. Most BLs provided optimal complexation with β-CD derivatives. In conclusion, the efficiency of combining sulfobutyl-β-CD and permethylated β-CD was superior to other single and dual CD systems applied. This method successfully separated each pair of stereoisomers investigated.

1. Introduction

β-Lactams (BLs) are in the center of interest of both medicine and organic chemistry. Due to the lethal inhibitory effect on the synthesis of pathogenic bacterial cell wall, β-lactams are widely applied as antibiotics [1,2]. An additional pharmaceutical importance of these chiral molecules and their derivatives is, that they play key role as intermediates in the stereoselective synthesis of various nitrogen containing heterocyclic compounds, β-amino acids, taxoid antitumor drugs, alkaloids and short chain peptides, or serine and cysteine protease inhibitors [3–8].

Since stereochemistry may have a significant effect on the biological activity of a drug and β-lactams are synthesized as racemates, development of effective chiral separation methods is needed. Resolution of BLs was achieved by high performance liquid chromatography or gas chromatography using chiral stationary phases bearing various functional groups including cyclodextrin (CD) derivatives [9–13].

Multiplicity of CD derivatives in the diameter of cavity and the substituents provides the possibility of enantiodiscrimination for a large scale of chiral compounds. CD derivatives are widely applied in capillary electrophoresis (CE) as chiral selectors due to their stability and low UV absorption.

In order to separate neutral compounds, like β-lactams by CE, a charged CD selector used to be applied to provide appropriate mobility to the uncharged compound. Furthermore, the neutral character of the analyte does not allow investigating the selectivity of neutral CD derivatives. To achieve improved resolutions as well as to investigate the efficiency of neutral selectors, dual systems containing a mixture of charged and uncharged CD derivatives were elaborated [14–24]. Anionic CDs slow down the mobility of the complexed species, while neutral CDs do not affect it. If the charged and neutral CDs applied simultaneously had opposite enantiomer recognition preference, better resolution could be predicted [14,21–24].

Previously, CE separation of a series of β-lactams by three negatively charged CD derivatives, e.g. sulfated-α-CD, sulfated-β-CD and carboxymethyl-β-CD was reported [25]. Sulfated-α-CD was most efficient in the separation of 10 out of 12 BL stereoisomers but this CD was used in relatively high concentrations.

This study aimed to select effective CD derivative(s) for chiral separations of 19 pairs of β-lactam stereoisomers by using CE. For this reason several single and dual CD systems were investigated and compared.

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2. Materials and methods

2.1. Chemicals

Background electrolyte buffer components, *Suprapure* sodium acetate, sodium dihydrogen phosphate, sodium hydroxide, glacial acetic acid, methanol and acetone were purchased from Merck GmbH (Darmstadt, Germany).

Cyclodextrin derivatives carboxymethyl-α-CD (CMACD); carboxymethyl-β-CD (CMBCD); carboxymethyl-γ-CD (CMGCD); sulfobutyl-β-CD (SBEBCD); β-cyclodextrin (BCD); hydroxypropyl-β-CD (HPBCD); randomly methylated β-CD (RAMEB) and permethylated β-CD (heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrin, TRIMEB) were products of CycloLab R&D Ltd. (Budapest, Hungary).

2.2. β-Lactams (BLs)

BL/1–7 (bicyclic), BL/8–10 (aromatic tricyclic) and BL/11–12 (aliphatic tricyclic): (1) *cis*-6-azabicyclo[3.2.0]heptan-7-one, (2) *cis*-7-azabicyclo[4.2.0]octan-8-one, (3) *cis*-7-azabicyclo[4.2.0]oct-3-en-8-one, (4) *cis*-7-azabicyclo[4.2.0]oct-4-en-8-one, (5) *cis*-8-azabicyclo[5.2.0]nonan-9-one, (6) *cis*-9-azabicyclo[6.2.0]decan-10-one, (7) *cis*-9-azabicyclo[6.2.0]dec-4-en-10-one, (8) *cis*-3,4-benzo-6-azabicyclo[3.2.0]heptan-7-one, (9) *cis*-4,5-benzo-7-azabicyclo[4.2.0]octan-8-one, (10) *cis*-5,6-benzo-8-azabicyclo[5.2.0]nonan-9-one, (11) *exo*-3-azatricyclo [4.2.1.01.5]nonan-4-one, (12) *exo*-3-azatricyclo[4.2.1.01.5]non-7-en-4-one) were prepared by cycloaddition of chlorosulfonyl isothiocyanate to the corresponding cycloalkenes and cycloalkadienes [26,27]. BL/13–19 (4-aryl-substituted): (13) 4-phenyl-2-azetidinone, (14) 4-(*p*-tolyl)-2-azetidinone, (15) 4-(*o*-chlorophenyl)-2-azetidinone, (16) 4-(*m*-chlorophenyl)-2-azetidinone, (17) 4-(*p*-chlorophenyl)-2-azetidinone, (18) 4-(*p*-fluorophenyl)-2-azetidinone, (19) 4-(*p*-bromophenyl)-2-azetidinone were synthesized according to the method published elsewhere [28]. (For structures refer to Tables 1–3.)

2.3. Capillary electrophoresis (CE)

CE was performed with an Agilent Capillary Electrophoresis ^{3D}CE system applying bare fused silica capillary of 33.5 cm total and 25 cm effective length with 50 μm I.D. (Agilent Technologies, Santa Clara, CA, USA). On-line UV absorption was detected at 200 nm by DAD UV-Vis detector. ChemStation software rev.A0903 (Agilent Technologies, USA) was used for data acquisition and handling. The capillary was thermostated at 25 °C. Sodium phosphate buffer (20 mM) at pH 2.5, sodium acetate buffer (20 mM) at pH 5.0 and sodium phosphate buffer (5 mM) at pH 8.0 were applied as background electrolyte (BGE), pH was adjusted by NaOH. Between measurements, the capillary was rinsed by 1 M NaOH, 0.1 M NaOH and distilled water subsequently for 2 min, then with BGE for 5 min. BL samples were dissolved in acetone (1 mg/ml corresponding to 5–10 mM), and were further diluted 5–10 times with 30% methanol and injected by 5×10^3 Pa pressure for 3 s. Some of the racemic samples (BL/2, BL/4, BL/7, BL/8, BL/9, BL/10 and BL/12) were spiked with the pure stereoisomer with known absolute configuration. Runs were performed by positive-polarity mode with 10 kV at pH 5.0 and 8.0 and by negative polarity mode with −15 kV at pH 2.5. The efficiency of the chiral separations was characterized by resolution (R_s) calculated by the following equation [29]:

$$R_s = \frac{1.18(t_2 - t_1)}{w(0.5)_1 + w(0.5)_2}$$

where $w(0.5)$ is the peak width at half height, t is the subsequent migration time of stereoisomers (1, 2 in lower index). In order to calculate the apparent complex stability constant (K_i') of β-lactams formed with CD derivatives, the effective mobilities of the analytes in the absence ($\mu_{0,i}$) and in the presence ($\mu_{x,i}$) of CDs in four concentrations (c_x) in the range of 2.5–20 mM were determined. By plotting $(\mu_{x,i} - \mu_{0,i})$ vs $(\mu_{x,i} - \mu_{0,i})/c_x$, the absolute value of slope of the regression line equals the stability constant [30]. The present setup did not allow us to achieve precision high enough to distinguish stereoisomer pairs. The apparent complex stability constants representing mean values of corresponding pairs were evaluated within 10% of standard deviation in average.

In case of determination of the complex stability constants and chiral separation efficiency in single CD systems, negatively charged CD derivatives, namely: CMACD, CMBCD, CMGCD or SBEBCD were used. On the other hand in dual CD systems, a negatively charged CD derivative (CMBCD (20 mM) or SBEBCD (20 mM)) and a neutral CD derivative (BCD, HPBCD, RAMEB or TRIMEB) were added together. In these setups the concentrations of HPBCD, RAMEB or TRIMEB were changed between 10 and 40 mM and for BCD between 5 and 15 mM. Due to the number of BLs and CDs applied in this study, the conditions of separations were not completely optimized.

3. Results and discussion

3.1. Stability of complexes of BLs with anionic CD derivatives

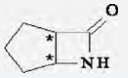
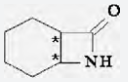
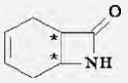
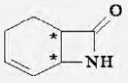
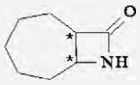
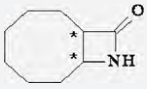
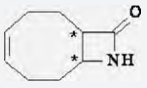
Apparent stability constants of BL complexes formed with anionic CD derivatives, namely sulfobutyl-β-CD or carboxymethyl-α-CD, carboxymethyl-β-CD and carboxymethyl-γ-CD have been determined and summarized in Tables 1–3. Comparison of the data obtained for α, β and γ series of carboxymethylated derivatives (CM-CDs) demonstrated that the relatively most stable complexes are formed with CMBCD proving that the size of β-CD ring is preferred by these analytes. Accordingly, the complex stability constants measured in case of SBEBCD were of the same magnitude.

The structural characteristics of BLs and the complex forming affinity with CMBCD and SBEBCD were correlated. In case of the bicyclic BLs (BL/1, BL/2, BL/5, BL/6) the rising ring size increased the stability of the complex both with CMBCD or SBEBCD (see Table 1) presumably due to more tight inclusion into the β-CD ring. The presence of a double bond (BL/3, BL/4, BL/7) weakened the stability of the complex.

The addition of a third, aromatic ring (BL/8, BL/9, BL/10) decreased the complex stability constants in case of CMBCD, while increased in case of SBEBCD (see Table 2). An explanation of this opposite effect could be a non-polar interaction between the butyl group of SBEBCD and the aromatic ring that is much weaker in case of CMBCD. Aliphatic tricyclic BLs (BL/11, BL/12) formed more stable complexes with CMBCD than the bicyclic ones, and the double bond (BL/12) in the tricyclic compound decreased again the stability similarly to the bicyclic subgroup.

Stability of 4-aryl-substituted BL complexes (BL/13–19) formed with each CD investigated depended on the kind and position of substituents on the aryl ring (see Table 3). Members of this subgroup were presumably inserted into the CD cavity with the substituent on the aromatic ring. This supposition is based on the realization that the electron withdrawing or donating character of para-substituents predicts the order of Me- H- F- Cl- Br- that is not seen in the stability of complexes indicating an order of H- F- Me- Cl- Br-. Instead, the size of the substituents increases in the same sequence as the complex stability. Furthermore, besides the stable complexes formed with BCD derivatives, BL/16, BL/17 and BL/19 presented strong affinity to CMACD, too. The smaller ring of this CD derivative may provide a more tight fit. Additionally, the position of chloride substituent affected the magnitude of the sta-

Table 1
Apparent complex stability constants of bicyclic BLs formed with different anionic CDs and resolution of BL enantiomer pairs in single CD systems and CMBCD or SBEBCD based dual systems.

β-Lactams			Single CD system				Dual CD system			
Subgroup	Number	Structure	Anionic CD derivatives	K′[a]	Rs[b]	Rs[c]	Rs[d]			
							15 mM BCD	40 mM HPBCD	40 mM RAMEB	40 mM TRIMEB
Bicyclic β-lactams	1		CMACD	19						
			CMBCD	176						0.78
			CMGCD	28						
			SBEBCD	144	0.87	0.52	0.90	1.40	1.10	1.93
	2		CMACD	32						
			CMBCD	428						0.78
			CMGCD	33						
			SBEBCD	343	1.00	0.51	0.70	1.32	1.45	1.52
	3		CMACD	27						
			CMBCD	202						0.59
			CMGCD	35						
			SBEBCD	176	1.03	0.51	0.80	1.26	1.60	1.74
	4		CMACD	27						
			CMBCD	238						1.68
			CMGCD	57						
			SBEBCD	216	1.21	0.61	0.94	1.74		2.93
	5		CMACD	34						
			CMBCD	787						0.92
			CMGCD	54						
			SBEBCD	425				1.68	1.52	1.94
	6		CMACD	41						
			CMBCD	1042						0.63
			CMGCD	101						
			SBEBCD	996						1.28
	7		CMACD	34						
			CMBCD	692						1.15
			CMGCD	73						
			SBEBCD	797				0.90		2.18

Centers of asymmetry are indicated by asterisks.
[a] Apparent complex stability constants in M^{-1} of BLs and CMACD, CMBCD, CMGCD at pH 8.0 or SBEBCD at pH 2.5.
[b] Resolution of BLs achieved by 20 mM CMACD, CMBCD, CMGCD at pH 8.0 or 20 mM SBEBCD at pH 2.5.
[c] Resolution of BLs achieved by 20 mM CMACD, CMBCD, CMGCD and SBEBCD at pH 5.0.
[d] Resolution of BLs achieved in dual CD systems containing the indicated neutral CD and 20 mM CMBCD (at pH 5.0) or 20 mM SBECD (at pH 2.5).

bility of both CMBCD and CMACD complexes (ortho < meta < para) indicating some steric strain for the inclusion.

3.2. Chiral separation of BLs in single CD systems

In general, SBEBCD was more effective than CM-CD derivatives in separation of BL stereoisomers (Tables 1–3). While SBEBCD separated 11 of 19 stereoisomers, CMACD, CMBCD and CMGCD could only separate 3, 5 and 2, respectively. Chiral resolution behavior of subgroups of BLs could be discussed on the basis of structural characteristics.

The smaller bicyclic BLs (BL/1–4) containing five- or six-membered rings could be resolved only by SBEBCD and not by CM-CD derivatives. However, larger bicyclic BLs (BL/5–7) forming more stable complexes than smaller ones were separated neither of these CD derivatives.

Chiral separations of aromatic tricyclic BLs (BL/8–10) have been achieved both by SBEBCD and β- as well as γ-CM-CD derivatives. The double bond containing aliphatic BL/12 (forming relatively less stable complex than the saturated analogue, BL/11) could be resolved by SBEBCD, only.

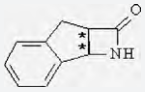
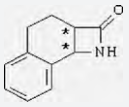
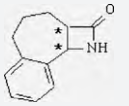
In case of 4-aryl-substituted BLs (BL/13–19) enantiodiscrimination by CDs depended on the position and kind of substituents on the phenyl ring. Resolution of 4-aryl BLs substituted in para position by halogens (BL/17–19) or by a methyl group (BL/14) could be achieved by SBEBCD but failed by CMBCD. On the contrary, ortho- and meta-substituted BL-s (BL/15–16) were resolved by CMBCD. Furthermore, CMACD was successful in chiral resolution of halogenated derivatives (BL/16–17, 19) forming complexes of $K' > 200\ M^{-1}$.

3.3. Optimization of single systems

The optimization of single systems included the elaboration of CD concentration and the selection of pH for optimal chiral resolution efficiency.

Resolution of BLs increased with the elevation of CMBCD concentration, but showed various kinds of dependence on the concentration of SBEBCD in the range of 2.5–20 mM. Literature recommends that SBEBCD should be used at pH 2.5–3.0 while CMBCD at pH 8.0 or at around pH 5.0 [14–16,22]. In order to optimize the conditions in this study, the chiral resolution efficiency was compared at pH 2.5 and 5.0 for SBEBCD and at pH 5.0 and 8.0 for CMBCD. Although, the numbers of resolved stereoisomer pairs were not dependent on changes of pH, individual differences in efficiency of resolution could be detected. In this work, slightly better reso-

Table 2
Apparent complex stability constants of tricyclic BLs formed with different anionic CDs and resolution of BL stereoisomer pairs in single CD systems and CMBCD or SBEBCD based dual systems.

β-Lactams			Single CD system				Dual CD system			
Subgroups	Number	Structure	Anionic CD derivatives	K′[a]	Rs[b]	Rs[c]	Rs[d]			
							15 mM BCD	40 mM HPBCD	40 mM RAMEB	40 mM TRIMEB
Aromatic tricyclic β-lactams	8		CMACD	49						
			CMBCD	96	1.83	1.95	2.13	1.80	2.19	4.88
			CMGCD	55	2.43	1.92				
			SBEBCD	213		0.93	1.92	2.65	3.55	5.00
	9		CMACD	53						
			CMBCD	268	0.88	1.42	0.94		1.41	1.31
			CMGCD	115	3.28	2.59				
			SBEBCD	513	1.28	1.21	1.80	1.70		2.03
	10		CMACD	44						
			CMBCD	164	1.43	1.60	0.55	1.54		6.45
			CMGCD	137						
			SBEBCD	564	2.38	3.43	5.77	5.52	9.52	5.61
Aliphatic tricyclic β-lactams	11		CMACD	32						
			CMBCD	773					0.82	1.88
			CMGCD	34						
			SBEBCD	338				1.13	1.23	2.53
	12		CMACD	16						
			CMBCD	363						1.07
			CMGCD	35						
			SBEBCD	344	1.01		0.73	1.75	2.28	1.56

Centers of asymmetry are indicated by asterisks.
[a] Apparent complex stability constants in M^{-1} of BLs and CMACD, CMBCD, CMGCD at pH 8.0 or SBEBCD at pH 2.5.
[b] Resolution of BLs achieved by 20 mM CMACD, CMBCD, CMGCD at pH 8.0 or 20 mM SBEBCD at pH 2.5.
[c] Resolution of BLs achieved by 20 mM CMACD, CMBCD, CMGCD and SBEBCD at pH 5.0.
[d] Resolution of BLs achieved in dual CD systems containing the indicated neutral CD and 20 mM CMBCD (at pH 5.0) or 20 mM SBECD (at pH 2.5).

lution could be achieved by CMBCD at pH 5.0 than at pH 8.0 (see Tables 2 and 3). It is worth mentioning that the pKa value of CMBCD is around pH 3.7 and in conditions below this pH (i.e. pH 2.5 in our system) this CD derivative would be uncharged and not able to provide mobility to the neutral BLs.

Since chiral resolution of BLs could not be achieved for all compounds in single systems, the efficiency of dual systems concerning the number of separated stereoisomer pairs were evaluated, as well.

3.4. Chiral separation of BLs in dual CD systems

Dual CD systems were applied in this work for two reasons. While enantiomers of uncharged chiral analytes can be recognized stereoselectively by a neutral CD, no chiral separation can be achieved by CE technique due to the lack of mobility difference between free and complexed enantiomers. Accordingly, the enantio-selective character of neutral CD derivatives could be investigated only in the presence of a charged additive providing mobility to neutral analytes [24].

Our aim was to search for the most effective combination (i.e. composition and ratio) of CDs separating BL stereoisomers. Hence, a negatively charged and a neutral CD derivative were used together. While the negatively charged CD decreased the mobility of the neutral analyte, the neutral CD alone did not affect it. Consequently, mobility changes of the analyte in a dual system are the resultant of the times spent in the different complexes formed with the two CD derivatives. The mixture of CDs could be more effective in chiral discrimination, if the two applied CDs have opposite enantio-recognition preference [14,21–23].

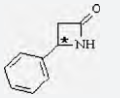
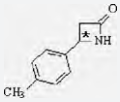
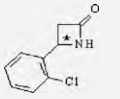
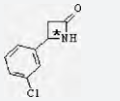
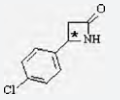
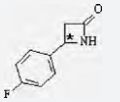
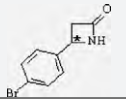
The elaboration of dual systems included variation of the composition and concentration of CDs and the pH of the BGE. We wished to select the system able to separate the greatest number of stereoisomer pairs. The most efficient anionic CDs in single systems were chosen as charged component of the dual systems. CMBCD and SBEBCD in concentration of 20 mM gave a high mobility to BLs due to their relatively high complex stability constants (cf. above) and therefore provided a wide separation window. In accordance with our findings that BLs preferred the β-CD ring, a series of BCD derivatives, namely BCD, HPBCD, RAMEB and TRIMEB were chosen as neutral additives.

3.5. Comparison of chiral resolution efficiency of dual and single CD systems

Changes of chiral resolution for individual BLs could be followed by the gradual addition of the second, uncharged CD derivative while maintaining constant concentration for the anionic CD. Concentrations varied between 5 and 15 mM in case of BCD and between 10 and 40 mM for the other BCD derivatives. In addition, the migration order of some stereoisomer pairs was determined by spiking the racemic analytes with pure stereoisomers of known configurations.

Various changes in chiral separation were presented by these series of analytes making the resolution poorer, better, or unchanged in dual systems compared to single ones (see Tables 1–3, Fig. 1). Variations in resolution are shown in Fig. 1. Structural subgroups of BLs will be discussed below. The decrease or disappearance of chiral resolution of BLs is associated with higher affinity of both (charged and neutral) selectors to the same stereoisomer,

Table 3
Apparent complex stability constants of 4-aryl-substituted BLs formed with different anionic CDs and resolution of BL enantiomer pairs in single CD systems and CMBCD or SBEBCD based dual systems.

β-Lactams			Single CD system				Dual CD system			
Subgroup	Number	Structure	Anionic CD derivatives	K′[a]	Rs[b]	Rs[c]	Rs[d]			
							15 mM BCD	40 mM HPBCD	40 mM RAMEB	40 mM TRIMEB
4-Aryl substituted β-lactams	13		CMACD	63						
			CMBCD	88			0.59			1.86
			CMGCD	43						
			SBEBCD	194						3.70
	14		CMACD	77						
			CMBCD	247			0.51		1.64	
			CMGCD	53						
			SBEBCD	382	1.38	0.58	1.87	0.55	1.00	1.21
	15		CMACD	78						
			CMBCD	126	0.61	1.27	0.79	1.10	1.49	2.46
			CMGCD	60						
			SBEBCD	330					1.39	7.69
	16		CMACD	226	2.02	1.96				
			CMBCD	210	0.71	1.33	0.83	1.01		3.34
			CMGCD	39						
			SBEBCD	376						6.61
	17		CMACD	494	1.75	0.87				
			CMBCD	259				1.11	1.90	
			CMGCD	46						
			SBEBCD	311	2.03	1.68	3.02	1.82	0.66	3.46
	18		CMACD	60						
			CMBCD	120						
			CMGCD	33						
			SBEBCD	102	1.73	0.92	1.64	1.49		3.32
	19		CMACD	1742	1.79	1.38				
			CMBCD	545				1.05	1.99	
			CMGCD	79						
			SBEBCD	447	2.08	1.55	2.25	1.61	1.67	3.01

Centers of asymmetry are indicated by asterisks.
[a] Apparent complex stability constants in M^{-1} of BLs and CMACD, CMBCD, CMGCD at pH 8.0 or SBEBCD at pH 2.5.
[b] Resolution of BLs achieved by 20 mM CMACD, CMBCD, CMGCD at pH 8.0 or 20 mM SBEBCD at pH 2.5.
[c] Resolution of BLs achieved by 20 mM CMACD, CMBCD, CMGCD and SBEBCD at pH 5.0.
[d] Resolution of BLs achieved in dual CD systems containing the indicated neutral CD and 20 mM CMBCD (at pH 5.0) or 20 mM SBECD (at pH 2.5).

namely, they competed for the same analyte. Resolution can be achieved even when the anionic CD alone is ineffective, but the dual system gains stereoselectivity from the neutral CD. Or else, the existing resolution can be improved by the addition of neutral CD to the charged one if the recognition patterns of the CDs are opposite.

Interestingly, in the case of BL/10 upon addition of TRIMEB to SBEBCD decreased resolution is observed until a reversal of

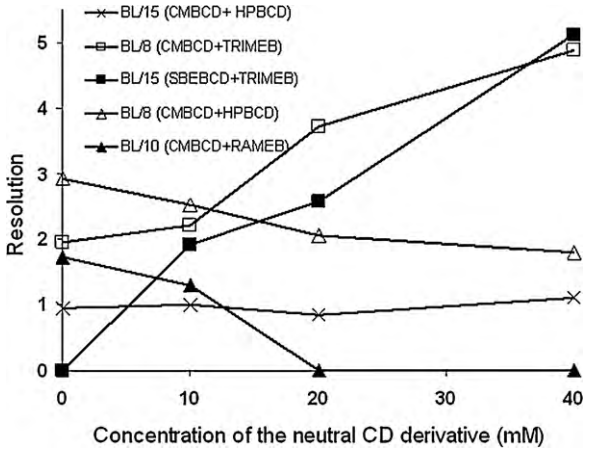


Fig. 1. Representative examples for different types of resolution changes in dual systems compared to single CD system; BGE: 20 mM sodium acetate (pH 5.0) containing 20 mM CMBCD, or SBEBCD.

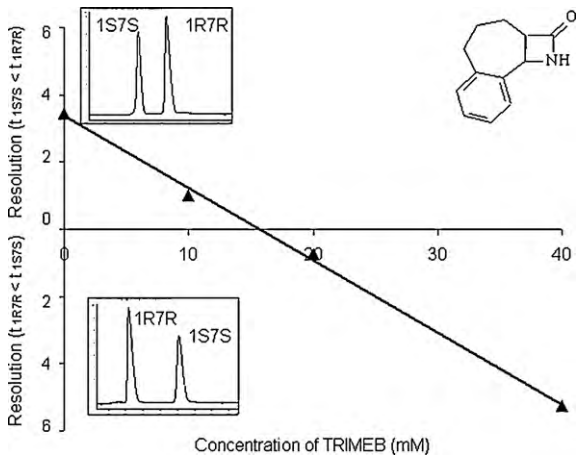


Fig. 2. Changes of chiral resolution of BL/10 in function of the concentration of TRIMEB; BGE: 20 mM sodium acetate (pH 5.0) containing 20 mM SBEBCD.

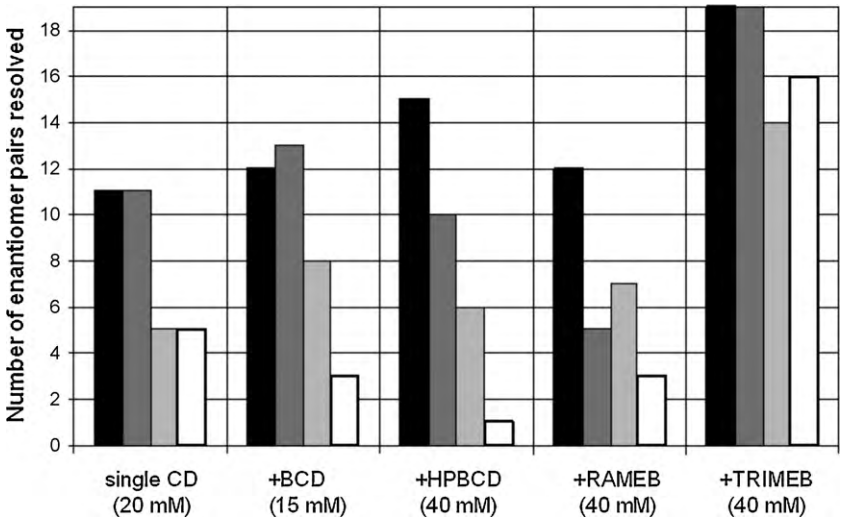


Fig. 3. Efficiency of chiral resolution (number of stereoisomer pairs resolved) of SBEBCD (at pH 2.5 (■) and at pH 5.0 (■)) and CMBCD (at pH 5.0 (■) and at pH 8.0 (□)) based single and dual CD systems; for more details see Section 2.3.

enantiomer migration order is seen (Fig. 2). Apparently, TRIMEB is more enantio-selective for the analyte than the charged SBE-BCD. Although the two CDs seemed to be antagonistic, the result was beneficial. Similar effect was reported previously by Lurie et al. [19].

3.6. Optimization of dual systems

In most cases, dual systems were more efficient than single ones at more acidic conditions (see Fig. 3). In particular, comparison of the number of chirally resolved stereoisomers by various dual CD systems showed that the lower pH is more favorable in case of HPBCD and RAMEB, and for BCD and CMBCD mixtures. Higher pH could be recommended for BCD and SBEBCD, or TRIMEB and CMBCD mixtures (see Fig. 3).

3.7. Comparison of chiral resolution efficiency of different dual systems

The applied single and dual CD systems could be characterized by the number of separated pairs of stereoisomers. The stereoselectivity of neutral CDs in combination with negatively charged CD derivatives depended on the type of BCD derivatives (see Tables 1–3). Enantiomers of bicyclic BLs (BL/1–7) could be separated by both SBEBCD–TRIMEB and CMBCD–TRIMEB mixtures. Members of smaller ring size in this subgroup could be separated by various SBEBCD based dual systems (Table 1). BL/8–10 of the aromatic tricyclic subgroup could be resolved by almost all combinations of CMBCD or SBEBCD based dual systems with the latter ones providing slightly more efficient enantioseparation (Table 2). Mixtures containing SBEBCD are the best choice for aliphatic tricyclic BL stereoisomers. The 4-aryl-substituted BL subgroup is the most heterogeneous (Table 3). Resolution behavior depended on the position and the kind of substituent on the aryl ring. Several members were resolved by CMBCD–BCD mixtures. Again, SBEBCD–TRIMEB based dual systems could resolve the whole 4-aryl-substituted series.

Our results indicated the neutral TRIMEB is the most efficient in combination with a charged one (Fig. 3). The seven times three methyl substituents on the β-CD ring provided optimal conditions for chiral resolution of the neutral BL stereoisomers investigated. In particular, the TRIMEB/SBEBCD dual system was found the most efficient composition.

4. Conclusions

The present work resulted in elaborating the most effective CD variant for chiral resolution of a series of BLs by CE. In general, the investigated BLs preferred the size of the β-CD ring. Comparing anionic carboxymethyl- and sulfobutyl-CD derivatives the latter ones proved to be more effective both in single and dual systems. In dual CD systems, TRIMEB was more efficient than HPBCD, RAMEB or BCD. Accordingly, mixtures of TRIMEB and the negatively charged SBEBCD separated all stereoisomer pairs investigated.

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References

[1] A. Mantagne, J. Lamotte-Brasseur, J.M. Frere, Catalytic properties of class A β-lactamases: efficiency and diversity, Biochem. J. 330 (1998) 581–598.
[2] J. Lamotte, G. Dive, J.M. Ghuysen, Conformational analysis of β- and γ-lactam antibiotics, Eur. J. Med. Chem. 26 (1991) 43–50.
[3] C. Palomo, J.M. Aizpurua, I. Ganboa, M. Oiaribe, β-Lactams as versatile intermediates in α and β-amino acid synthesis, Synlett 12 (2001) 1813–1826.
[4] C. Palomo, I. Ganboa, M. Oiaribe, G.T. Sciano, J.L. Miranda, A β-lactam route to short peptide segments to angiotensin-converting enzyme (ACE) inhibitors, ARKIVOC 5 (2002) 8–16.
[5] E. Juaristi, Enantioselective Synthesis of β-Amino Acids, Wiley–VCH, New York, 1997.
[6] H.H. Wasserman, H. Matsuyama, R.P. Robinson, β-Lactams as building blocks in the synthesis of macrocyclic spermine and spermidine alkaloids, Tetrahedron 58 (2002) 7177–7190.
[7] B. Alcaine, P. Almendros, J.M. Alonso, M.F. Aly, C. Padro, E. Saez, M.R. Torres, Efficient entry to highly functionalized β-lactams by regio- and stereoselective 1,3-dipolar cycloaddition reaction of 2-azetidinone-tethered nitrones, synthetic application, J. Org. Chem. 67 (2002) 7004–7013.
[8] K. Achilles, T. Schirmeister, H.H. Otto, β-Lactam derivatives as enzyme inhibitors: 1-peptidyl derivatives of 4-phenylazetidin-2-one as inhibitors of elastase and papain, Arch. Pharm. Pharm. Med. Chem. 333 (2000) 243–253.
[9] K. Huang, D.W. Armstrong, E. Forró, F. Fülöp, A. Péter, Separation of enantiomers and control of elution order of β-lactams by GC using cyclodextrin-based chiral stationary phases, Chromatographia 69 (2009) 331–337.
[10] R. Berkecz, R. Török, I. Ilisz, E. Forró, F. Fülöp, D.W. Armstrong, A. Péter, LC enantioseparation of β-lactam and β-amino acid stereoisomers and a comparison of macrocyclic glycopeptide- and β-cyclodextrin-based columns, Chromatographia 63 (2006) S37–S43.
[11] P. Sun, C. Wang, D.W. Armstrong, A. Péter, E. Forró, Separation of enantiomers of β-lactams by HPLC using cyclodextrin-based chiral stationary phases, J. Liq. Chromatogr. Relat. Technol. 29 (2006) 1847–1860.

[12] A. Péter, A. Árki, E. Forró, F. Fülöp, D.W. Armstrong, Direct high-performance liquid chromatographic enantioseparation of β-lactam stereoisomers, Chirality 17 (2005) 193–200.
[13] Z. Pataj, I. Ilisz, R. Berkecz, E. Forró, F. Fülöp, A. Péter, Comparison of separation performances of amylase- and cellulose-based stationary phases in the high-performance liquid chromatographic enantioseparation of stereoisomers of β-lactams, Chirality 22 (2010) 120–128.
[14] M. Fillet, P. Hubert, J. Crommen, Enantiomeric separations of drugs using mixtures of charged and neutral cyclodextrins, J. Chromatogr. A 875 (2000) 123–134.
[15] S.-I. Izumoto, H. Nishi, Enantiomer separation of drugs by capillary electrophoresis using mixtures of β-cyclodextrin sulphate and neutral cyclodextrins, Electrophoresis 20 (1999) 189–197.
[16] M. Fillet, B. Chankvetadze, J. Crommen, G. Blaschke, Designed combination of chiral selectors for adjustment of enantioseparation selectivity in capillary electrophoresis, Electrophoresis 20 (1999) 2691–2697.
[17] P. Dubsky, J. Svobodova, B. Gas, Model of CE enantioseparation systems with a mixture of chiral selectors. Part I: Theory of migration and interconversion, J. Chromatogr. B 875 (2008) 30–34.
[18] M.J. Sepeniak, C.L. Copper, K.W. Whitaker, V.C. Anigbogu, Evaluation of dual-cyclodextrin phase variant of capillary electrokinetic chromatography for separation of nonionizable solutes, Anal. Chem. 67 (1995) 2037–2041.
[19] I.S. Lurie, R.F.X. Klein, T.A. Dal Cason, M.J. LeBelle, R. Brenneisen, R.E. Weinberger, Chiral resolution of cationic drugs of forensic interest by capillary electrophoresis with mixtures of neutral and anionic cyclodextrins, Anal. Chem. 66 (1994) 4019–4026.
[20] I.S. Lurie, Separation selectivity in chiral and achiral capillary electrophoresis with mixed cyclodextrins, J. Chromatogr. A 792 (1997) 297–307.
[21] A.M. Abustoffa, M. Fillet, P. Hubert, J. Crommen, Prediction of selectivity for enantiomeric separations of uncharged compounds by capillary electrophoresis involving dual cyclodextrin systems, J. Chromatogr. A 948 (2002) 321–329.
[22] M. Fillet, L. Fotsing, J. Crommen, Enantioseparation of uncharged compounds by capillary electrophoresis using mixtures of anionic and neutral β-cyclodextrin derivatives, J. Chromatogr. A 817 (1998) 113–119.
[23] S. Surapanemi, K. Ruterbories, T. Lindstrom, Chiral separation in capillary electrophoresis using dual neutral cyclodextrins: theoretical models, J. Chromatogr. A 761 (1997) 249–257.
[24] S. Terabe, H. Ozaki, K. Otsuka, T. Ando, Electrokinetic chromatography with 2-O-carboxymethyl-β-cyclodextrin as a moving "stationary" phase, J. Chromatogr. 332 (1985) 211–217.
[25] C. Jiang, D.W. Armstrong, A. Péter, F. Fülöp, Enantiomeric separation of a series of β-lactams using capillary zone electrophoresis, J. Liq. Chromatogr. Relat. Technol. 30 (2007) 1709–1721.
[26] F. Fülöp, The chemistry of 2-aminocycloalkanecarboxylic acids, Chem. Rev. 101 (2001) 2181–2204.
[27] E. Forró, J. Árva, F. Fülöp, Preparation of (1R,8S)-9-azabicyclo[6.2.0]-dec-4-en-10 one: potential strating compounds for synthesis of anatoxin, Tetrahedron-Asymmetry 12 (2001) 643–649.
[28] R. Berkecz, I. Ilisz, E. Forró, F. Fülöp, D.W. Armstrong, A. Péter, LC enantioseparation of aryl-substituted β-lactams using variable-temperature conditions, Chromatographia 63 (2006) S29–S35.
[29] S. Fanali, Controlling enantioselectivity in chiral capillary electrophoresis with inclusion-complexation, J. Chromatogr. A 792 (1997) 227–267.
[30] M.S. Bellini, Z. Deyl, G. Manetto, M. Kohlicková, Determination of apparent binding constants of drugs by capillary electrophoresis using β-cyclodextrin as ligand and three different linear plotting methods, J. Chromatogr. A 924 (2001) 483–491.