

Chiral Separation of Basic Compounds on Sulfated β -Cyclodextrin-Coated Zirconia Monolith by Capillary Electrochromatography

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Sulfated β -cyclodextrin (SCD)-coated zirconia monolith was used as the chiral stationary phase in capillary electrochromatography for enantiomeric separation of basic chiral compounds. SCD adsorbed on the zirconia surface provided a stable chiral stationary phase in reversed-phase eluents. Retention, chiral selectivity and resolution of a set of six basic chiral compounds were measured in eluents of varying pH, composition of methanol and buffer. Optimum mobile phase condition for the separation of the compounds was found to be methanol content of 30%, buffer concentration of 30 mM and pH of 4.0.

Key Words : Chiral separation, Capillary electrochromatography, Zirconia monolith, Sulfated β -cyclodextrin

Introduction

Capillary electrochromatography (CEC) on monolithic columns are becoming attractive alternative to particle-packed columns in HPLC and electrochromatography.¹⁻³ Monolithic columns are devoid of problems and difficulties associated with packed capillary columns, including burdensome packing of stationary phase particles in a capillary and frits that cause formation of air bubbles during the analysis which results in reduction of separation efficiency, and break easily.⁴⁻⁷ The monolithic columns allow fast mass transfer at lower pressure drops, enabling much faster separations. The continuous monolithic bed in the capillary column also allows high linear velocities that enable high throughput screening and fast separations of enantiomers.⁸ CEC has been increasingly utilized in studies on the development and evaluation of separation methods including chiral separations⁹⁻¹¹ as it provides high efficiency because of the flat profile of electroosmotic flow (EOF) to pump the mobile phase and ability to separate charged as well as uncharged compounds through electrophoresis and chromatographic separation.¹¹ Several reviews have been reported on enantioseparations using CEC as a separation technique.^{10,12,13}

Zirconia is a viable alternative to silica as the support, due to its unique and extraordinary chemical, mechanical and thermal stability.¹⁴⁻¹⁶ Zirconia particles are stable over the entire pH range and have been used for prolonged periods at temperatures up to 200 °C. The unique surface chemistry of zirconia extends different applications for its use in chromatography.^{14,17} A number of zirconia-based CSPs have been evaluated in HPLC¹⁸⁻²² and CEC.²³⁻²⁹

Charged cyclodextrins have been widely used as chiral resolving agents in CE.³⁰ Among them sulfated β -cyclodextrin (SCD) has been widely employed in chiral separation by CE,^{31,32} HPLC^{33,34} and CEC.³⁵ Ye *et al.* reported a SCD-modified silica particle-packed column for chiral separation in CEC in which SCD was dynamically adsorbed on a strong anion-exchanger packing based on silica.³⁶

In this work, we report preliminary results of enantiomer separation by CEC on SCD-adsorbed zirconia monolithic column (SCD-ZM). On the zirconia surface Brönsted acid sites, Brönsted base sites, and Lewis acid sites are present.¹⁴ The Lewis acid sites, which are not found on silica, are utilized for adsorbing strong Lewis-basic sulfate groups of SCD to obtain a stable adsorbed layer of the chiral selector. This allows the SCD coating on the surface of ZM without modifying the surface with cationic functional moieties as in silica-based packing materials.³⁶ Adsorbed layer of Lewis-basic ligands on the zirconia has been shown to be quite stable in typical reversed-phase eluents.³⁷ It is expected the cathodic EOF generated by the combined negative charges from the sulfate groups of adsorbed SCD and the dissociated zirconol groups of the ZM will provide faster chiral resolutions. We have investigated chiral separation of a set of six basic compounds on the SCD-ZM in aqueous organic eluents of varying pH, organic and electrolyte compositions to evaluate the performance of the column.

Experimental

Materials. Fused silica capillaries (75 μ m I.D., 365 μ m O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Zirconium butoxide, acetic acid (AA), triethylamine (TEA), polyethylene glycol (PEG) (MW = 10,000 g mol⁻¹), sodium hydroxide and sulfated β -cyclodextrin (SCD) were purchased from Aldrich (Milwaukee, WI, USA). All reagents used were reagent grade or better having higher than 99% purity. HPLC-grade methanol (MeOH) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Water was purified with an Elgastat UHQ water purification system (Bucks, UK). Chiral compounds including atropine (ATR), homatropine (HOM), propranolol (PRO), nadolol (NAD), oxprenolol (OXp) and benalaxyl (BEN) were of the highest-purity available from Aldrich (Milwaukee, WI, USA) or TCI (Tokyo, Japan).

Instrumentation. An Agilent HP 3DCE System (Palo Alto,

CA, USA) equipped with a diode-array UV detector, a ± 30 kV high voltage power supply and an external nitrogen pressure was used for the CEC separations. Instrument control and data collection were performed with the ChemStation software. The morphology of the zirconia monoliths was examined by a field emission scanning electron microscope (FE-SEM S-4100, Hitachi, Japan). A syringe pump from Cole-Parmer (Vernon Hills IL, USA) was used to inject the SCD solution into the zirconia monolithic capillary.

Column Preparation. Zirconia monolithic capillary column with total length of 35 cm and monolithic bed length of 25 cm was prepared and characterized by SEM according to the method reported earlier.²⁵ The detection window was created right after the monolith bed by removing the protective coating with a razor blade. To perform the SCD coatings on the surface of ZM bed, the ZM capillaries were initially washed with MeOH and then with water. Then, an aqueous SCD solution with a concentration of 100 mg/mL was passed through the capillaries for 2 h at a flow rate of $5 \mu\text{L min}^{-1}$ using a syringe pump to coat the entire zirconia monolithic bed of the capillary column. The capillary was left in a GC oven at 90°C overnight for drying. The capillary was then rinsed with methanol and mobile phase, respectively.

Chromatography. CEC separations were carried out at 25°C with an applied voltage of 5 kV and monitored at 214, 254 and 280 nm. An external pressure of 10 bars was applied to both buffer reservoirs. The mobile phases were mixtures of MeOH and AA-TEA buffer of varying pH in different compositions. In order to prevent loss of the adsorbed SCD by dissolution the eluents SCD was added to the eluent at concentration of 20 mg/mL. These mobile phases were filtered through a nylon membrane filter of $0.2\text{-}\mu\text{m}$ pore size and degassed prior to use. The monolithic capillary columns were equilibrated for 8–10 h in order to reduce baseline noise before CEC runs. Samples dissolved in the mobile phase were injected electro-kinetically at 15 kV for 3 s. Separations were done at the applied voltage of 15 kV. Migration times of two consecutive injections were in agreement within 3%. Fresh mobile phase was replenished after each run of sample. The dead time was measured by injecting acetone.

Results and Discussion

Zirconol groups on the surface of zirconia monolith (ZM) can undergo Brönsted acid-base reactions [$\text{ZrOH} \rightleftharpoons \text{ZrO}^- + \text{H}^+$ (1); $\text{ZrOH}_2^+ \rightleftharpoons \text{ZrOH} + \text{H}^+$ (2)]. Net zero charge on the zirconia surface is observed at pH between 5 and 6,¹⁶ and thus the direction of EOF can be either cathodic above this pH or anodic below this pH.³⁸ Figure 1 shows variation of electroosmotic mobility (μ_{eo}) measured by acetone on the native and SCD-ZM with pH. Cathodic EOF was invariably observed for both bare and modified zirconia regardless of pH, indicating adsorption of anionic SCD on the ZM surface. The magnitude of EOF increases with pH as more zirconol groups dissociate according to Eq. (1) to increase negative surface charges, resulting in an increasing EOF. The magni-

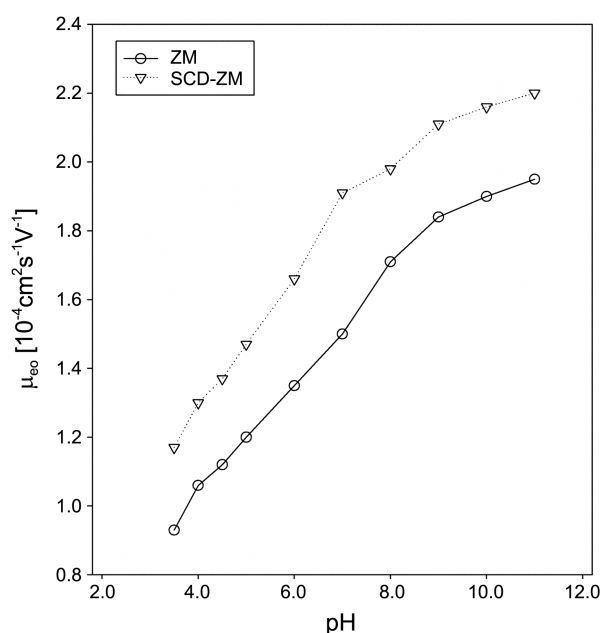


Figure 1. Electroosmotic flow with pH. Conditions: mobile phase, 30/70 (v/v) MeOH/AA-TEA buffer (30 mM, pH 4.0) containing 20 mM SCD; column; $50 \mu\text{m ID} \times 35 \text{ cm}$ length, 25 cm monolith bed; reservoir pressure, 10 bar; voltage, 15 kV; injection, 15 kV, 3 sec; temperature, 25°C .

tude of EOF on SCD-ZM is greater than that on the native ZM due to the additional negative charges from the sulfate groups of adsorbed SCD.

The effect of MeOH content in the eluent on chiral separation was examined by varying the MeOH content from 20 to 35% for a typical analyte, ATR, and resulting chromatograms are shown in Figure 2 along with enantioselectivity (α), resolution factor (R_s) and the number of the theoretical plate for the first-eluting enantiomer (N_1). Migration time

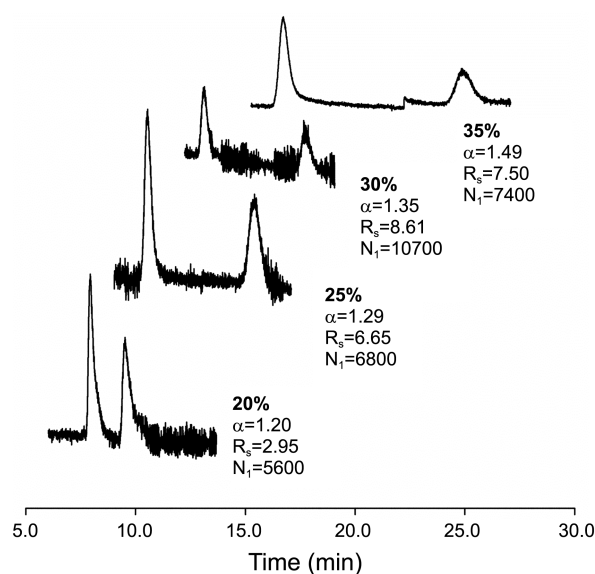


Figure 2. Influence of methanol content on enantioseparation of atropine. Mobile phase, MeOH/AA-TEA buffer (30 mM, pH 4.0) with 20 mM SCD. Other conditions are as shown in Figure 1.

and α for the analyte were increased as the MeOH content was increased. Migration time was increased due to decreasing ϵ/η ratio of the eluent with increasing MeOH content, thereby causing EOF to decrease. The value of this ratio computed using the ϵ^{39} and η^{40} data decreases with increasing MeOH content. The value of μ_{eo} was decreased from 2.54 to $1.42 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1} \text{ V}^{-1}$ as the MeOH content was varied from 20 to 35%. Resolution factors and theoretical plate counts increased as the MeOH content was increased up to 30% and then decreased with a further increase in MeOH content. While variation of α is marginal variation of resolution factor is much greater. The much greater variation of R_s with MeOH composition is likely due to the much bigger changes in the plate counts, according to the equation, $R_s = 1/4 \cdot N^{1/2} \cdot (\alpha - 1)$.⁴¹ Similar trends were observed for the separations of the remaining compounds. It is thought that MeOH content of 30% is optimal when both migration time and resolution are taken into consideration.

Enantioseparation of a typical analyte, ATR, was studied by varying the concentration of AA-TEA buffer from 20 to 35 mM, and resulting chromatograms and values for α , R_s and N_1 are shown in Figure 3. Migration time was increased with buffer concentration as expected. As the buffer concentration was increased, the magnitude of EOF was decreased due to the reduced double-layer thickness.⁴² Enantioselectivity and resolution were the highest at 30 mM and decreased with a further increase in the electrolyte concentration, which is most likely due to the increased Joule heating that causes molecular diffusion to increase. The plate count did not change significantly with the buffer concentration above 25 mM. Similar trends were observed for the remaining compounds. Buffer concentration of 30 mM was thus chosen for further separations.

The pH of the mobile phase will affect the electromig-

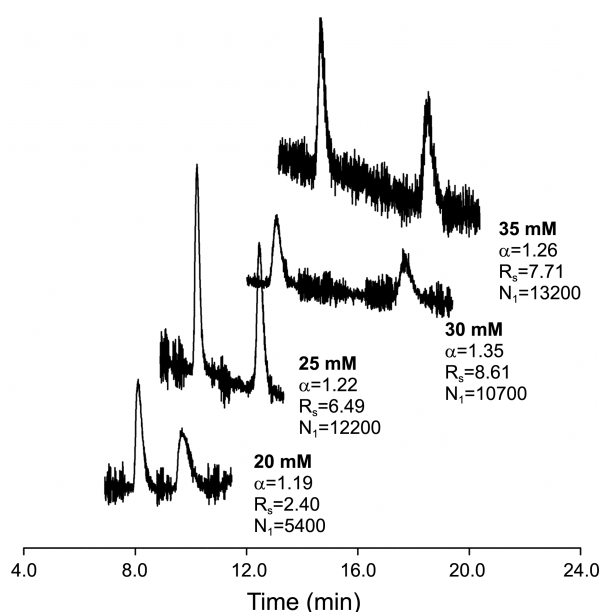


Figure 3. Influence of buffer concentration on enantioseparation of atropine. Mobile phase, 30/70 (v/v) MeOH/AA-TEA buffer (pH 4.0) with 20 mM SCD. Other conditions are as shown in Figure 1.

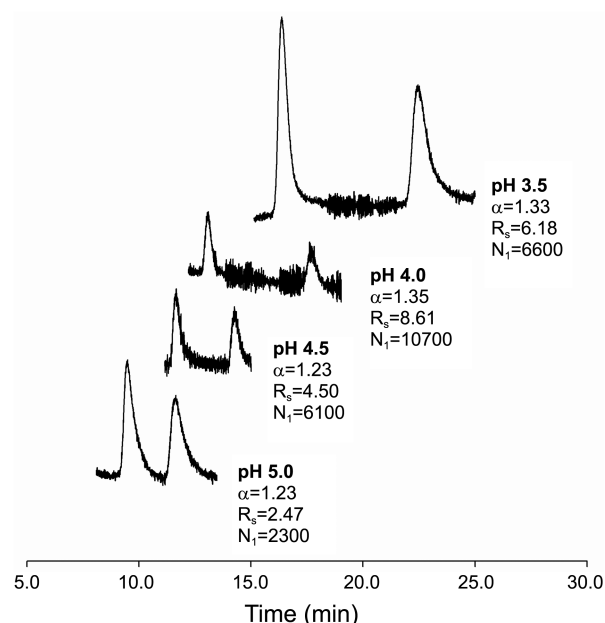


Figure 4. Influence of pH on enantioseparation of atropine. Mobile phase, 30/70 (v/v) MeOH/AA-TEA buffer (30 mM) with 20 mM SCD. Other conditions are as shown in Figure 1.

ration behavior of a basic analyte as it determines the degree of ionization of the analyte and hence the electrophoretic migration behavior. The separation of ATR has been examined at different pH, and the chromatograms are shown in Figure 4 along with α , R_s and N_1 values. As pH was increased from 3.5 to 5 the migration time of ATR was decreased monotonically due to increasing EOF as seen in Figure 2. The fraction of protonated basic molecules will decrease with pH to reduce their electrophoretic migration which is co-directional with EOF, but its extent seems not so big to influence the overall migration driven by the much greater EOF. The highest enantioselectivity and resolution were obtained at pH 4.0, and further increase in pH produced lowered selectivity and resolution. Similar trends were observed for the other compounds.

Chromatograms for the enantioseparation of the six analytes on SCD-ZM are shown in Figure 5. Enantiomers of ATR and HOM were baseline separated. For BEN, OXP and POR partial separations were achieved. BEN has a somewhat shorter migration time than the remaining analytes. BEN has a carboxylic acid group that partially dissociates to give a carboxylate anion at pH of the eluent. Electrostatic repulsion of the carboxylate group against the sulfate group of SCD is likely to hinder inclusion of the analyte into the cavity of SCD, thereby giving a decreased migration time. NAD has three stereogenic centers and is expected to have eight stereoisomers. As the two hydroxyl groups on its cyclohexane ring are conformationally locked in the *cis*-form,⁴³ only four stereoisomers are possible (RSR, SRS, RRS, and SSR). For NAD partial separation with three peaks was obtained. This is favorably compared with the separation of NAD on a chiral stationary phase incorporating multiple chiral-selectors such as crown ether-capped β -cyclodextrin

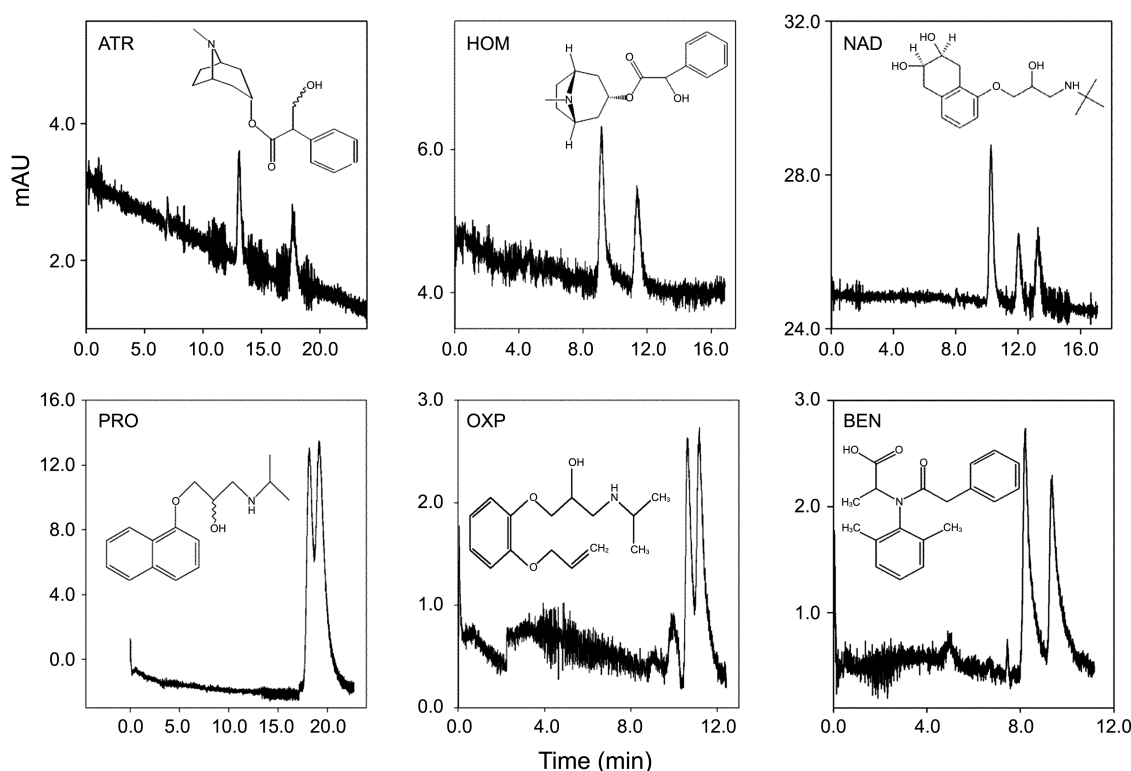


Figure 5. Chromatograms for the chiral separation of the six compounds on SCD-ZM. Mobile phase, 30/70 (v/v) MeOH/AA-TEA buffer (pH 4.0, 30 mM) with 20 mM SCD. Other conditions were the same as in Figure 1.

bonded silica where only two peaks were obtained.⁴⁴ No appreciable decline in resolution and retention after over fifty injections, and run-to-run and day-to-day repeatability of the column of less than 3% indicate that the SCD-ZM column is practically stable in the eluents with added SCD.

Conclusion

Sulfated β -cyclodextrin was adsorbed on zirconia monolith without pretreatment to add cationic moieties as required for silica, and SCD-adsorbed zirconia monolithic capillary was used for the separation of enantiomers of a set of six basic chiral compounds by CEC. The EOF behavior of bare and SCD-ZM column was studied in MeOH/AA-TEA buffer of varying pH, which showed increasing cathodic EOF with pH. Influences of the MeOH and buffer composition, and pH of the eluent on enantiomer separation on SCD-ZM were investigated. A mobile phase containing 30% MeOH and 30 mM buffer of pH 4 provided the best chiral resolutions for the analytes studied.

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