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Chiral separation by a monofunctionalized cyclodextrin derivative: From selector to permethyl-β-cyclodextrin bonded stationary phase

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ABSTRACT

Preparation of (6-monoureido-6-monodeoxy) permethylated β-cyclodextrin bonded chiral stationary phase from permethylated 6-monoamino-6-monodeoxy-β-cyclodextrin is described. The optimized chiral stationary phase was evaluated by using HPLC separation of racemates of coumarin derivatives. Column characterization was performed by solid-state ^{13}C, ^{15}N, ^{29}Si NMR using cross-polarization at the magic angle spinning. The development process was supported by CE experiments where the complex formation between cyclodextrins and warfarin was investigated. The results demonstrate good enantio-discrimination for coumarin derivatives.

1. Introduction

Cyclodextrin (CD) technology offers various solutions for the separation of drug enantiomers in chemical, pharmaceutical and biological research [1]. Yet, there appears to be a continuous challenge in finding optimal conditions for the separation within reasonable analysis time. Most CD derivatives used in chiral separations are mixtures of high variety of randomly substituted homologues and regioisomers. Hence, reproducibility is often related to batch-to-batch reproducibility of chemical synthesis of the applied CDs. Even small differences in the degree of substitution or isomer distribution can influence the result of the separations [2–6]. Application of single isomer cyclodextrin derivatives turns advantageous in the batch-to-batch reproducibility of reactions. However, in the preparation of single isomer CD derivatives it is difficult to achieve the desired purity at reasonable yield in a series of selective, multi-step reactions [7].

Our aim was to apply permethylated 6-monoamino-6-monodeoxy-β-cyclodextrin [8] (PMMABCD) as chiral selector for the development of a permethylated 6-monoureido-6-monodeoxy β-cyclodextrin (UPMBCD) silica-bonded chiral stationary phase (CSP) and demonstrate its chromatographic properties. Warfarin and related coumarin derivatives (see Fig. 1) were chosen to test enantiomeric separation properties of the new CSP.

2. Experimental

2.1. Materials

Sodium azide-1-^{15}N, 3-isocyanatopropyltriethoxysilane, hexamethyldisilazane, n-hexane, trimethylchlorosilane, methanol, DMF, toluene, acetone, tetrahydrofurane, pyridine, racemic warfarin sodium-chlatrate and achiral test substances (phenol, toluene, aniline, ethyl-benzene) were purchased from Sigma–Aldrich (Milwaukee, USA). Pd/C (10% Pd), acetonitrile (HPLC gradient grade), phosphoric acid, boric acid, acetic acid and triethylamine (analytical grade) were from Merck (Darmstadt, Germany). Hypersil Silica 5 μm mean pore diameter 100 Å, surface area 280 m^2/g, pore volume 0.61 cm^3/g (high-purity, "B"-type silica) was from Thermo Hypersil Keystone (Runcorn, Cheshire, UK). Nucleodex-β-PM column was from Macherey-Nagel (Düren, Germany). CD-Screen column (containing 4-nitrophenyl groups) is a product of ChiroQuest Ltd. (Budapest, Hungary). Empty 250 mm × 4.0 mm I.D. HPLC columns were from Sugiyama Shoji Co., Ltd. (Yokohama, Japan). 6^I-O-tosyl-β-cyclodextrin, permethylated 6-monoamino-6-monodeoxy-β-cyclodextrin and heptakis (2,3,6-tri-O-methyl)-

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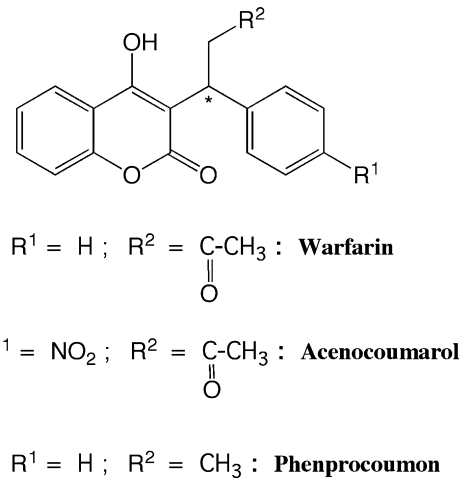

Fig. 1. The molecular structure of coumarin-type anticoagulants.

β-cyclodextrin (TRIMEB) are products of CycloLab Ltd. (Budapest, Hungary). *rac*-Acenocumarol and *rac*-phenprocoumon were from Promochem (Wesel, Germany). (*S*)-warfarin and (*R*)-warfarin enantiomers were prepared as previously described [9].

2.2. Synthesis of UPMBCD-silica

The preparation of ^{15}N-labelled PMMABCD has been described [10]. In order to overcome sensitivity problems in natural abundance for ^{15}N NMR experiments, 50 at% ^{15}N-labeling of the selector was performed starting from Na^{15}N=N=N. The silylating agent was prepared by the reaction of PMMABCD (2.1 g, 1.5 mmol) with equimolar 3-isocyanatopropyltriethoxysilane (0.37 ml, 0.37 g, 1.5 mmol) in distilled, dry tetrahydrofurane (10 ml) at room temperature. Elemental analysis of silylating agent found: C, 52.64; H, 8.15; N, 1.61; Si, 1.59%. Calculated: C, 52.04; H, 8.01; N, 1.69; Si, 1.69%. After removing the solvent the product was used without further purification in the bonding process.

6.6 g silica was suspended in 50 ml toluene with ultrasonication, traces of water were removed by azeotropic destillation. 1 g of the silylating agent was dissolved in 5 ml pyridine and this solution was added dropwise to the cool suspension of the silica, while vigorous stirring was applied. The reaction mixture was gently stirred at 105 °C for 20 h. The product was filtered, washed with toluene, acetone and methanol, in this order. The surface coverage of the obtained dry silica gel (7.06 g) was 0.30 μmol/m^2. In order to improve peak shape an end-capping procedure was performed by applying hexamethyldisilazane (0.7 ml, 0.54 g, 3.3 mmol) and trimethylchlorosilane (0.7 ml, 0.59 g, 5.4 mmol) in dry tetrahydrofurane (50 ml) as silylating agents at 50 °C for 1 h. The filtrate was washed with acetone, 25% (v/v) methanol and acetone again in order to remove ammonium chloride.

2.3. Chromatographic experiments

The isomeric purity of the PMMABCD selector was determined by HPLC with Evaporative light scattering detector (Polymer Laboratories Ltd., Church Stretton, UK). A special HPLC column developed for cyclodextrin analysis (CD-Screen) thermostated to 25 °C was used. The mobile phase was acetonitrile—0.1 M triethylamine formate buffer (pH 4) with solvent gradient: the acetonitrile content increased from 5% to 90% during 20 min. The flow rate was 1 ml/min. The isomeric purity of PMMABCD was 96.5%, the detected impurity was identified as permethyl-β-cyclodextrin. UPMBCD column performance was evaluated under reversed phase conditions. Agilent 1100 HPLC system with diode array detector at 283 nm was used for the liquid chromatographic measurements. The test solution for analytical separation was racemic warfarin (3×10^6 M) dissolved in 20% aqueous methanol. The (*S*)-warfarin peak was identified with the pure (*S*)-enantiomer.

2.4. Capillary electrophoresis

Capillary electrophoresis was performed with an Agilent Capillary Electrophoresis ^{3D}CE system applying bare fused silica capillary of 64.5 cm total and 56 cm effective length with bubble cell and 50 μm I.D. (Agilent Technologies, Santa Clara, CA, USA). On-line UV absorption at 209 and 308 nm was detected by DAD UV–vis detector. The capillary was thermostated at 20 °C. Britton–Robinson buffer prepared from 40 mM borate, 40 mM acetate and 40 mM phosphate in a mixture of 1:2:2 (v/v/v) was applied as background electrolyte (BGE) at pH values adjusted by NaOH. Between measurements, the capillary was rinsed by 1 M NaOH, 0.1 M NaOH and distilled water, subsequently for 1 min and with BGE for 8 min. Warfarin samples were dissolved in absolute ethanol and further diluted with distilled water. Racemic warfarin (2×10^{-6} M) was spiked with the pure (*R*) enantiomer (10^{-6} M) and injected by 5×10^3 Pa pressure for 3 s. Runs were performed in the positive-polarity mode with 30 kV. The quality of the selector can be characterized by selectivity (α) and resolution (R_S) of the enantiomers to be separated. The mentioned parameters are given by the following equations [11]:

$$\alpha_{1,2} = \frac{\mu_1}{\mu_2} \quad (1)$$

$$R_S = \frac{1.18(t_1 - t_2)}{w(0.5)_1 + w(0.5)_2} \quad (2)$$

where μ is the apparent mobility of the enantiomers (1,2 in lower index), $w(0.5)$ is the peak width at half height, t is the migration time. In order to calculate the apparent complex stability constant (K_i) of warfarin enantiomers to the selector CD, the mobilities of the analytes in the absence ($\mu_{0,i}$) and in the presence ($\mu_{x,i}$) of CD in five concentrations in the range of 5–20 mM and 15–40 mM (c_x) for PMMABCD and TRIMEB, respectively, 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 [12].

2.5. Nuclear magnetic resonance spectroscopy

Solid-state ^{13}C, ^{15}N, ^{29}Si NMR experiments were performed on a 600 MHz Varian NMR SYSTEM^{TM} using zirconia rotors in Varian/Chemagnetics narrow bore 3.2 mm HXY triple resonance MAS probe operated in HX double resonance mode. Cross-polarization at the magic angle spinning (CP/MAS) was applied to enhance ^{13}C, ^{15}N and ^{29}Si sensitivity. MAS rates of 7 kHz were chosen and proton high power decoupling (SPINAL scheme) was applied during 20 ms acquisition time. CP contact times of 1, 2, 3 ms were applied for the ^{13}C, ^{15}N and ^{29}Si experiments, respectively. Repetition delay was 5 s. Spectral windows of ^{13}C: 125 kHz, ^{15}N: 69 kHz, ^{29}Si: 62.5 kHz were used and digital resolutions varied in the range 1.9–3.8 Hz. Carbon chemical shifts are referenced to adamantane (δ = 38.6, 29.5 ppm). Nitrogen-15 shifts are referenced to the nitromethane chemical shift scale (δ = 0 ppm) using solid ^{15}N-glycine as a secondary reference (δ = −350 ppm). ^{29}Si shifts are referred to tetramethylsilane (δ = 0 ppm).

The solid-state NMR measurements have been performed on dried silica samples.