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Short communication

Enantiomer separations of basic chiral compounds by capillary electrochromatography on a phosphated β-cyclodextrin-modified zirconia monolith

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ABSTRACT

Phosphated β-cyclodextrin (PCD)-coated zirconia monolith was used as the chiral stationary phase in capillary electrochromatography (CEC) for separation of four basic chiral compounds including metoprolol (MET), sertraline (SER), citalopram (CIT) and atenolol (ATE). Migration, chiral selectivity and resolution data were measured in reversed-phase mobile phases of varying pH, buffer and organic modifier compositions. Optimum mobile phase conditions for CEC separation of the compounds studied were found to be a 15-mM aqueous buffer of pH 5.0 with 5 mM PCD. Baseline separations of enantiomers of CIT, MET and SER, and partial separation for ATE were achieved with the optimal mobile phase.

1. Introduction

Monolithic columns are becoming an attractive alternative to particle-packed columns in HPLC and electrochromatography [1–3]. The monolithic capillary columns are devoid of the problems and difficulties associated with packed columns, including burdensome packing of stationary phase particles in a capillary and preparation of frits by sintering a zone of the packing that tend to break easily and cause formation of air bubbles [4–7]. The monolithic columns allow faster separations due to high linear velocity and fast mass transfer at lower pressure drops [8].

Cyclodextrins (CDs) and derivatives are among the most commonly used chiral selectors. Recent developments in enantioseparation on the CD-based stationary phases by electromigration methods have been reviewed [9,10]. Charged CDs have been widely used for enantioseparations by capillary electrophoresis (CE) of acidic and basic chiral compounds as electrostatic interactions can be exploited to assist the diastereomer formation between these compounds and charged CDs, which helps further spatially orientated intermolecular interactions through dipolar forces as well as through hydrogen bonding, steric and π–π interactions for chiral recognition [11]. Both positively andnegatively charged CDs have been employed for the chiral separations of acidic, and basic as well as neutral compounds [12]. While negatively charged CDs with sulfoalkyl, sulfato and carboxy groups have been used for the chiral separation by CE [13], phosphated β-CD (PCD) has rarely been used [14–17].

Zirconia is a viable alternative to silica, due to its chemical, mechanical and thermal stabilities [18–20]. Zirconia-based chiral stationary phases (CSPs) have been evaluated in HPLC [21–27] and capillary electrochromatography (CEC) [28–32] in particle-packed and monolithic columns.

In this work PCD was dynamically coated on zirconia monolith (ZM) in the capillary to provide a CSP (PCDZM). Phosphate groups on the PCD are present as anions even in the run buffers of even strongly acidic pH (<2). Lewis basic phosphate groups on PCD would bind strongly onto the Lewis acid sites of the zirconia [33,34]. to provide a stable layer of PCD-modified CSP on the zirconia monolith. A PCDZM column was used for the enantiomer separation by CEC of a set of four basic chiral compounds in aqueous organic mixtures containing PCD as the eluent. Dual chiral recognition systems employing the chiral selectors in both the stationary and mobile phases have been utilized in a bid to attain enhanced enantioselectivity [35–37]. It was shown that when the negatively charged chiral selectors present in both stationary and mobile phase impart the same enantioselectivity on the analyte the selectivity enhancement has been observed [35,36]. The use of the PCD-based CSP in CEC has not been reported. Influences of pH and the content of PCD, organic modifier, buffer in the eluent on enantioseparation were investigated.

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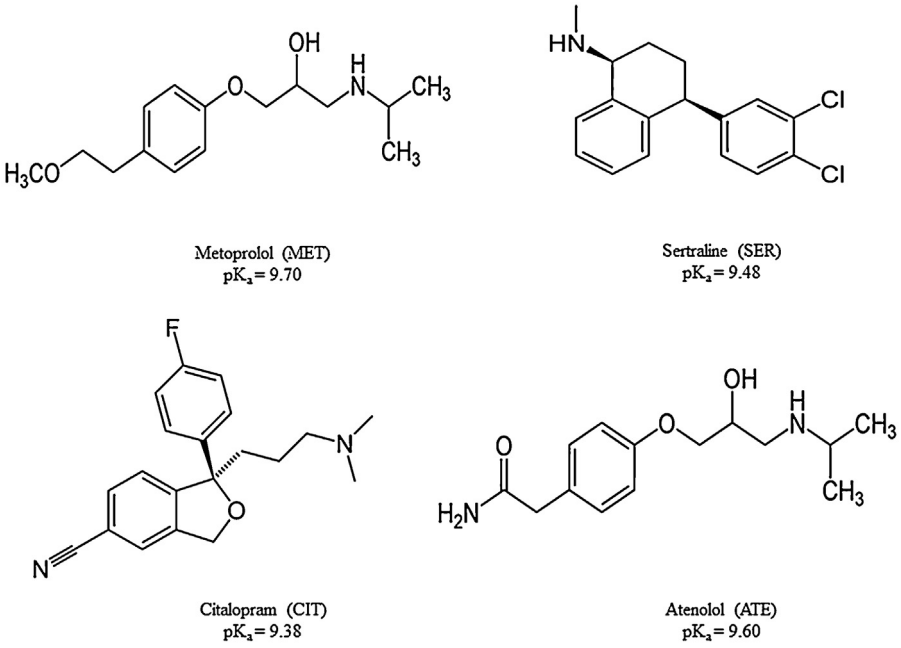

Fig. 1. Structures of chiral compounds studied.

2. Experimental

2.1. Materials

Fused silica capillaries (50 μm I.D., 365 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). β-Cyclodextrin, phosphorus oxychloride, anhydrous pyridine, zirconium butoxide in *n*-butanol, acetic acid, polyethylene glycol (PEG) (MW = 10,000 g mol^{-1}), potassium dihydrogen phosphate and sodium hydroxide were obtained from Sigma-Aldrich (Milwaukee, WI, USA) or TCI (Tokyo, Japan). All reagents used were reagent grade or better. HPLC-grade acetonitrile (ACN) and methanol (MeOH) were obtained from J.T. Baker (Phillipsburg, NJ, USA). Water was purified with an Elgastat UHQ water purification system (Bucks, UK). Basic chiral compounds including metoprolol (MET), sertraline (SER), citalopram (CIT) and atenolol (ATE) were of the highest-purity available from Aldrich (Milwaukee, WI, USA) or TCI (Tokyo, Japan). Structures of the chiral compounds studied are shown with their pK_a values in Fig. 1. PCD was prepared according to the procedure in the literature [15].

2.2. Instrumentation

An Agilent HP ^{3D}CE 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. An external pressure of 9 bar was applied to both reservoirs. Instrument control and data collection were performed with the ChemStation software. Separations were carried out at 25 °C and monitored at 200, 214, 234, 254 and 280 nm. 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 PCD solution into the zirconia monolithic capillaries.

2.3. Column preparation

Zirconia monolithic capillary column with total length of 35 cm and monolithic bed length of 25 cm was prepared according to the method reported earlier [30]. PCDZM column was obtained by washing the zirconia monolithic capillary with MeOH and then by passing the PCD solution in water (30 mg mL^{-1}) through the capillary for 2 h at a flow rate of 5 μL min^{-1} using a syringe pump. The coated capillary was then rinsed with the mobile phase. The SEM image is given in the Supplementary information (Fig. S1).

2.4. Chromatography

The eluents used for the enantioseparation were phosphate buffers or phosphate buffers mixed with ACN or MeOH. Varying amounts of PCD were added to the mobile phase in order to investigate the effect of the PCD on the enantioseparation on the PCDZM. The mobile phases were then filtered through a nylon membrane filter of 0.2-μm pore size and degassed prior to use. The PCDZM capillary was flushed with the run buffer for 8–10 h prior to CEC runs. Samples dissolved in the eluent were injected electro-kinetically at 10 kV for 3 s. Migration times of two consecutive injections were in agreement within 3%. Fresh eluent was replenished after each run of sample.

3. Results and discussion

3.1. Influence of PCD concentration

The PCDZM showed cathodic EOF as seen in Fig. 2, which shows variation of electroosmotic mobility (μ_{eo}) with PCD concentration in the buffer. Cathodic EOF was observed due to the negative surface charges from dissociated surface zirconol groups and PCD ions adsorbed on the surface [30,38]. The magnitude of EOF decreased with an increase in the PCD concentration in the buffer, which resulted in increased ionic strength and hence reduced zeta potential.

Fig. 3 shows chromatograms for the chiral separation of two typical analytes, MET and SER, in buffers with varying PCD content, along with resolution (R_s) and apparent enantioselectivity ($\alpha = t_2/t_1$, where t_1 and t_2 are migration times of the first and second eluting enantiomer, respectively). Resolution and selectivity increased as the PCD concentration was increased from 2