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Enantiomer separation by pressure-supported electrochromatography using capillaries packed with a permethyl-β-cyclodextrin stationary phase

D. Wistuba*, H. Czesla, M. Roeder, V. Schurig*

Institute of Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

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Abstract

Efficient enantiomer separation by pressure-assisted, micro-packed capillary electrochromatography (CEC) has been carried out using a permethyl-β-cyclodextrin-modified silica support (PM–β-CD–silica). When comparing this method with micro-packed-high-performance liquid chromatography in the single-column-mode, CEC displays higher column efficiencies (about three times higher theoretical plate numbers at comparable elution times). The pressure support (about 10 bar), applied to avoid bubble formation, has a negligible influence on elution times in CEC. The influence of the type and composition of organic modifiers is described.

Keywords: Enantiomer separation; Electrochromatography; Chiral stationary phases, LC; Barbiturates

1. Introduction

Since its inception by Pretorius et al. in 1974 [1], capillary electrochromatography (CEC) has developed into a promising contemporary separation technique. Yet, in comparison to achiral separations, only a limited number of chiral separations have been reported thus far.

Enantiomer separation by CEC can be performed in two modes:

(i) In open-tubular electrochromatography (OT-EC), the internal capillary wall is coated with the chiral stationary phase (CSP). The first enantiomer separation of a number of non-steroidal anti-inflammatory drugs (NSAIDs) by OT-EC was demonstrated by Mayer and Schurig [2–5] with open tubular columns (50 μm I.D.) coated with immobilized Chirasil-Dex, a chiral dimethylpolysiloxane consisting of covalently linked permethyl-β-cyclodextrin. Subsequently, a ''unified enantioselective chromatographic'' approach involving gas chromatography (GC), supercritical fluid chromatography, OT-LC and OT-EC, using a single Chirasil-Dex-coated column, was realized [6–8]. A related approach was subsequently applied by Armstrong et al. [9] for the enantiomer separation of mephobarbital by OT-EC. Likewise, Sezemán and Ganzler [10] reported on the separation of the enantiomers of epinephrine on a γ-cyclodextrin-coated capillary. Neat cellulose derivatives were employed as solid non-immobilized CSPs by Francotte and Jung [11]. Unfortunately, the column lifetime was very short.

(ii) In micro-packed-CEC, capillaries are filled with typical chiral high-performance liquid chroma-

*Corresponding author.

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tography (HPLC) packing materials. Thus, enantiomer separation by micro-packed-CEC was performed by Li and Lloyd [12] using protein stationary phases such as α_1-acid glycoprotein (AGP) and by Lloyd et al. [13] using human serum albumin (HSA). Cyclodextrin-immobilized stationary phases were also employed in CEC. Lelievre et al. [14] separated chlorthalidon and mianserin using capillaries packed with hydroxypropyl-β-cyclodextrin-bonded silica. The enantiomers of hexobarbital and benzoin (neutral solutes) and amino acid derivatives (anionic solutes) were resolved on a β-cyclodextrin stationary phase by Li and Lloyd [15]. Several researchers also used imprinted polymers as chiral monoliths in CEC [16–18]. Recently, Wolf et al. [19] immobilized naproxen-derived and Whelk-O CSPs on silica and used these CSPs with excellent chiral separation factors, α, for enantiomer separation in CEC. Cyclodextrin-derived selectors have also been used as mobile-phase additives in CEC for enantiomer separation [14,20].

Here, we report on the enantiomer separation by pressure-supported CEC using capillaries packed with permethylated β-cyclodextrin-modified silica and compare the results obtained with those using micro-packed HPLC. The influence of organic modifier and the pressure support is demonstrated.

2. Experimental

2.1. Material and methods

Dimethoxy-(3-mercaptopropyl)methylsilane and 9-borabicyclo[3.3.1]nonane (9-BBN) (0.5 *M* in tetrahydrofuran) were purchased from Fluka (Deisenhofen, Germany). Silica (Nucleosil, 5 μm, 300 Å pore size) was obtained from Grom (Herrenberg, Germany) and derivatized with dimethoxy-(3-mercaptopropyl)-methylsilane to (3-mercaptopropyl)-methyl-silica according to ref. [21]. The degree of substitution of the silica, as determined by elemental analysis, was typically 0.7 nmol/g. Mono-6-(octen-7-enyl)-permethyl-β-cyclodextrin was synthesized as described previously [22] and was attached to (mercaptopropyl)methyl-silica as follows. To a solution containing 53.4 mg (0.035 mmol) of mono-6-(octen-7-enyl)-permethyl-β-cyclodextrin in 5 ml of dry toluene, 50 mg of (3-mercaptopropyl)methyl-silica were added at 0°C under nitrogen. A 25-μl volume of 9-BBN was added and the mixture was allowed to warm up to room temperature. After 24 h, an additional amount of 9-BBN (25 μl) was added. After 3 h, the mixture was filtered and the modified silica was washed successively with diethyl ether, methanol, *n*-hexane, methanol and diethyl ether. After drying, the permethyl-β-cyclodextrin-modified silica support (PM–β-CD–silica) shows a substitution degree of 0.1 mmol selector per g silica, as judged from elemental analysis. Fused-silica capillaries (100 μm I.D., Ziemer, Mannheim, Germany) were packed and conditioned with a HPLC pump (Sykamp, Gilching, Germany) as described by Behnke et al. [23]. The capillaries were packed in the following steps. The first step in the preparation is to produce an initial frit by tapping one end of the capillary into silica (10 μm) wetted with water, drying for 12 h at room temperature and sintering with a laboratory-made heater. A slurry of PM–β-CD–silica in methanol (20 mg packing material in 60 μl methanol) was ultrasonicated and pumped into the capillary (placed in an ultrasonic bath) at 380 bar for 1 h using a HPLC pump (Sykamp). After flushing with water, the final outlet frit is made by sintering the packing material at a distance of approximately 15 cm from the end of the capillary. The initial end frit is cut off and the capillary emptied up to the outlet frit by flushing with water. Finally, the inlet frit was sintered. A window for on-column UV detection was prepared using conc. nitric acid.

2.2. Pressure supported micro-packed-CEC and micro-packed-HPLC

Pressure supported micro-packed-CEC was performed with a capillary electrophoresis system (Grom) combined with a gradient pump (Sykamp) for flushing and pressurizing the packed capillaries according to ref. [22]. The UV detector was operated at 230 nm. To avoid bubble formation, all runs were performed with a pressure of 10–15 bar at the injection buffer vial. Micro-packed-CEC and micro-packed-HPLC were carried out with 100 μm I.D. capillaries packed with PM–β-CD–silica at an effective length (to the detection window) of 25 or 23.5

cm. The overall length of the capillaries was 40 cm. Samples were injected by the pressure mode (5–10 s, 100 bar). Mobile phases were prepared from HPLC-grade methanol and acetonitrile (Merck, Darmstadt, Germany) and phosphate buffer (5 m*M*, pH 7.0) and were degassed using helium.

3. Results and discussion

Enantiomer separation by micro-packed-CEC and micro-packed-HPLC has been performed on permethyl-β-cyclodextrin that was covalently bonded via a thioether-spacer to silica (cf. Fig. 1). PM–β-CD–silica was prepared by the addition of the thiol group of a modified silica to the double bond of mono-6-(oct-7-enyl)-permethyl-β-cyclodextrin (it should be noted that, depending on the reaction conditions, a competitive monoalkylation of cyclodextrin may also occur at the 2-position. Therefore, the structure depicted in Fig. 1 is putative).

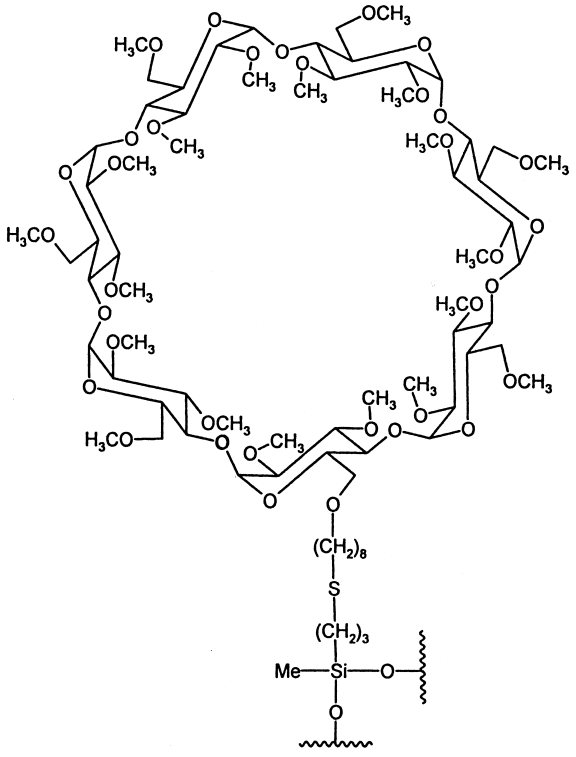

Fig. 1. PM–β-CD-silica.

Permethyl-β-cyclodextrin is well established as an excellent chiral selector in GC or HPLC. Its use in micro-packed-CEC has not been reported previously. In Fig. 2, a representative example is shown. One of the main obstacles in micro-packed-CEC is bubble formation inside the column. This effect usually leads to the breakdown of the current and the electroosmotic flow (EOF) and it can be circumvented by pressurizing the flow system using a HPLC pump coupled to the inlet buffer vial. In this way, micro-packed-CEC can be performed under stable and reproducible conditions. Another advantage of pressurizing is the possibility of flushing the column, e.g. for changing the eluent. Fig. 3 shows the influence of the pressure support on the enantiomer separation of mephobarbital by micro-packed-CEC. The elution time in enantiomer separation by pressure-assisted micro-packed-CEC is clearly dominated by a substantial contribution of the EOF. This is demonstrated in the following way. Comparing micro-packed-HPLC at 10 bar (without applied

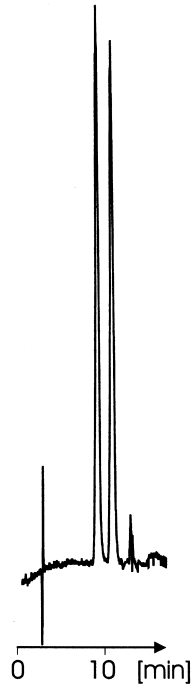

Fig. 2. Enantiomer separation of hexobarbital by CEC on PM–β-CD–silica. Conditions: 25 cm (overall length, 40 cm)×100 μm; phosphate buffer (5 m*M*, pH 7.0)–acetonitrile (9:1, v/v); 20 kV; 10 bar; UV detection at 230 nm.

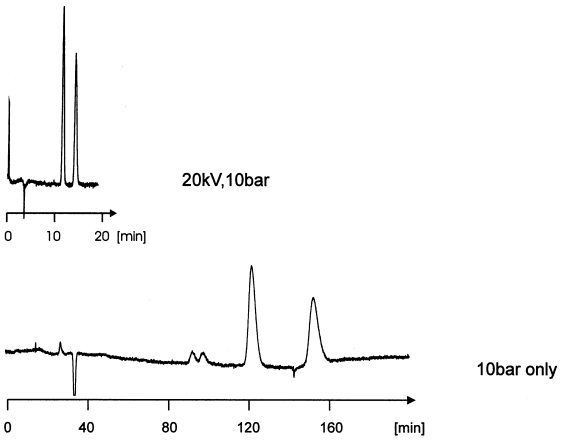

Fig. 3. Influence of pressure support on the enantiomer separation of mephobarbital. Conditions: 23.5 cm (overall length, 40 cm)× 100 μm capillary packed with PM–β-CD–silica; phosphate buffer (5 m*M*, pH 7.0)–methanol (4:1, v/v); UV detection, 230 nm; upper chromatogram, 20 kV, 10 bar; lower chromatogram, 10 bar (the small peaks before mephobarbital were caused by a memory effect).

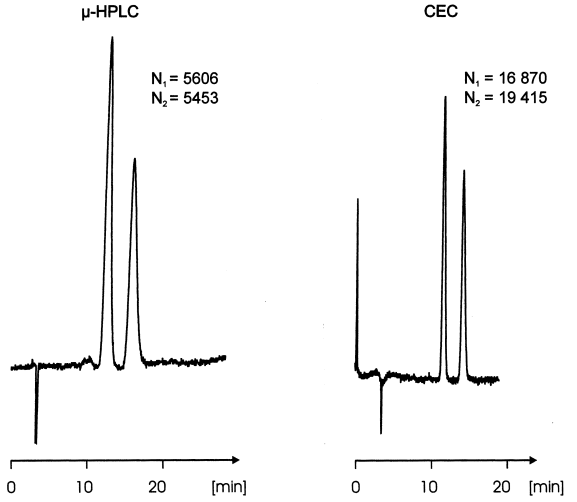

Fig. 4. Enantiomer separation of mephobarbital by micro-packed-HPLC and CEC. Conditions: 23.5 cm (overall length, 40 cm)×100 μm capillary packed with PM–β-CD–silica; phosphate buffer (5 m*M*, pH 7.0)–methanol (4:1, v/v); UV detection, 230 nm; CEC, 20 kV; 10 bar; micro-packed-HPLC, 140 bar.

voltage, Fig. 3, bottom) with pressure-assisted micro-packed-CEC at 10 bar and a voltage of 20 kV (Fig. 3, top) a ten-times-longer elution time was found for the first method for comparable chiral separation factors (α_{LC}=1.35; α_{CEC}=1.31) and resolutions ($R_{S\ (LC)}$=3.7; $R_{S\ (CEC)}$=3.39).

A further advantage of coupling a HPLC pump to the capillary electrophoresis system is the possibility of switching between the micro-packed-CEC and the micro-packed-HPLC mode. This allows for the direct comparison of the two methods with one single capillary column (unified approach). A comparison of the enantiomer separation of mephobarbital by micro-packed-CEC and micro-packed-HPLC using the same column is shown in Fig. 4. Both efficiency and resolution are clearly higher in the CEC mode at comparable elution times. For the enantiomers of mephobarbital, 5606 and 5453 theoretical plates per metre were obtained by micro-packed-HPLC, compared to 16 870 and 19 415 theoretical plates per metre by micro-packed-CEC. While the chiral separation factor, α, remained nearly unchanged, the resolution R_S increased from 2.08 in micro-packed-HPLC to 3.39 in micro-packed-CEC.

The amount of modifier in the mobile phase strongly influences the elution time, the resolution, R_S, and the theoretical plate number, N. Methanol and acetonitrile were used as organic modifiers, with the best enantioselectivity being observed with the former (e.g., α=1.48 vs. 1.27 for mephobarbital). Furthermore, by adjusting a comparable elution time for the enantiomer separation of mephobarbital, a higher resolution, R_S, was achieved for methanol

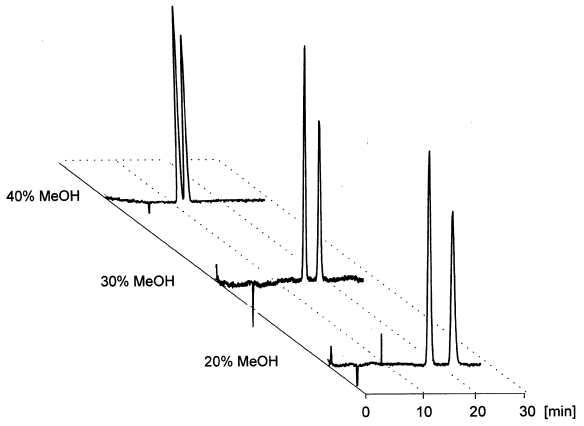

Fig. 5. Enantiomer separation of mephobarbital by CEC. Conditions: 23.5 cm (overall length, 40 cm)×100 μm capillary packed with PM–β-CD–silica; buffer: phosphate (5 m*M*, pH 7.0), 15 kV, 10–15 bar; UV detection, 230 nm; modifier, 20, 30 and 40% methanol.

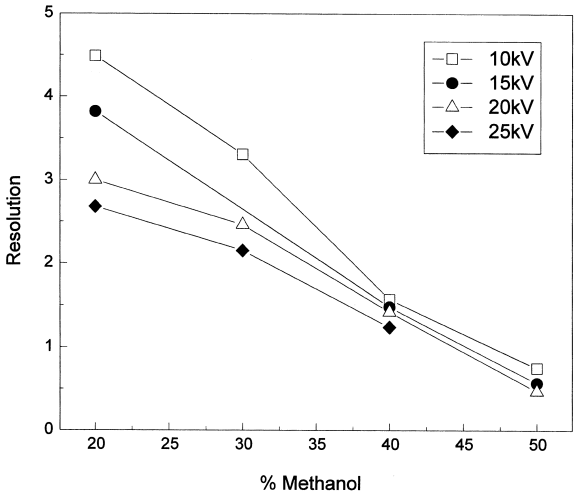

Fig. 6. Dependence of the resolution, R_S, of mephobarbital on the amount of methanol at various voltages. Chromatographic conditions as in Fig. 5.

than acetonitrile. Thus, with a 25-cm packed capillary, 20% methanol produces an R_S of 3.68 (elution time of the first enantiomer, 9.8 min), while 10% acetonitrile leads to a resolution of only $R_S=2.57$ (elution time of the first enantiomer, 9.1 min). As shown in Fig. 5 and Fig. 6, the enantiomer separation of mephobarbital was studied with methanol–phosphate buffer systems of different compositions: 20:80, 30:70, 40:60 and 50:50 (% methanol:%

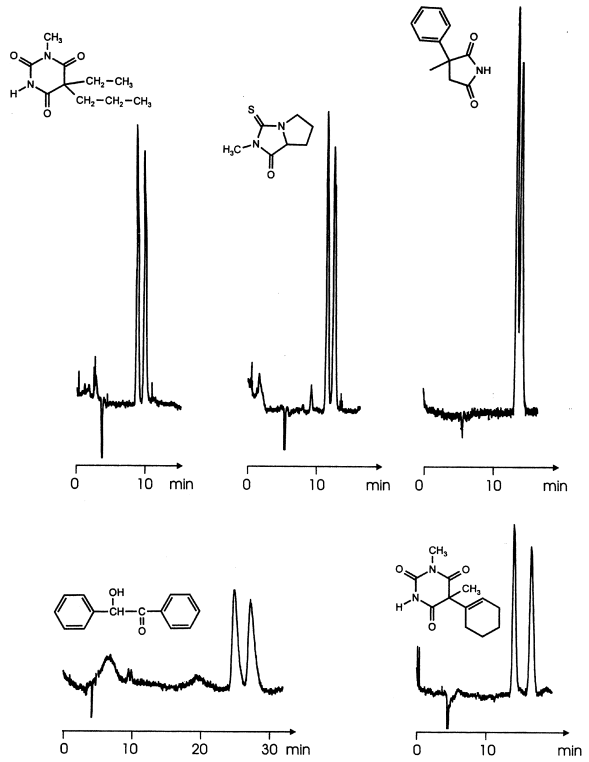

Fig. 7. Enantiomer separation of 5-ethyl-1-methyl-5-(*n*-propyl)barbituric acid, MTH-proline, α-methyl-α-phenylsuccinimide, benzoin and hexobarbital on a 23.5-cm (overall length, 40 cm)× 100 μm capillary packed with PM–β-CD–silica by CEC. Conditions: phosphate buffer (5 m*M*, pH 7.0)–methanol (4:1, v/v); 10–15 bar, 15 kV (benzoin, 20 kV).

Table 1
Enantiomer separation by pressure supported micro-packed CEC on PM–β-CD–silica

Substrate	Voltage (kV)	t_1/t_2 [a]	N_1/N_2 [b]	α	R_S
Mephobarbital	20	10.6/13.2	17 565/17 695	1.31	3.00
Hexobarbital	15	14.0/16.3	20 068/21 890	1.25	2.56
Pentobarbital	15	21.0/22.0	25 215/27 849	1.06	0.89
1-Methyl-5-(2-propyl)-5-(*n*-propyl)barbituric acid	15	19.9/21.0	21 013/15 894	1.07	0.85
5-Ethyl-1-methyl-5-(*n*-propyl)barbituric acid	15	13.1/15.1	18 734/19 714	1.24	2.3
Benzoin	20	24.5/26.7	18 739/16 156	1.11	1.37
α-Methyl-α-phenylsuccinimide	15	13.0/13.5	35 075/28 354	1.09	1.01
Glutethimide	20	18.7/20.3	17 425/15 543	1.11	1.35
MTH-proline	15	11.0/11.9	23 341/21 351	1.17	1.52
Methyl mandelate	10	12.6/13.2	43 509/40 653	1.09	0.78

Conditions: 23.5 cm capillary (total length, 40 cm)×100 μm; phosphate buffer (5 m*M*, pH 7.0)–methanol (4:1, v/v); 10–15 bar.
[a] *t* (min); [b] per metre.

buffer) and voltages (10, 15, 20 and 25 kV). As expected, the decrease in the methanol content caused an increase in the elution time, t_R, the theoretical plate number, N, the chiral separation factor, α, and the resolution, R_S. An exception to this finding was obtained using 40% methanol at a voltage of 10 kV. In this case, surprisingly, the first peak of the enantiomeric pair was the broader one and, thus, the theoretical plate number was much lower than for the second peak. To rationalize this phenomenon, further investigations will be required. A 20% methanol content produced the highest resolution, $R_S=4.49$, at a voltage of 10 kV and an elution time of 26.4 min for the first-eluted enantiomer. To shorten the elution time, a higher voltage can be applied. A voltage of 20 kV leads to an elution time for the first enantiomer of 10.6 min and a R_S of 3.0. The above-mentioned conditions were chosen for the enantiomer separation of various chiral compounds, such as mephobarbital, hexobarbital, pentobarbital, 5-ethyl-1-methyl-5-(*n*-propyl)-barbituric acid, 1-methyl-5-(2-propyl)-5-(*n*-propyl)-barbituric acid, benzoin, α-methyl-α-phenylsuccinimide, glutethimide, methylthiohydantoin (MTH)-proline and methyl mandelate. Chromatographic data are summarized in Table 1 and representative examples are shown in Fig. 7.

4. Conclusions

Micro-packed capillary electrochromatography employing PM–β-CD–silica as the chiral stationary phase has been applied successfully for the efficient enantiomer separation of various racemates. The separation efficiency in pressure-assisted micro-packed-CEC is always higher than that with micro-packed-HPLC at similar elution times. The full potential of micro-packed-CEC may be realized in the future with a smaller diameter particle packing. The micro-packed-CEC and micro-packed-HPLC mode of operation can be performed with the same column and the same equipment (unified approach).

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