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Enantioseparation of chiral (benzylsulfinyl)benzamide sulfoxides by capillary electrophoresis using cyclodextrins as chiral selectors

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ABSTRACT

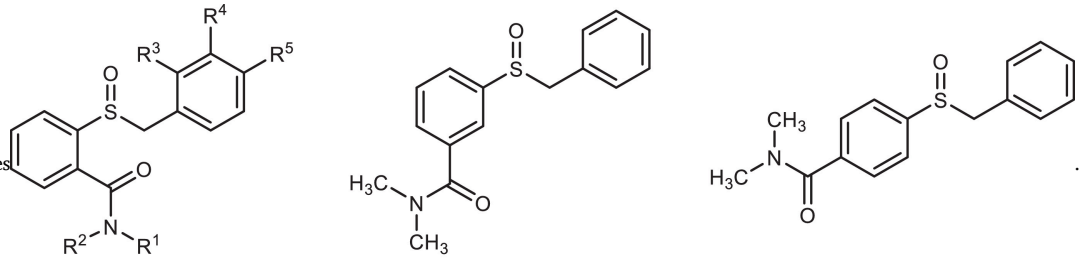
Sulfur as a stereogenic center can be found in synthetic compounds and natural products. The current study evaluated the enantioseparation of 16 chiral (benzylsulfinyl)benzamides compounds by capillary electrophoresis using charged cyclodextrins (CDs) as chiral selectors in 50 mM sodium acetate buffer, pH 5.5. The sulfoxides varied in the type and position of the substituent of the benzyl moiety as well as the position and methylation of the amide group. Typically, randomly substituted CDs separated the majority of the model analytes in contrast to single isomer CDs. In case of random substitution, γ-CD derivatives displayed higher resolution ability toward the set of model compounds followed by β-CD and α-CD derivatives. Except for a few examples, the (+)-enantiomer of the analytes migrated before the (–)-isomer irrespective of the type of the CD so that the chiral recognition appeared to be also mostly independent on the structure of the sulfoxides. Evaluation of complexation constants and complex mobilities of selected CD-analyte pairs revealed that the separations were based on the stereoselective complexation by the CD expressed as complexation constants but examples for complex mobilities as the determining factor for the enantiomer migration order were also found. In case of 2-(4-bromobenzylsulfinyl)-*N*-methyl benzamide in the presence of *heptakis*(2,3-di-*O*-methyl-6-*O*-sulfo)-α-CD reversal of the enantiomer migration order as a function of the CD concentration was observed. Using neutral CD derivatives in the presence of sodium dodecyl sulfate-base micelles at pH 9.0 only few sulfoxides could be enantioseparated.

1. Introduction

In most cases, the stereogenic centers of chiral molecules are carbon atoms. However, other atoms such as sulfur or phosphor in the appropriate oxidation status such as sulfonate and sulfoxide and phosphine or phosphonate, respectively, can also be stereogenic centers. Many pharmacologically active substances are chiral sulfoxides including pharmaceuticals such as proton pump inhibitors [1] or cyclooxygenase inhibitors [2] or plant secondary metabolites [3]. Furthermore, sulfoxides originate from the oxidation of sulfide groups of compounds. Because the pharmacological activity is often attributed to one of the sulfoxide enantiomers, their separation has been of great interest. This is especially true for proton pump inhibitors which have been analyzed by HPLC, sub/supercritical fluid chromatography (SFC) or capillary electrophoresis (CE) as recently summarized [1].

Furthermore, structurally simple aliphatic or aromatic sulfoxides have served as model compounds for the evaluation of various chiral separation techniques. In this context, Chankvetadze et al. noted an extremely high separation factor α larger than 110 for the enantioresolution of 2-(benzylsulfinyl)benzamide (Table 1, compound 1) on a cellulose tris(3,5-dichlorophenylcarbamate) column using propan-2-ol as mobile phase [4]. Unmodified sulfoxides [5,6] as well as 2-(benzylsulfinyl)benzamide derivatives [7–10] were studied on several polysaccharide-based chiral columns under various mobile phase conditions. Most recently, molecular modeling provided a rationale for the separation of 2-(benzylsulfinyl)benzamides on polysaccharide columns [11]. The enantioseparation of simple unmodified chiral aliphatic/aromatic sulfoxides as well as 2-(benzylsulfinyl)benzamide derivatives was also studied by SFC using polysaccharide-based chiral stationary phases

Table 1

Structures of sulfoxides 

1-3, 6-16 **4**[a] **5**[b]

#	IUPAC name	R^1	R^2	R^3	R^4	R^5
1	2-(benzylsulfinyl)benzamide	H	H	H	H	H
2	2-(benzylsulfinyl)-*N*-methyl benzamide	CH_3	H	H	H	H
3	2-(benzylsulfinyl)-*N,N*-dimethyl benzamide	CH_3	CH_3	H	H	H
6	2-(3-bromobenzylsulfinyl)benzamide	H	H	H	Br	H
7	2-(4-bromobenzylsulfinyl)benzamide	H	H	H	H	Br
8	2-(4-methylbenzylsulfinyl)benzamide	H	H	H	H	CH_3
9	2-(4-tertbutylbenzylsulfinyl)benzamide	H	H	H	H	$C(CH_3)_3$
10	2-(3-bromobenzylsulfinyl)-*N*-methyl benzamide	CH_3	H	H	Br	H
11	2-(4-bromobenzylsulfinyl)-*N*-methyl benzamide	CH_3	H	H	H	Br
12	2-(3-methoxybenzylsulfinyl)-*N*-methyl benzamide	CH_3	H	H	OCH_3	H
13	2-(3-methylbenzylsulfinyl)-*N*-methyl benzamide	CH_3	H	H	CH_3	H
14	2-(2-methylbenzylsulfinyl)-*N,N*-dimethyl benzamide	CH_3	CH_3	CH_3	H	H
15	2-(3-methylbenzylsulfinyl)-*N,N*-dimethyl benzamide	CH_3	CH_3	H	CH_3	H
16	2-(4-methylbenzylsulfinyl)-*N,N*-dimethyl benzamide	CH_3	CH_3	H	H	CH_3

[a]IUPAC name: 3-(benzylsulfinyl)-*N,N*-dimethyl benzamide.
[b]IUPAC name: 4-(benzylsulfinyl)-*N,N*-dimethyl benzamide.

[6,12,13], by HPLC with chiral columns based on teicoplanin [14–16] or cyclodextrin (CD) columns [17], respectively.

CE has been established as a suitable alternative to HPLC for liquid phase enantioseparations primarily due to the high-resolution ability and flexibility of the technique [18–20]. Consequently, CE has been used for the separation of the enantiomers of many compounds in various analytical fields including pharmaceuticals [21–24], bioanalysis [25] food [26,27] or environmental analysis [28]. However, while the CE enantioseparations of chiral sulfoxide drugs such as proton pump inhibitors have been frequently explored [1], the resolution of the enantiomers of model sulfoxide compounds as described for HPLC above has been scarcely studied by CE. The enantioseparation of "simple" aliphatic/aromatic sulfoxides is generally challenging because these structures cannot be ionized and do not contain many groups that can interact with cyclodextrins, which are the most often applied chiral selectors in CE [29–31]. To the best of our knowledge, there is only one publication of the enantioseparation of chiral model sulfoxides and sulfinate esters by capillary electrophoresis using sulfated β-cyclodextrin (S-β-CD) and carboxymethyl-β-CD (CM-β-CD) as chiral selectors and 10 mM sodium phosphate buffer, pH 8.3 as background electrolyte (BGE) [32]. Almost all analytes could be enantioresolved in the presence of S-β-CD, while CM-β-CD was less effective. Obvious structure-separation relationships could not be established but the type and position of the substituents in the aromatic ring affected analyte resolution. Because the enantioseparation of 2-(benzylsulfinyl)benzamides had not been investigated by CE previously, the aim of the present study was the evaluation of charged α-CD, β-CD and γ-CD derivatives as chiral selectors for this purpose. A set of 16 chiral (benzylsulfinyl)benzamides was used (Table 1) differing in the position of the amide substituent and *N*-methylation as well as the type and position of the substituent in the benzylsulfinyl moiety. Moreover, the enantioseparation of the sulfoxides by neutral CDs in the micellar electrokinetic chromatography (MEKC) mode was also briefly addressed.

2. Materials and methods

2.1. Chemicals

Sulfobutylether-β-CD (SBE-β-CD, degree of substitution (DS) ~6.4) was from Cydex (San Diego, CA, USA), *heptakis*(6-*O*-sulfo)-β-CD (HS-β-CD) *heptakis*(2,3-di-*O*-methyl-6-*O*-sulfo)-β-CD (HDMS-β-CD), *heptakis* (2,3-di-*O*-acetyl-6-*O*-sulfo)-β-CD (HDAS-β-CD), succinyl-β-CD (Suc-β-CD, DS ~3.5), *hexakis*(2,3-di-*O*-methyl-6-*O*-sulfo)-α-CD (HDMS-α-CD), *octakis*(2,3-di-*O*-methyl-6-*O*-sulfo)-γ-CD (ODMS-γ-CD) and carboxymethyl-γ-CD (CM-γ-CD, DS ~3.5), Succinyl-γ-CD (Suc-γ-CD, DS ~3.5), *heptakis*(6-deoxy-6-amino)-β-CD heptahydrochloride (HA-β-CD), *octakis*(6-deoxy-6-amino)-γ-CD octahydrochloride (OA-γ-CD), (2-hydroxy-3-*N,N,N*-trimethylamino)propyl-α-CD chloride (TMA-α-CD, DS ~2–4.5), (2-hydroxy-3-*N,N,N*-trimethylamino)propyl-γ-CD (TMA-γ-CD, DS ~2 - 5), α-CD, methylated β-CD (M-β-CD, DS ~12), 2,6-di-*O*-methyl-β-CD 50% purity (DM-β-CD50, DS ~11–14), 2,6-di-*O*-methyl-β-CD 95% purity (DM-β-CD95, DS 14), 2-hydroxypropyl-β-CD (HP-β-CD, DS ~4.5), *heptakis*(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), methylated α-CD (M-α-CD, DS ~11), 2-hydroxypropyl-α-CD (HP-α-CD, DS ~4.5), carboxymethyl-α-CD (CM-α-CD, DS = ~3.5) and 2- hydroxypropyl-γ-CD (HP-γ-CD, DS ~4.5) were from Cyclolab Ltd (Budapest, Hungary). Sulfated β-CD (S-β-CD, DS ~12–15) and sulfated α-CD (S-α-CD, DS ~8–11) were obtained from Sigma-Aldrich Chemie GmbH (Munich Germany), carboxymethyl-β-CD (CM-β-CD, DS ~3.5), (2-hydroxy-3-*N,N,N*-trimethylamino)propyl-β-CD chloride (TMA-β-CD, DS ~5) and β-CD were from Wacker Chemie (Munich Germany) and sulfated γ-CD (S-γ-CD, DS ~13–15) was obtained from Cyclodextrin Shop (Tilburg, The Netherlands).

The chiral sulfoxides 2, 6, 8, 9, 12–16 (Table 1) were supplied by the group of Prof. B. Chankvetadze, Tbilisi, Georgia [33]. Sulfoxides 1, 3–5, 7,10 and 11 were synthesized using the methods as described in [34], see supplementary material for details. All other chemicals were of ana-

lytical grade. Water was purified using a TKA Genpure UV-TOC from Thermo Scientific (Waltham, USA). BGE and sample solutions were filtered through 0.22 μm polypropylene syringe filters from BGB Analytik (Schloßböckelheim, Germany).

2.2. Capillary electrophoresis

Experiments were performed on a Beckman P/ACE MDQ CE system (AB Sciex, Darmstadt, Germany) equipped with a UV-Vis diode array detector and controlled by 32 KARAT software for system control, data acquisition and processing. 50 μm I.D., 365 μm O.D. fused-silica capillaries with a total length of 50.2 cm and an effective length of 40.0 cm were from CM Scientific (Silsden, UK). All rinsing steps were conducted at a pressure of 138 kPa (20 psi). A new capillary was treated subsequently with 0.1 M NaOH for 20 min, water for 10 min, 0.1 M NaOH for 10 min, 0.1 M phosphoric acid for 10 min and water for 10 min. Between the analyses, the capillaries were washed with 0.1 M NaOH for 2 min and with the BGE for 3 min. The applied voltage was 25 kV, and the capillary temperature was maintained 20 °C. UV detection was performed at 220 nm at the cathodic end of the capillary in case of normal polarity and at the anodic end when polarity was reversed. Dimethyl sulfoxide (DMSO) was used as marker of the electroosmotic flow (EOF).

CE separations were performed in 50 mM sodium acetate buffer prepared on a daily basis, while a 50 mM sodium borate buffer, pH 9.0, was used for MEKC. Both electrolyte solutions contained 10% (v/v) methanol. The pH of the BGEs was adjusted after the addition of the CDs. The buffers were filtered (0.22 μm) and degassed by sonication before use. Sample solutions of the sulfoxides (100 μg/mL prepared in water/methanol, 1:1, v/v) were introduced at a pressure of 3.5 kPa (0.5 psi) for 5 s. The migration order was confirmed by spiking with the individual sulfoxide enantiomers obtained by HPLC as described in Section 2.3.

Viscosity measurements of the buffers for the determination of complexation constants were performed using the CE instrument as a viscosimeter and 0.1% (m/v) riboflavin-5′-phosphate as boundary marker according to [35]. Electrophoretic mobilities were measured in triplicate and viscosity measurements were determined four-fold.

Analyte resolution (R_S value) was calculated according to

$$R_S = \frac{2\left(t_2 - t_1\right)}{W_1 + W_2} \quad (1)$$

where t_1 and t_2 are the migration times of the first and second migrating enantiomers and W_1 and W_2 are the peak width or the respective peaks.

2.3. HPLC fractionation of sulfoxide enantiomers

HPLC for obtaining the sulfoxide enantiomers was performed on a Shimadzu instrument composed of LC-10AT and LC-10AS pumps, a SPD-10A UV-VIS detector, a SIL-10A auto injector, a DGU-20A3R degassing unit, a CTO-20AC temperature controller and a SCL-10A system controller (Duisburg, Germany). The LCsolution software was used for instrument control and data acquisition. A Lux i-Cellulose 5 column (150 × 4.6 mm, 5 μm, Phenomenex, Aschaffenburg, Germany) containing cellulose tris(3,5-dichlorophenylcarabamate) as chiral selector in combination with methanol as mobile phase was used. The flow rate was 1.0 mL/min, the temperature was set at 20 °C and detection was carried out at 254 nm. 50 μL of the solutions of the sulfoxides prepared at a concentration of 1,2 mg/mL in methanol, were injected. A minimum of 20 runs were performed for each sulfoxide and the eluate containing the respective enantiomers were pooled followed by evaporation of the solvent under reduced pressure. The compounds were obtained as amorphous off-white to yellow solids. The purity of the isolated enantiomers was estimated by HPLC analysis using the same experimental set-up. The optical rotation of the purified enantiomers was determined in ethanol using a P2000 polarimeter from Jasco (Pfungstadt, Germany).

2.4. Software

The CEVal software [36] was used for non-linear curve fitting for the determination of the complexation constants and the mobilities of the sulfoxide-CD complexes.

3. Results and discussion

The structures of the 16 chiral (benzylsulfinyl)benzamides are summarized in Table 1. They differ in the position of the amide substituent and *N*-methylation as well as the type and position of the substituent in the benzylsulfinyl moiety. The enantiomers were obtained by HPLC. In case of all sulfoxides, the first eluted enantiomer displayed dextrorotary optical rotation (data not shown). The separation of the enantiomers of 2-(benzylsulfinyl)benzamide (compound 1) on cellulose tris(3,5-dichlorophenylcarbamate) using methanol, ethanol or propan-2-ol as mobile phases with the (+)-enantiomer eluting first has been reported [4]. Moreover, Carradori et al. showed that the enantiomers of sulfoxide 1 and the *N*-methyl derivative 2, which eluted first from a cellulose *tris*(3,5-dichlorophenylcarbamate) column using ethanol as eluent, possess the (*R*)-configuration [37]. All sulfoxides studied here also displayed the elution order (+)-enantiomer before the (–)-enantiomer on the cellulose tris(3,5-dichlorophenylcarbamate) column using ethanol as eluent (data not shown). Because the different substitution patterns of compounds 1 to 16 do not affect the absolute configuration of the enantiomers at the chiral sulfur atom (the priorities of the respective substituents according to the Cahn-Ingold-Prelog rules do not change), it is safe to assume that for all 16 sulfoxide analytes the (*R*)-configuration can be assigned to the (+)-enantiomer and the (*S*)-configuration to the levorotary enantiomer, although this has been experimentally proven only for sulfoxides 1 and 2 [37].

Because the sulfoxides cannot be ionized, charged CDs were evaluated for the separation of the enantiomers. In addition, MEKC conditions using neutral CDs as well as SDS as surfactant were briefly studied.

3.1. CE using charged CD derivatives

For the separation of neutral analytes, the carrier ability of charged CDs can be exploited [38]. Initial separations using compounds 1, 2, 6 and 8 as well as CM-β-CD or CM-γ-CD as chiral selectors were attempted at pH 2.5. However, at this pH the analytes could not be detected either at the cathode or at the anode, when reversing the polarity of the separation voltage. Consequently, the pH was increased in order to increase the magnitude of the electroosmotic flow (EOF) so that the analytes can be separated after the EOF. Using 50 mM phosphate and acetate buffers and CE concentrations of 10 mg/mL, the pH range 3.5 to 7.0 was subsequently studied. The best enantioresolution was observed for 50 mM sodium acetate buffer, pH 5.5, so that this pH was selected for further studies. Addition of 10% (v/v) methanol resulted in narrower peaks. Therefore, all screening experiments were performed in a 50 mM sodium acetate buffer, pH 5.5 containing 10% (v/v) methanol at a separation voltage of 25 kV. Under these conditions, negatively charged CDs containing sulfate, sulfonate or carboxyl groups migrate to the anode so that uncharged analytes are detected after the EOF when detection is carried out at the cathodic end. In case of positively charged CDs containing amino or quaternary ammonium groups, the polarity of the voltage was reversed, and the analytes were detected at the anodic end. The separations were not further optimized as the development of an analytical method for a specific sulfoxide was not the aim of the present study. The results are summarized for randomly substituted CDs in Table 2 and for single isomer CDs in Table 3.

Table 2
Enantiomeric resolution values and migration order of sulfoxides under standardized conditions in presence of randomly substituted charged CDs. The faster migrating enantiomer is indicated in brackets.

Sulfoxide	SBE-β-CD	S-α-CD	S-β-CD	S-γ-CD	CM-α-CD	CM-β-CD	CM-γ-CD	Suc-β-CD	Suc-γ-CD	TMA-α-CD	TMA-β-CD	TMA-γ-CD
1	0.3 (-)	0.6 (+)	1.7 (+)	3.0 (+)	0.3 (-)	4.1 (+)	3.5 (+)	1.7 (+)	1.6 (+)	0.3 (-)	2.5 (+)	4.3 (+)
2	0.9 (+)	0.2 (+)	0.9 (+)	3.2 (+)	ns	4.4 (+)	3.5 (+)	5.3 (+)	2.8 (+)	0.2 (+)	7.5 (+)	7.8 (+)
3	0.2 (+)	0.9 (+)	0.2 (+)	0.8 (+)	ns	2.0 (+)	4.2 (+)	1.9 (+)	2.2 (+)	0.3 (+)	2.5 (+)	5.9 (+)
4	ns	ns	ns	1.2 (+)	ns	1.0 (+)	1.2 (+)	1.1 (+)	1.0 (+)	ns	ns	ns
5	1.1 (-)	ns	0.2 (+)	10.7 (+)	ns	1.6 (-)	1.4 (+)	1.3 (+)	1.0 (+)	ns	ns	0.2 (-)
6	2.4 (-)	2.9 (+)	1.0 (+)	16.0 (+)	2.2 (+)	0.6 (+)	4.5 (+)	1.8 (+)	1.9 (+)	3.0 (+)	2.6 (+)	14.9 (+)
7	8.9 (-)	5.4 (+)	2.1 (+)	13.3 (+)	7.6 (-)	8.9 (+)	20.6 (-)	1.4 (+)	6.5 (+)	1.1 (+)	5.4 (+)	10.7 (+)
8	1.6 (-)	ns	1.0 (+)	7.9 (+)	ns	3.2 (-)	12.3 (+)	2.8 (+)	3.2 (+)	ns	2.1 (+)	11.5 (+)
9	nd	2.4 (+)	2.4 (+)	12.8 (+)	ns	nd	9.4 (+)	5.2 (+)	1.9 (+)	2.3 (+)	ns	12.7 (+)
10	1.6 (-)	1.2 (+)	2.8 (+)	10.2 (+)	ns	3.5 (+)	1.6 (+)	2.1 (+)	2.3 (+)	3.5 (+)	5.1 (+)	11.7 (+)
11	2.4 (-)	7.5 (+)	4.9 (+)	10.4 (+)	3.5 (-)	6.1 (+)	13.1 (+)	2.4 (+)	4.3 (+)	0.8 (+)	5.8 (+)	ns
12	0.9 (-)	ns	ns	3.6 (+)	ns	ns	1.7 (+)	1.6 (+)	2.0 (+)	0.8 (+)	1.6 (+)	4.1 (+)
13	2.0 (+)	0.6 (+)	ns	8.0 (+)	ns	1.9 (-)	13.7 (+)	1.6 (+)	1.9 (+)	0.2 (+)	0.5 (+)	2.6 (+)
14	0.5 (+)	1.1 (+)	0.2 (+)	0.6 (+)	ns	3.2 (+)	7.0 (+)	2.8 (+)	3.1 (+)	0.2 (-)	1.9 (+)	5.3 (+)
15	1.0 (+)	0.7 (+)	1.3 (+)	2.0 (+)	0.6 (+)	0.9 (+)	3.2 (+)	0.8 (+)	2.0 (+)	0.9 (+)	1.7 (+)	4.4 (+)
16	0.2 (+)	1.0 (+)	ns	0.5 (+)	ns	2.0 (+)	6.8 (+)	0.9 (+)	2.1 (+)	ns	2.1 (+)	2.4 (+)

nd, not detected within 60 min; ns, not separated.
CE conditions: 50 mM sodium acetate buffer, pH 5.5, 50 μm, 50.2/40 cm fused-silica capillary; 20 °C, 25 kV; detection at 220 nm at the cathodic end in the presence of negatively charged CDs and at the anodic end in the presence of cationic CDs. CD concentrations: 5 mg/mL SBE-β-CD; 10 mg/mL TMA-α-CD, TMA-β-CD, TMA-γ-CD and S-γ-CD; 20 mg/mL CM-α-CD, CM-β-CD, CM-γ-CD, S-α-CD, Suc-β-CD and Suc-γ-CD; 40 mg/mL S-β-CD.

Table 3
Enantiomeric resolution values and migration order of sulfoxides under standardized conditions in the presence of single isomer charged CDs. The faster migrating enantiomer is indicated in brackets.

Sulfoxide	HS-β-CD	HDAS-β-CD	HDMS-α-CD	HDMS-β-CD	ODMS-γ-CD	HA-β-CD	OA-γ-CD
1	0.8 (+)	0.8 (-)	2.2 (+)	ns	ns	2.3 (+)	0.3 (-)
2	1.9 (+)	0.2 (-)	0.9 (+)	ns	ns	3.5 (+)	ns
3	1.6 (+)	ns	ns	ns	ns	1.9 (+)	ns
4	1.5 (+)	0.3 (+)	ns	ns	ns	ns	0.5 (+)
5	ns	ns	0.5 (+)	ns	ns	0.9 (+)	0.3 (-)
6	1.8 (+)	0.4 (-)	0.7 (-)	1.0 (+)	ns	1.7 (+)	ns
7	7.3 (+)	ns	5.6 (+)	1.1 (-)	0.9 (-)	6.5 (+)	1.8 (-)
8	2.6 (+)	ns	2.2 (+)	ns	ns	3.4 (+)	0.7 (+)
9	6.0 (+)	0.5 (-)	ns	2.3 (-)	1.6 (-)	ns	1.1 (-)
10	2.4 (+)	0.7 (-)	2.2 (-)	0.8 (+)	ns	9.3 (+)	0.3 (+)
11	6.7 (+)	3.2 (-)	1.6 (+)	1.8 (+)	ns	12.0 (+)	0.7 (-)
12	0.2 (+)	ns	0.5 (+)	ns	ns	0.2 (+)	1.1 (-)
13	0.4 (+)	1.7 (-)	1.2 (+)	2.2 (+)	2.4 (+)	ns	ns
14	1.0 (+)	ns	0.2 (-)	ns	Ns	1.8 (+)	ns
15	1.1 (+)	ns	1.2 (+)	ns	ns	3.3 (+)	ns
16	1.2 (+)	ns	ns	ns	ns	1.1 (+)	ns

ns - not separated.
CE conditions: 50 mM sodium acetate buffer, pH 5.5, 50 μm, 50.2/40 cm fused-silica capillary; 20 °C, 25 kV; detection at 220 nm at the cathodic end in the presence of negatively charged CDs and at the anodic end in the presence of cationic CDs. CD concentrations: 20 mg/mL HS-β-CD, HA-β-CD and OA-γ-CD; 40 mg/mL HDMS-α-CD, HDMS-β-CD, ODMS-γ-CD and HDAS-β-CD.

3.1.1. Effect of the CD structure and substitution pattern

With the exception of CM-α-CD, randomly substituted CDs proved to be effective chiral selectors for the sulfoxides (Table 2). In the presence of S-γ-CD, CM-γ-CD, Suc-β-CD and Suc-γ-CD the enantiomers of all analytes were resolved and in the vast majority of the cases even baseline separated. Especially the γ-CD derivatives yielded high resolution with R_S values up to 20.6 (sulfoxide 7 and CM-γ-CD). β-CD derivatives were somewhat less effective closely followed by α-CD derivatives except for CM-α-CD as stated above. It may be speculated that the large cavity of the γ-CD derivatives provided a better fit for the sulfoxides as compared to the smaller cavities of β-CDs and α-CDs. The type of substituent appeared to play a minor role. For example, S-γ-CD, CM-γ-CD and Suc-γ-CD displayed comparable efficiency resolving the enantiomers of all sulfoxide analytes, although R_S values were typically lower in case of Suc-γ-CD compared to the two other γ-CD derivatives. Furthermore, negatively charged CDs appeared to have an advantage over positively charged CDs although the number of positively charged CDs in this study was limited so that this observation may not be generalized at this point. It is interesting to note, that in most cases the (+)-enantiomers migrated first independent of the structure or charge of the CD. An exception was SBE-β-CD, where in a little over half the sulfoxides displayed the elution order (–) before (+). In case of the negatively charged CDs, separation was obtained after the EOF. Because the negatively charged CDs migrate toward the anode the weaker complexed enantiomer of the uncharged sulfoxide analytes will migrate first under normal polarity of the applied voltage and detection at the cathodic end of the capillary. The same scenario applies for positively charged CDs. In this case the polarity of the applied voltage was reversed because of the adsorption of the positively charged CDs to the capillary wall, and, consequently, the direction of the EOF changed from the cathode to the anode. Thus, detection was carried out at the anodic end of the capillary. As in the presence of negatively charged CDs, the analytes migrated after the EOF while the positively charged CDs migrated toward the cathode. Thus, the weaker complexed enantiomer will also migrate first under these circumstances so that the chiral recognition did not generally change as a function of the charge of the CDs.

In few cases, the enantiomer migration order depended on the cavity size of the CDs. For example, in case of compound 7 in the presence of carboxymethylated CDs the (–)-enantiomer migrated first in the presence of CM-α-CD and CM-γ-CD, while (+)-7 migrated first when CM-β-CD was used as chiral selector (Fig. 1A). The migration order was (+)-11 before (–)-11 using CM-β-CD or CM-γ-CD, while it was (–)-11 before

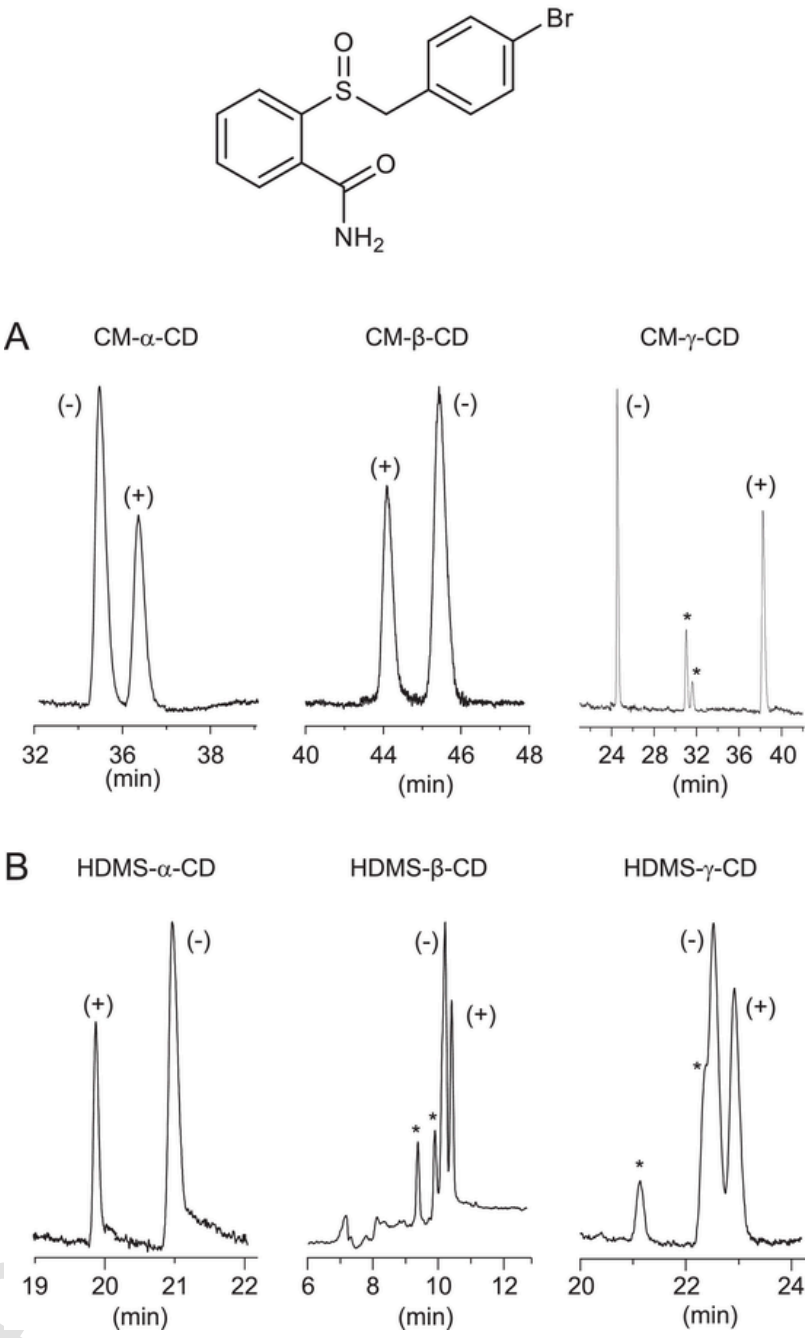


Fig. 1. Electropherograms of the separation of the enantiomers of 2-(4-bromobenzylsulfinyl) benzamide (compound 7) in the presence of (A) 20 mg/mL CM-α-CD, CM-β-CD, CM-γ-CD and (B) 40 mg/mL HDMS-α-CD, HDMS-β-CD and HDMS-γ-CD. Other experimental conditions: 40/50.2 cm, 50 μm I.D. fused-silica capillary; 50 mM sodium acetate buffer, pH 5.5; 20 °C; 25 kV; detection at 220 nm. * synthetic impurity.

(+)-11 in the presence of CM-α-CD. Further examples can be found in Table 2. A dependence of the enantiomer migration order on the cavity size of the CD has been observed for many non-sulfoxide enantiomers as summarized, for example in [39].

Single isomer CDs were less efficient for the enantioseparation of the sulfoxides under the standardized conditions (Table 3). Only HS-β-CD enantioseparated 15 out of the 16 sulfoxides, followed by HA-β-CD (13) and HDMS-α-CD (12). It is interesting to note that single isomer γ-CD derivatives were less effective than the corresponding β-CDs or α-CDs. In case of CDs containing a sulfate moiety in position 6 and methyl groups in positions 2 and 3, the order was HDMS-α-CD (12) > HDMS-β-CD (6) > ODMS-γ-CD (3). Derivatization of the wider secondary rim appeared to reduce the enantioseparation ability of the CDs as HS-β-CD featuring only sulfate groups at C6 separated more enantiomers compared to HDAS-β-CD or HDMS-β-CD, which contain acetyl substituents and methyl groups, respectively, in position 2 and 3 of the D-glucopyranose units of the CDs. As observed for randomly substituted CDs, in most cases the migration order of the sulfoxide enantiomers was (+) before (–) for CDs, except for HDAS-β-CD and OA-γ-CD, where the (–)-enantiomer migrated before the (+)-enantiomer in most cases. It should be noted that the polarity of the voltage was reversed in case of OA-γ-CD and detection was performed at the anodic end of the capillary. As seen in case of the randomly substituted CDs, the cavity size of the CDs affected the enantiomer migration order for some analytes. For example, the (+)-enantiomer of compound 7 migrated first in the presence of HDMS-α-CD, while it was the (–)-enantiomer in the presence of

HDMS-β-CD and HDMS-γ-CD (Fig. 1B). Further examples can be found in Table 3. See also the discussion in Section 3.1.2 below.

3.1.2. Effect of the structure of the sulfoxides

The benzamide sulfoxides differ in the position of the amide substituent and *N*-methylation as well as the type and position of the substituent in the benzylsulfinyl moiety (Table 1). Substituents in the benzylsulfinyl residue were bromine, methoxy, methyl or tert.-butyl groups. The parent compound 1 (2-(benzylsulfinyl)benzamide) was at least partially resolved by all but two CDs, i.e., HDMS-β-CD and HDMS-γ-CD. As stated above, in the presence of most negatively charged CDs the (+)-enantiomer of the sulfoxides migrated first. As also stated above, it is reasonable to assume from the known absolute configuration of compounds 1 and 2 [37] and the elution order of the other sulfoxide analytes from a cellulose tris(3,5-dichlorophenylcarbamate) column that the (*R*)-configuration can most likely be assigned to the (+)-enantiomer of all compounds, because none of the substitution patterns of the sulfoxides changes the absolute configuration at the chiral sulfur atom. Therefore, the chiral recognition in the vast majority of cases is independent of the structure of the sulfoxides, i.e., the type or position of the substituents. Nonetheless, a few exceptions were observed. For example, the position of the substituent in the benzyl moiety affected the migration order when HDMS-α-CD was used as chiral selector. Thus, the migration order was opposite for the compound pairs 6 and 7 as well as 10 and 11, which feature a bromine substituent in position 3 (compounds 6 and 10) or position 4 (compounds 7 and 11), respectively. Electropherograms of the enantioseparations of compounds 10 and 11 in the presence of HDMS-α-CD are shown in Fig. 2. Reversal of the enantiomer migration order was also observed for the pair of compounds 14 and 15, which bear a methyl group in position 2 or 3, respectively (Table 3). Opposite migration order was also found in case of compounds 10 and 11 in the presence of OA-γ-CD (Table 3). *N*-methylation of the benzamide group also affected the chiral recognition by CDs as the opposite migration order was observed for the compound pairs 7 and 11 in the presence of HDMS-β-CD (Figs. 1B and 2B) or for compounds 1 and 2, when randomly substituted SBE-β-CD was the chiral selector (Table 2). An effect of the type of substituent was found for OA-γ-CD and compounds 7 (bromine), 8 (methyl) and 9 (tert.-butyl), which feature the substituents in position 4 of the benzyl moiety (Table 3). Finally, the position of the benzamide group played a role for compound 1 (position 2), 4 (position 3) and 5 (position 4) in the presence of SBE-β-CD or TMA-α-CD (Table 2).

3.1.3. Determination of apparent complexation constants and complex mobilities

In order to rationalize the opposite enantiomer migration order of some analytes as a function of the position of the substituents or *N*-methylation, the complexation constants as well as the mobilities of the diastereomeric analyte-CD complexes were determined for compounds 7, 10 and 11 in the presence of HDMS-α-CD and HDMS-β-CD. The data were obtained as best fit parameters of the dependence of the effective mobility on the CD concentrations in the range of 5 to 30 mM at pH 5.5

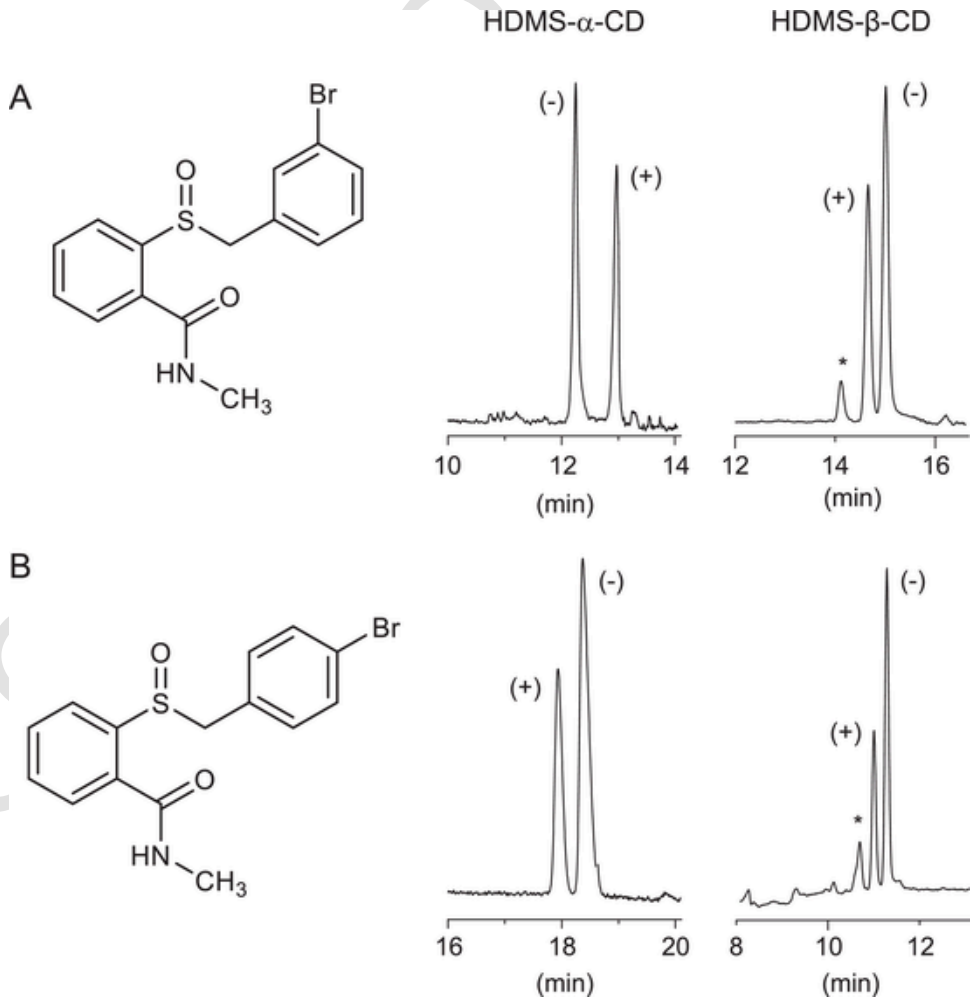


Fig. 2. Electropherograms of the separation of the enantiomers of (A) 2-(3-bromobenzylsulfinyl)-*N*-methylbenzamide (compound 10) and (B) 2-(4-bromobenzylsulfinyl)-*N*-methylbenzamide (compound 11) in the presence of 40 mg/mL HDMS-α-CD and HDMS-β-CD. For other experimental conditions see Fig. 1.

assuming the formation of 1:1 sulfoxide CD-complexes according to Eq. (2)

$$\mu_{eff} = \frac{\mu_f + \mu_c \cdot K \cdot [CD]}{1 + K \cdot [CD]} \quad (2)$$

where μ_{eff} is the effective mobility, μ_f the mobility of the free analyte, μ_c the limiting mobility of the CD-analyte complex, K the complexation constant, and [CD] is the molar concentration of the CDs. The software CEVal was used for data fitting because this software allows correction of the migration times in case of tailing peaks according to the Haarhoff-van der Linde equation [36]. Moreover, the observed mobilities were corrected for the increasing viscosity of the BGE upon increasing CD concentrations.

In this process it was noted that in case of compound 11 the enantiomer migration order changed as a function of the concentration of HDMS-α-CD as illustrated in Fig. 3. At concentrations up to 15 mM, the (–)-enantiomer migrated before (+)–11, while at concentrations of 20 mM and above, the (+)-isomer was detected first. Comigration of the enantiomers occurred at an approximate concentration of 17.5 mM HDMS-α-CD. The dependence of the enantiomer migration order as a function of the CD concentration has been observed for several analytes as, for example, summarized in [39,40].

The mobility and complexation data are summarized in Table 4. It should be noted that these data are apparent constants and mobilities rather than thermodynamic values because the ionic strength of the buffers is not known, and CD concentrations referred to the volume and not to the mass of the solvent. Although differences between the respective values are typically not statistically significant based on the overlapping 95% confidence intervals, the trend of the data can still be used to rationalize the migration behavior of the analytes. As the complexes migrate toward the anode, the mobility values have a minus sign by definition. In the presence of HDMS-α-CD, the (+)-enantiomer of the three sulfoxides always displayed the higher complexation constant. Thus, the chiral recognition of the CD is identical toward the enantiomers of the three analytes and the initially assumed dependence of the chiral recognition as a function of the position of the bromo substituent is not supported by the complexation data. Nonetheless, different enantiomer migration orders were observed. In case of compound 11, opposite migration sequence of the enantiomers was noted in the initial experiments with relatively high concentrations of the CDs (Table 3 and Fig. 3). However, as described above, the order was (+)–11 before (–)–11 at high concentrations, while the (+)-enantiomer migrated slower than the (–)-enantiomer at lower concentrations as observed for compound 10 over the entire concentration range. It is well known that there are two enantioselective mechanisms in CE [19,41]. The first one is based on the different affinities of the enantiomers toward a chiral selector as reflected in differences in the complexation constants (chromatographic or affinity-dependent mechanism). The so-called electrophoretic enantioselective mechanism (mobility-dependent mechanism) is due to differences in the mobilities of the analyte-selector complexes. Both mechanisms, i.e., differences in complexation constants and complex mobilities may cooperate or counteract each other in a given chiral separation in CE [41]. Thus, the enantioseparation of compound 11 is dominated by the complexation constants at low concentrations (as in the case of compound 10) with the stronger bound (+)-enantiomer migrating second. In contrast, at high CD concentrations, when a larger fraction of the enantiomers is complexed, the migration order is determined by the (anodic) complex mobilities resulting in a reversal of the sequence. Similar result has been reported earlier for some basic drugs [42]. It is worth mentioning that as in [42], the mobility-dependent separation of enantiomers was characterized by a higher separation factor α compared to affinity-dependent separation (Fig. 3). Comparing the mobilities of the complexes of the respective compounds, the absolute mobility in case of compound 10 is lower compared to compound 11 and the difference between the mobilities of

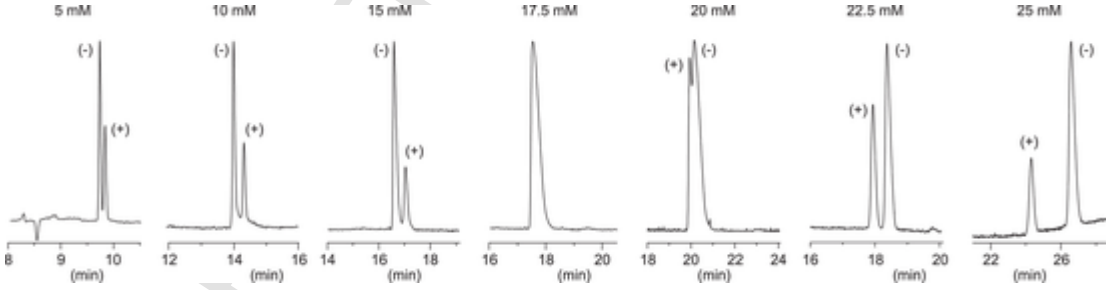


Fig. 3. Electropherograms of the separation of the enantiomers of 2-(4-bromobenzylsulfinyl)-*N*-methylbenzamide (compound 11) as a function of the concentration of HDMS-α-CD in the background electrolyte. For other experimental conditions see Fig. 1.

Table 4
Apparent complexation constants (K) and mobilities of the free analyte (μ_f) as well as analyte-CD complexes (μ_c) and enantiomer migration order (EMO). The numbers in brackets refer to the 95% confidence interval.

Sulfoxide		HDMS-α-CD				HDMS-β-CD			
		K(M^{-1})	μ_f($10^{-9}m^2V^{-1}s^{-1}$)	μ_c($10^{-9}m^2V^{-1}s^{-1}$)	EMO	K(M^{-1})	μ_f($10^{-9}m^2V^{-1}s^{-1}$)	μ_c($10^{-9}m^2V^{-1}s^{-1}$)	EMO
7	(+)	28.6	0.004	–0.525	1	63.6	0.005	–0.098	2
		(20.5 / 36.3)	(0.003 / 0.005)	(–0.205 / –0.712)		(48.7 / 80.1)	(0.002 / 0.009)	(–0.075 / –0.121)	
	(-)	20.2		–0.706	2	50.1		–0.109	1
		(15.6 / 26.2)		(–0.301 / –1.223)		(40.1 / 63.5)		(–0.088 / –0.137)	
10	(+)	60.6	0.007	–0.082	2	160.1	0.008	–0.046	1
		(49.0 / 72.1)	(0.003 / 0.103)	(–0.051 / –1.202)		(132.8 / 180.9)	(0.005 / 0.012)	(–0.022 / –0.069)	
	(-)	38.9		0.101	1	127.4		–0.054	2
		(19.8 / 53.3)		(–0.505 / –1.435)		(111.3 / 142.5)		(–0.036 / –0.078)	
11	(+)	24.4	0.012	–0.523	2 → 1	93.9	0.011	–0.086	1
		(15.9 / 28.7)	(0.005 / 0.015)	(–0.252 / –0.884)		(78.9 / 115.6)	(0.009 / 0.018)	(–0.062 / –0.102)	
	(-)	19.6		–0.611	1 → 2	113.3		–0.079	2
		(12.8 / 25.4)		(–0.331 / –1.109)		(89.7 / 136.8)		(–0.055 / –0.098)	

CE conditions: 50 mM sodium acetate buffer, pH 5.5, 50 μm, 50.2/40 cm fused-silica capillary; 20 °C, 25 kV; detection at 220 nm at the cathodic end.

the complexes of the (+)-enantiomer and (–)-enantiomer is much smaller in case of compound 10. It becomes apparent, that the effect of the mobilities on the observed enantioseparation will be much smaller for sulfoxide 10 so that no dependence of the migration order on the CD concentration is observed for this analyte. Moreover, the complexation constants of the enantiomers of sulfoxide 11 are lower compared to compound 10 which also rationalizes a smaller effect of the complexation strength on the enantioseparation. In case of compound 7 a large difference between the mobilities of the enantiomer-CD complexes was found, which explains the fact that the weaker complexed (–)-enantiomer migrated second because the higher anodic mobility of this complex.

HDMS-β-CD displayed opposite chiral recognition toward compounds 7 and 11 as a function of methylation of the benzamide group (Table 4). In case of sulfoxide 7 the (+)-enantiomer was bound stronger than the (–)-enantiomer, while for the *N*-methylated analog 11 the (–)-enantiomer was complexed stronger than (+)−11. Because the differences between the mobilities of the diastereomeric analyte-CD complexes were rather small, the enantioseparation of both sulfoxides is due to the opposite chiral recognition (binding strength) of the analytes by the CD as a function of the methylation of the benzamide nitrogen.

A different scenario applies comparing enantioseparations of compounds 10 and 11. The (+)-enantiomer of compound 10 with the bromo substituent in position 3 is complexed stronger than (–)−10, so that in comparison to compound 11 with the substituent in position 4 the chiral recognition of the respective enantiomers by HDMS-β-CD is opposite to each other. However, identical migration order was observed for both compounds. Thus, in case of sulfoxide 10, the observed enantiomer migration order is primarily due to the higher anodic mobility of the weaker bound (–)-enantiomer. Therefore, the overall enantioseparation is determined by the affinity of enantiomers towards the chiral selector in case of sulfoxides 7 and 11, while in the case of compound 10 it is determined by the mobilities of the transient diastereomeric associates. This illustrates one more time that both enantioselective separation mechanisms are effective in chiral CE.

3.2. MEKC using neutral CD derivatives

MEKC has the advantage that it enables to study the resolution ability of neutral chiral selectors such as uncharged CDs toward uncharged analytes as the sulfoxides in the present study. Separations were carried out in fused-silica capillaries applying 50 mM sodium borate butter, pH 9.0 as electrolyte solution. Using β-CD and γ-CD as selectors at concentrations of 20 and 50 mg/mL, SDS concentrations were varied between 20 and 100 mM. Based on the higher resolution observed at 100 mM SDS this concentration was selected for further experiments. Peaks were sharper upon addition of 10% (v/v) methanol. Subsequently, the neutral CDs were added at concentrations of 50 or 60 mg/mL, while carboxymethylated CDs were studied at 20 mg/mL. Native α-CD could not be investigated because a precipitate was formed when this CD was added to a BGE containing 100 mM SDS. The separation system of the individual CDs was not further optimized.

The results of the screening are summarized in Table S1. Only γ-CD and its derivatives HP-γ-CD and CM-γ-CD proved to be effective chiral selectors, while β-CD and its derivatives only resolved few sulfoxide enantiomers. Under the experimental conditions of the screening, HP-γ-CD and CM-γ-CD were the most universal selectors as these CDs at least partially separated most analytes. Nonetheless, the highest resolution of R_S = 5.5 was achieved in case of compound 9 and M-β-CD. None of the analytes was enantioseparated in the presence of HP-α-CD or HP-β-CD. It is interesting to note, that in the presence of β-CD derivatives the (+)-enantiomer of the sulfoxides migrated first except for compound 16 using CM-β-CD, while the (–)-enantiomer was detected first when γ-CD or one of its derivatives was applied as chiral selector.

4. Conclusions

The chiral (benzylsulfinyl)benzamides could be effectively separated by capillary electrophoresis in a pH 5.5 background electrolyte using CDs as chiral selectors. In contrast, neutral CD derivatives under MEKC conditions resolved only few of the analytes. Interestingly, randomly substituted charged CDs were by far more efficient compared to single isomer CDs with the exception of HS-β-CD, which separated even more analytes that its randomly substituted counterpart S-β-CD. Especially in the case of random substitution, the larger cavity of γ-CD derivatives appeared to favorably accommodate the analytes because the enantiomers of all analytes were separated in the presence of S-γ-CD, CM-γ-CD and Suc-γ-CD and 14 out of 16 in the case of TMA-γ-CD. Nonetheless, except for CM-α-CD, the other α-CD derivatives also effectively resolved the analyte enantiomers. In the vast majority of cases, the (+)-enantiomers migrated before the (–)-isomers independent of the CD or the structure of the analytes. Thus, structure-separation relationships could not be concluded in the present study. Further studies should enlarge the variety of the substituents as well as multiple substitutions on the (benzylsulfinyl)benzamide core structure. In addition, larger *N*-alkyl or *N*-aryl substituents in the benzamide moiety could be evaluated.

Analysis of the complexation constants and complex mobilities of selected analyte-CD pairs revealed that the (+)-enantiomers of compounds 7, 10 and 11 were always bound stronger by HDMS-α-CD, while the weaker (–)-enantiomer-CD complex exhibited the higher anodic mobility. The same was found for compounds 7 and 10 in the presence of HDMS-β-CD. This resulted in opposite enantiomer migration order in case of compounds 7 and 10 in the presence of both CDs illustrating that the effective migration order may result from either stereoselective complexation or differences of the mobility between the diastereomeric enantiomer-CD complexes. This may also lead to a reversal of the migration order of the enantiomers as a function of the CD concentration as observed for compound 11 in the presence of HDMS-α-CD. HDMS-β-CD complexed the (–)-enantiomer of sulfoxide 11 stronger resulting in the opposite migration order compared to compound 10. Summarizing, as observed for other analytes [39,41], the enantiomer migration order may be dominated by either stereoselective complexation (expressed as complexation constants) or by the mobilities of the diastereomeric complexes. A general prediction of the mechanism responsible for analyte migration in enantioselective CE cannot be concluded from the migration order alone.

CRediT authorship contribution statement

Mari-Luiza Konjaria: Methodology, Investigation, Formal analysis, Writing – original draft. **Rusudan Kakava:** Investigation, Writing – review & editing. **Alessandro Volonterio:** Writing – review & editing. **Bezhan Chankvetadze:** Conceptualization, Writing – review & editing. **Gerhard K.E. Scriba:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463027.

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